ISSC

Interstate Shellfish Sanitation Conference

SUMMARY OF ACTIONS

2011 Biennial Meeting

Seattle, Washington
October 1 - 7, 2011

Proposal Subject: *Vibrio vulnificus* Risk Management Plan for Oysters exemption for licensed shellfish harvesters and certified dealers who produce fewer than 1.5 million raw oysters per year and/or sell all of their oysters directly to retailers.

Specific NSSP Guide Reference: Section II, Chapter II Risk Assessment and Risk Management @.04 *Vibrio vulnificus* Risk Management for Oysters, New B.

Text of Proposal/ Requested Action Add a new section; Section II, Chapter II Risk Assessment and Risk Management @.04 B. *Vibrio vulnificus* Risk Management for Oysters.

A. For states having 2 or more etiologically confirmed shellfish-borne *Vibrio vulnificus* illnesses since 1995 traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state (Source State), the Authority shall develop and implement *a Vibrio vulnificus* Management Plan.

B. **Exemptions. This section does not apply to licensed shellfish harvesters and certified shellfish dealers who produce fewer than 1.5 million raw oysters per year and/or sell all of their oysters directly to retailers.**

~~B.~~ **C.** The Source State's *Vibrio vulnificus* Management Plan shall define the administrative procedures and resources necessary to accomplish (i.e. establish and maintain) involvement by the state in a collective illness reduction program. The goal of the *Vibrio vulnificus* Management Plan will be to reduce the rate of etiologically confirmed shellfish-borne *Vibrio vulnificus* septicemia illnesses reported collectively by California, Florida, Louisiana, and Texas, from the consumption of commercially harvested raw or undercooked oysters by 40 percent for years 2005 and 2006 (average) and by 60 percent for years 2007 and 2008 (average) from the average illness rate for the years 1995 -1999 of 0.303/million. The list of states (California, Florida, Louisiana, Texas) used to calculate rate reduction may be adjusted if after a thorough review, epidemiological and statistical data demonstrates that it would be appropriate. The illness rate shall be calculated as the number of illnesses per unit of population. The goal may be reevaluated prior to the year 2006 and adjusted in the event that new science, data, or information becomes available. State's compliance with the Plan will require States to maintain a minimum of 60% reduction in years subsequent to 2008. Determination and compliance after 2008 will be based on two-year averages beginning in 2009.

Public Health Significance: The *Vibrio vulnificus* Risk Management Plan for Oysters was introduced to the ISSC as being modeled after the U.S. Egg Safety Action Plan. The NSSP which has been in existence since 1925 is far more restrictive than FDA's October 2004 proposed rule for Egg Safety and the Prevention of Salmonella Enteritidis in Shell Eggs During Production and certain egg producers.

The most recent data from the Centers for Disease Control and Prevention (CDC) show that SE illnesses have essentially remained steady for the past several years. CDC estimated that 118,000 illnesses were caused by consumption of SE-contaminated eggs in 2001. Accordingly, FDA believes that further actions to improve egg safety--building upon the safe consumer handling labeling and egg refrigeration at retail rule of 2000--are the most effective way to achieve our public health goals of a 50% reduction in overall salmonellosis and a 50% reduction in SE outbreaks by 2010.

	In comparison to an annual average of less than 40 *V.v.* infections to high-risk consumers that are attributed to shellfish, approximately half of those persons infected die, there are approximately 40,000 cases of salmonellosis reported in the United States annually. Because many milder S.E.cases are not diagnosed or reported, the actual number of S.E. infections may be thirty or more times greater. It is estimated that approximately 600 persons die each year with acute salmonellosis. Just as with *V.v.* infections, Salmonellosis infections are more common in the summer than winter. Young children, the elderly, and the immunocompromised are the most likely to have severe S.E. infections. Since the FDA has proposed a rule that exempts certain egg producers from the rule and the rule is far less burdensome to the egg industry than the *Vibrio vulnificus* Risk Management Plan for Oysters is on the Gulf oyster industry, an exemption should be given to oyster producers as suggested.
Cost Information (if available):	None
Action by 2005 Task Force I	Recommended referral of Proposal 05-100 to the appropriate committee as determined by the Conference Chairperson.
Action by 2005 General Assembly	Adopted recommendation of 2005 Task Force I.
Action by USFDA	Concurred with Conference action.
Action by 2007 Vibrio Management Committee	Recommended adoption of Proposal 05-100 as a research need. More data is needed on the number of small harvesters and the number of small dealers; the percentage of all harvesters and dealers in the affected states that are in this category; the number of illnesses attributable to these small harvesters or dealers; other food commodities that allow exemptions from public health requirements based on the small size of the harvester/producer/processor; and the pathogens of concern with these other foods.
Action by 2007 Task Force I	Recommended adoption of the Vibrio Management Committee recommendation on Proposal 05-100.
Action by 2007 General Assembly	Adopted recommendation of 2007 Task Force I.
Action by USFDA	December 20, 2007 Concurred with Conference action.
Action by 2009 Research Guidance Committee	Recommended no action. Rationale: No data presented.
Action by 2009 Task Force I	Recommended referral of Proposal 05-100 to the Executive Board. The Task Force stongly urges the Executive Board to identify approaches to gather the information necessary for further deliberation of the issue.

Action by 2009 General Assembly	Adopted recommendation of 2009 Task Force I on Proposal 05-100.
Action by Executive Board 10/23/2009	Approved referral of Proposal 05-100 to the *Vibrio* Management Committee. The *Vibrio* Management Committee will be asked to hold a conference call within the next 30 days to identify the types of information needed and who best can acquire that data.
Action by USFDA 02/16/2010	Concurred with Conference action on Proposal 05-100 with the following comments and recommendations for ISSC consideration. While FDA agrees to participate in Vibrio Management Committee discussions to identify approaches for gathering information that may further deliberation on Proposal 05-100, it is the Agency's current thinking that exemption of any harvester or dealer, regardless of operational size, from NSSP *Vv* controls is not an appropriate public health approach. FDA considers it essential that all harvesters and all dealers employ NSSP *Vv* control measures. Any allowance for exemption would be contrary to the food safety and public health protection initiatives of the NSSP. In consideration of the ongoing and developing efforts to address *Vv* illnesses and deaths, FDA believes it would be more prudent for the Executive Board to take no action on Proposal 05-100, or at least table further consideration pending consideration of Proposal 00-201.
Action by 2011 Task Force I	Recommended no action on Proposal 05-100. Rationale – Adequately addressed in the Model Ordinance.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 05-100.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 05-100.

Proposal Subject: Rapid Screening Method for ASP

Specific NSSP Guide Reference Section II. Model Ordinance Chapter III Laboratory @.02 Methods
ISSC Constitution, ByLaws, and Procedures
Procedure XVI. Procedure for Acceptance and Approval of Analytical Methods for the NSSP.

Text of Proposal/ Requested Action For many years, there has been an expression of need by regulatory agencies and industry to develop a test to monitor ASP levels with precision and accuracy.

The method developed by Jellett Rapid Testing Ltd has been presented to the ISSC and other regulatory bodies over the past several years. In cooperation with individuals, governments and those organizations, the analytical method has been refined and improved. The Rapid Test kits have been tested in several states and foreign countries, and JRT has some internal papers, including one done by Mike Quilliam, that are now in preparation and should be submitted/in press by the time of the ISSC meeting. There are some talks coming up ICMSS, CWHMA where the ASP test will be presented, and from which there will be proceedings later this year or early next year.

It should be noted that this test is built on the same platform by the same company, and uses a similar format to the Jellett Rapid Test for PSP that is already accepted by the ISSC.

The CONSTITUTION BY-LAWS and PROCEDURES of the INTERSTATE SHELLFISH SANITATION CONFERENCE allow the ISSC, through the Laboratory Methods Review Committee, to accept analytical methods that are sufficiently validated but are not AOAC or APHA methods. This is defined in the Constitution, PROCEDURE XVI. PROCEDURE FOR ACCEPTANCE AND APPROVAL OF ANALYTICAL METHODS FOR THE NSSP. Two possible reasons for considering a method are found in Subdivisions i and ii.

Subdivision i. Meets immediate or continuing need;
Subdivision ii. Improves analytical capability under the NSSP as an alternative to other approved or accepted method(s)

Currently, Table 4 of Chapter II.10 allows the use of any "Peer recognized HPLC Methods with or without clean up." for ASP analysis. The need for standard methods has been expressed by regulatory agencies, governmental organizations and industry for many years. The Jellett Rapid Test for ASP has been validated over a wide geographic area to demonstrate its simplicity, reliability, precision and accuracy. As a result of ongoing improvements and demonstrations of efficacy, and the need that has been expressed by industry and state agencies, the Jellett Rapid Test for ASP is presented as a screening method for the NSSP as a Type III or Type IV method.

Please see attached additional information.

Suggested wording:
Section II, Chapter III Laboratory @.02 Methods

C. Biotoxin. Methods for the analyses of shellfish and shellfish harvest waters shall be:
(1) The current AOAC and APHA methods used in bioassay for paralytic

shellfish poisoning toxins; and

(2) The current APHA method used in bioassay for Karemia breve toxins.

(3) The Jellett Rapid Test for ASP may be used as a screening method for ASP toxins by regulatory and industry laboratories.

Public Health Significance:

Currently, only data from certified laboratories conducting ASP analyses using any "Peer recognized HPLC Methods with or without clean up" are considered reliable and acceptable. Because of many significant constraints, in practical terms, this means that only state laboratories (in the US, governmental laboratories in other countries) can provide acceptable data at this time using methods not specifically defined by the ISSC. Acceptance of the Jellett Rapid Test for ASP would allow harvesters, processors, and regulatory agencies to screen for ASP with an accepted standardized method that provides valid useable data.

The Jellett Rapid Test for ASP was developed over several years in answer to the oft-stated need for a rapid, reliable, non-animal analytical method. The Jellett Rapid Test for ASP is not meant to be a definitive "Standard Method", but rather to augment "Peer recognized HPLC Methods…" by providing an additional tool that is currently not available.

Possible applications for The Jellett Rapid Test for ASP include:

- as a method of screening out negative samples in shellfish regulatory labs;
- as a harvest management tool at aquaculture facilities or in wild shellfish harvest areas (especially nearshore areas) to determine if shellfish are free of ASP and safe to harvest; as a quality control tool for shellfish processing plants, distributors and wholesalers to ensure incoming shellfish are free of ASP toxins before processing or further distribution (this test could become part of the plant's HACCP program);
- as a tool for water classification for biotoxins;
- to assist in site selection for aquaculture activity;
- as a screening tool for toxic phytoplankton in seawater to provide an early warning for shellfish growers; and
- as a research tool for broad scale ecological monitoring.

The rationale for using the Jellett Rapid Test for ASP is that the kits provide a cost-effective screen (especially in low-volume laboratories) for ASP that can provide a standardized test for screening and substantially reduce the cost of analyses. The same extract is used for the Rapid Test that is used for HPLC, so the Jellett Rapid Method extract can easily be sent for a confirmation in another lab if necessary. As a harvest management tool, the use of the Jellett Rapid Test for ASP will supplement regulatory agency efforts and help prevent the harvest of contaminated product. Having the ability to conduct tests using an accepted standardized method will allow those processors who choose to use this test to demonstrate that they are truly controlling for ASP hazards in the harvested shellfish.

The Jellett Rapid Test for ASP could be used to build long-term databases on a broader scale than a regulatory lab can afford and, by using a standardized method, will provide consistent results. These databases could be supplemented with industry testing in areas where there is no testing currently. This would extend, augment and strengthen the current food safety system broadening and refining the food safety net by increasing the number of testing sites and generating long term data in more areas.

HPLC is expensive and highly technical, requiring a large capital and personnel investment. HPLC machines, like other analytical equipment, also break down regularly. Therefore there needs to be backup HPLC machines OR other methods available.

A simple, rapid, effective, reliable test, available to all harvesters, regulators, and processors, would increase the monitoring and reduce the chance that shellfish containing ASP toxins above the regulatory limit would be harvested or marketed.

Cost Information (if available): Each test kit costs $20 (**€18)**. It has been reported that each analysis using the HPLC costs approximately $140 per test. History has shown that large numbers of ASP monitoring samples are negative. The costs cited do not take into account the costs associated emergency closures, recalls, or providing medical care to those affected by toxic shellfish. Also, some states are interested in the test because they do not have to invest in HPLC technology if they have the Rapid Test as an alternative.

Action by 2005 Laboratory Methods Review Committee Recommended that Proposal 05-109 be referred to the appropriate committee as determined by the Conference Chairman.

Action by 2005 Task Force I Recommended adoption of the Laboratory Methods Review Committee recommendation on Proposal 05-109.

Action by 2005 General Assembly Adopted recommendation of 2005 Task Force I.

Action by USFDA Concurred with Conference action.

Action by 2007 Laboratory Methods Review Committee Recommended no action on Proposal 05-109. Rationale – Method needs modification because of changes to the antibody. In addition, there is insufficient data to demonstrate acceptability to the Conference. The submitter is requested to provide data to the Executive Office for approval.

Action by 2007 Task Force I Recommended referral of Proposal 05-109 to an appropriate committee as determined by the Conference Chairman.

Action by 2007 General Assembly Adopted recommendation of 2007 Task Force I.

Action by USFDA December 20, 2007

Concurred with Conference action with the following comments and recommendations for ISSC consideration.

The Conference has made considerable progress in its efforts to recognize new and developing analytical methods for the detection of indicators, pathogens, and marine toxins. Much credit goes to the Laboratory Methods Review Committee and its leadership for ensuring a scientifically defensible process for adopting analytical methods under the NSSP.

At the 2007 meeting numerous analytical methods were proposed for ISSC adoption. However, many of these methods were lacking the validation and associated data needed by the Laboratory Methods Review Committee to make a final determination regarding their efficacy for use in the NSSP. As a result the General Assembly voted "No Action" on analytical method Proposals 05-107, 05-108, 05-109, 05-111, 05-113, and 05-114. It is FDA's understanding that the intent of the "No Action" vote was not to remove these Proposals from ISSC deliberation as "No Action" normally suggests, but rather to maintain them before the Conference pending submission of additional data for further consideration. The Voting Delegates, by requesting the Proposal submitters provide additional data to the Executive Office for methods approval consistent with Procedure XVI, clearly recognized the importance and utility of these methods and intended to maintain them before the Conference for possible adoption following additional data submission. FDA requests that the ISSC Executive Board confirm FDA's understanding of this outcome. FDA fully supports such a Conference action and encourages the Executive Office to pursue submission of additional data as necessary to move forward with acceptance of these methods.

Action by 2009 Laboratory Methods Review Committee

Recommended no action on Proposal 05-109. Rationale: Requested additional information has not been submitted.

Action by 2009 Task Force I

Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 05-109.

Action by 2009 General Assembly

Referred Proposal 05-109 to the Laboratory Methods Review Committee.

Action by USFDA 02/16/2010

Concurred with Conference action on Proposal 05-109.

Action by 2011 Laboratory Methods Review Committee

Recommended no action on Proposal 05-109.

Rationale: The submitter informed the ISSC that he was no longer pursuing approval of this method.

Action by 2011 Task Force I

Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 05-109.

Action by 2011 General Assembly

Adopted recommendation of 2011 Task Force I on Proposal 05-109.

Action by FDA February 26, 2012

Concurred with Conference action on Proposal 05-109.

Laycock, Maurice V., Joanne F. Jellett, W. Hywel Morgan. 2004. Characteristics and Applications of the Jellett Rapid Tests for PSP and ASP. *In:* Holland, Patrick and Michael A. Quilliam, (Eds.) Proceedings 2nd HABTech 2003 Workshop, Nelson, New Zealand. Nov 26-30, 2003.

Characteristics and Applications of the Jellett Rapid Tests for PSP and ASP

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Abstract
The Jellett Rapid Tests for PSP and ASP toxins were tested with calibration standards to investigate sensitivities to individual toxins spiked into mussel extracts at concentrations around the regulatory limits. PSP test strips showed their highest sensitivity to saxitoxin (Stx) and gonyautoxins-2 and -3 (Gtx2/3) and were least sensitive to Gtx1/4 and neosaxitoxin (Neo). Sensitivities were intermediate to mixtures of Stx with Neo and to Gtx1/4 with Gtx2/3, which are more typical of naturally occurring PSP toxin profiles. All of the PSP toxins that were tested gave positive responses at or below the regulatory limit. The ASP test detected domoic acid at around 5 $\mu g.g^{-1}$, well below the regulatory limit. Uses for the Rapid Tests for screening in regulatory laboratories and testing in field conditions for PSP toxins and domoic acid in shellfish and phytoplankton are discussed.

Key words
Paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), lateral flow immunochromatography (LFI), saxitoxin, domoic acid, test kits.

Introduction

Shellfish toxicity and food safety have been monitored successfully by mouse bioassays (AOAC, 1999) for more than fifty years. The current trend toward replacement methods has resulted in the development of more sophisticated methods such as liquid chromatography with mass spectrometric or fluorescence detectors. They not only provide a higher degree of accuracy and sensitivity but individual toxins can be identified in complex mixtures. However, aside from the high capital cost of the instruments, their maintenance and requirement for a well equipped laboratory and trained staff, sample clean up has been an on going problem. Antibody methods, such as ELISA require little sample preparation and equipment is relatively inexpensive. However, ELISA methods are slow and cannot be easily carried out outside the laboratory, or in unskilled hands.

Lateral flow immunochromatography (LFI) is an alternative format for antibody detection of shellfish toxins. The self-contained simplicity and reliability of these test strips has found applications in many areas such as screening for illicit drugs and home pregnancy testing. They are essentially yes/no tests engineered to indicate a specific analyte concentration. We have developed LFI tests for PSP and ASP toxins and one for DSP toxins is being developed. The absence of a coloured test line on the strip indicates that the sample contained the toxin at a concentration around half the regulatory limit. Because most samples tested by regulatory agencies are negative, LFI tests can be used to screen a large number of samples quickly and only those with toxin concentrations above or approaching regulatory limits need to be tested further, thereby speeding through-put, reducing costs and the number of mice used in bioassays. In addition to growing acceptance of the PSP and ASP test strips by regulatory agencies, they are also being tested in isolated communities, by shellfish farmers and for phytoplankton monitoring.

The Jellett Rapid Test for PSP (formerly, MIST Alert) is based on antibodies that recognise all of the saxitoxin (Stx) and neosaxitoxin (Neo) analogues, but not equally. Our first publication (Laycock et al., 2001) describing the characteristics of the PSP test showed relative sensitivities to a range of purified PSP toxins. All fell within the regulatory limit. Sensitivities to Neo and its 11-sulphated gonyautoxin analogues (Gtx1/4) were about five fold less than to Stx and its analogues. Detection levels for the sulfamate analogues of Stx (C1/2 and B1) fell

between the two (Gtx2/3 and Gtx1/4) extremes. The PSP test has been subjected to extensive field trials (Jellett et al., 2002; MacIntosh et al., 2002) which showed no false negatives in over two thousand samples. Extracts containing only Gtx1/4 or Neo are rare but if encountered at concentrations close to the regulatory limit, would they fall within the detection limit of the test? We have examined this question with spiked samples containing only Gtx1/4 and Neo and the effect of the presence of other PSP toxins in the profile.

The ASP test has also been subjected to independent testing and shown to be easy to use and reliable (MacIntosh and Smith, 2002). The detection limits of the ASP test were examined in a similar manner to the PSP test with a calibration standard and the data are presented.

Materials and Methods

The LFI test strips are manufactured by Jellett Rapid Testing Ltd. with stringent quality control to ensure reproducibility. Test strips are contained in plastic cassettes with a sample well and a window. A test line (T-line) and a control line (C-line) can be seen in the window about 15 min after applying a sample. In the absence of toxin, both lines can be seen. For samples containing toxin in concentrations greater than the regulatory limit, no T-line appears, and only the C-line is seen. No clean-up is necessary but extracts must be diluted to 20% (1:5) for PSP and to 10% (1:10) for ASP with a buffer solution supplied with the tests to ensure the proper solution conditions for the test to function. This is indicated by the formation of a visible C-line.

Non-toxic mussels were homogenised and extracted by the AOAC extraction procedures for PSP with 0.1 N HCl (AOAC, 1999). Samples of this control extract were spiked with purified PSP toxin calibration solutions obtained from the National Research Council of Canada. The total molar concentration of separate or mixed toxins was the same for each spiked extract. A series of dilutions was prepared from the highest concentration of 3200 nM with control extract. The prepared samples were then diluted 1:5 with buffer solution. Test units were removed from their sealed pouches and 100 µl of the buffered samples was applied to each sample well. After 15 min, test and control lines were fully developed and the results digitised using a conventional computer scanner. T-line intensities were measured using Softmax Pro software (Molecular Devices, CA). Five replicate measurements were taken and each converted to percent of the maximum line intensity at zero toxin concentration.

For ASP, a non-toxic mussel homogenate was extracted into four volumes (1:5) of 50% aqueous methanol. A sample of this methanolic extract was spiked with a calibration standard of domoic acid to equivalent of 20 $\mu g.g^{-1}$ tissue and a dilution series was prepared by serial dilution using the non-toxic, control extract. A running buffer solution designed for the ASP test was then added (1:10) to the different concentrations in the series. Samples (100 µl) at each concentration were applied to the test strips and the results recorded by scanning.

Results

PSP

The five values for T-line colour were plotted against toxin concentration in spiked extracts before dilution 1:5 with the running buffer. The slopes and positions of the different curves reflect the proportions of toxins recognised differently by the antibodies. Plots of T-line intensities against toxin concentrations showed a lower sensitivity to Neo than to Stx, so that a weak T-line persisted with samples containing Neo alone at 1300 nM. This is approximately at the PSP regulatory limit of 80 µg per 100 g tissue (calculated for Stx as the free base) in an AOAC extract. The test showed the highest sensitivity to Stx and the plot from samples containing only Stx is shown together with that for Neo in Fig. 1A to illustrate the range of sensitivities.

Data for the sensitivities to Gtx2/3 and Gtx1/4 are plotted together in Fig. 1B. The PSP test had the lowest sensitivity to Gtx1/4. At the regulatory limit for Stx (1300 nM), T-line intensity was reduced to about 60% of that obtained with a non-toxic sample and 90% at twice that concentration. At 1300 nM Gtx2/3 reduced the T-

line by 95%. Responses to equimolar mixtures of Stx with Neo and Gtx1/4 with Gtx2/3 are shown in Fig. 1C. Both curves indicate 90% reduction of T-line intensity for total toxin concentrations at the regulatory limit. A reduction of T-line intensity of 50% is interpreted as positive. Toxin concentrations at 50% decrease in T-line intensity are shown on the graphs by narrow vertical lines.

ASP

The sensitivity of the ASP test was well within the regulatory limit of 20 $\mu g.g^{-1}$. Figure 2 shows that in samples containing 5 $\mu g.g^{-1}$ in a methanol extract, the T-line intensity was 80% reduced, and 90% at 10 $\mu g.g^{-1}$, from that obtained with non-toxic extracts. The domoic acid concentration in methanolic extracts that resulted in a 50% decrease in T-line intensity, which is interpreted as positive, was 2.5 $\mu g.g^{-1}$. Spiked AOAC extracts were also tested. The tissue concentration in an AOAC extract is 2.5 times that in a methanolic extract and the 50% T-line was around 1.0 $\mu g.g^{-1}$. The ASP test was found to be more susceptible to a matrix effect with higher concentrations of tissue causing a decrease in C and T-line intensities. This difference between extraction methods was common with 1:5 dilutions in running buffer but not at with 1:10 dilutions. The latter dilution therefore was adopted for the ASP test.

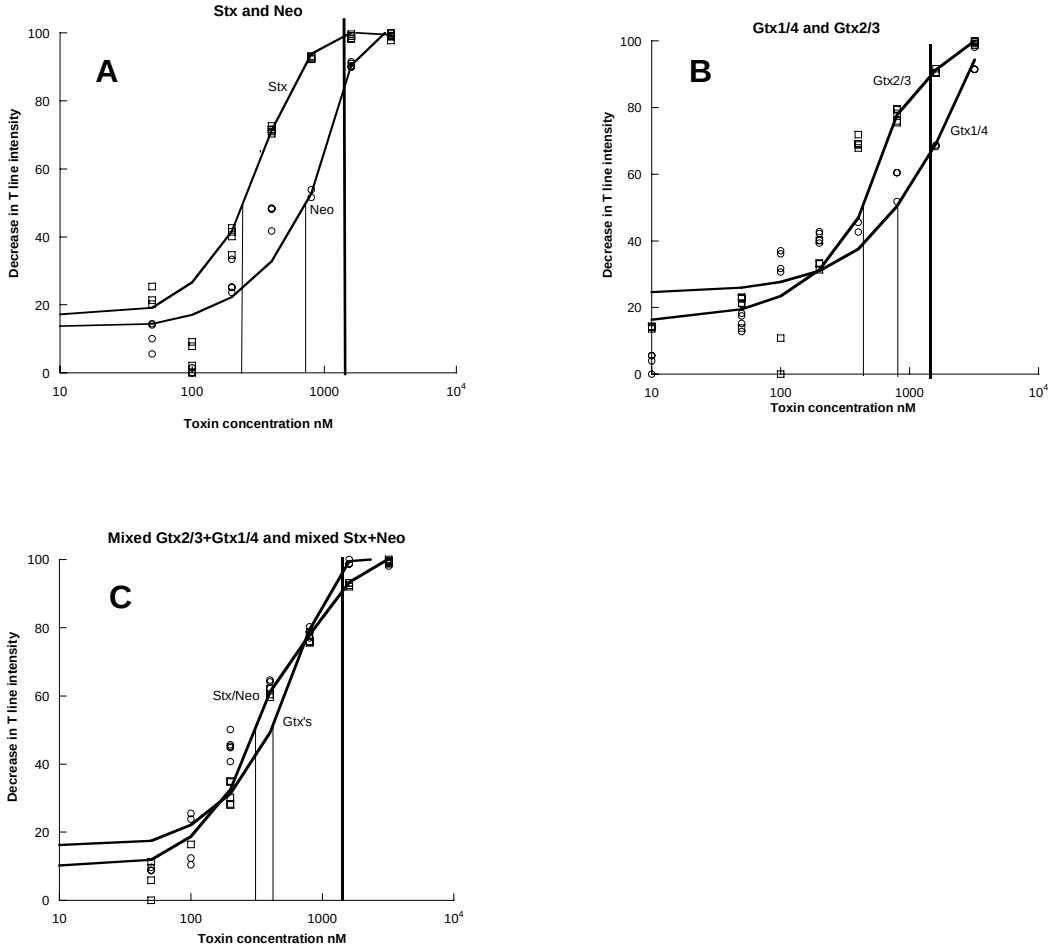

Figure 1.

Non-toxic mussel homogenate was extracted by the AOAC method into an equal volume of 0.1 M HCl. Samples were spiked with NRC certified toxin standards to 3200 nM. Dilution series were prepared by mixing

with non-toxic extract. The extracts containing different toxin concentrations were then mixed 1:5 with PSP running buffer solution and 100 µl applied to the test strips. After 20 min. T line intensities were measured by scanning into a computer and digitising (Softmax, Molecular devices, CA). The regulatory limit of 80 µg/100 g is indicated by the heavy vertical line and fine vertical lines indicate toxin concentrations at 50% decrease in T-line intensity.

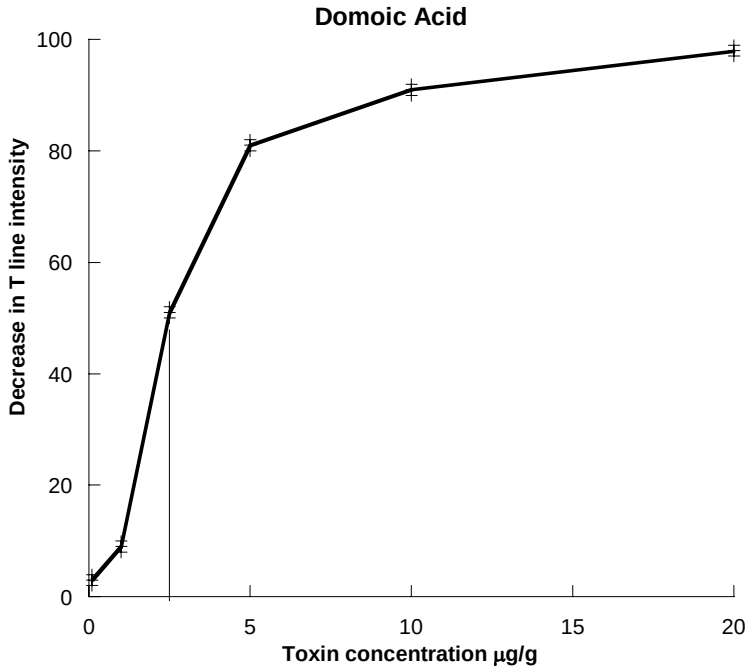

Figure 2.
Non-toxic mussel homogenate was extracted into four volumes of 50% methanol a sample spiked with domoic acid to 20 µg/g homogenate. Serial dilutions were made with non-toxic extract and mixed with ASP running buffer solution. A sample (100 µl) of each solution was applied to each test strip. Line intensities were measured as described in the legend to Fig. 1. The regulatory limit for ASP is 20 µg/g. The vertical line indicates the toxin concentration at 50% decrease in T-line intensity.

Discussion
The Jellett Rapid Tests for PSP and ASP are designed to indicate the presence of toxins in shellfish and phytoplankton at concentrations around half the regulatory limit for Stx and domoic acid in shellfish. Experiments with purified PSP toxins show that responses to different analogues are not equal (Laycock, et al., 2001). Also, at toxin concentrations around the regulatory limit T-line intensities may be intermediate. At lower and higher concentrations the T-line is either equal in intensity to the control line or it is absent. The recommended way to interpret tests that show T-lines of intermediate intensity is by comparison with the C line. In the absence of toxin T and C-line intensities are equal. If the T-line appears to be 50% or less intense than the C-line the test is considered to be positive, indicating that the extract contained significant amounts of the toxin. If no T-line appears, toxin concentrations may be well above the regulatory limit. In this case, concentrations

may be estimated by making serial dilutions with non-toxic extract. The recommended dilution with running buffer solution (1:5 for PSP and 1:10 for ASP) should be maintained and serial dilutions are prepared with non-toxic extract. A lower ratio of buffer to extract will increase the concentration of toxin in the sample but, depending on the extracted tissue, a matrix effect may be seen by diminished control line intensity.

The PSP test is least sensitive to Gtx1/4 and Neo. However, these analogues rarely occur in the absence of Stx, and more especially Gtx2/3, which is the most common of all the PSP toxins found in shellfish. The Rapid Test for PSP has shown the highest sensitivity for both of these toxins. Experiments to examine test responses to samples containing toxin profiles such as those for which the test is least sensitive were possible only with samples spiked with purified toxins of known concentrations. The results presented here show that only for extracts containing Gtx1/4 alone, at concentrations close to the regulatory limit, the test response may be intermediate between clearly positive or negative. The effect of mixed toxins increased sensitivity to samples containing Gtx1/4 and Neo. This is illustrated in Fig. 3 in which equimolar concentrations of Gtx2/3 with Gtx1/4 and Stx with Neo resulted in responses well within the regulatory limit. In an earlier publication (Laycock et al., 2001) the test was called MIST Alert but is now the Jellett Rapid Test for PSP. It should be noted that the earlier data were presented as toxin concentration before dilution (1:5) with running buffer solution. Current test strips are similar to those produced earlier with comparable sensitivities to the different PSP toxin analogues. Sensitivities to the sulfamate toxins C1/2 and B1 are not presented here but as shown earlier they fall between Neo and Stx. The decarbamoyl analogues of Stx have also been tested and responses were very similar to their corresponding carbamates.

Both the PSP and ASP tests have been subjected to extensive independent field trials (Jellett et al., 2002; MacIntosh et al., 2002; MacIntosh and Smith, 2002) with naturally occurring toxic shellfish. Based on the encouraging results of these trials the Rapid Tests for shellfish toxins are being adopted for routine use in monitoring programs. The test strips provide a reliable screening tool for regulatory agencies, costing significantly less than alternatives for shellfish monitoring, such as the mouse bioassay or HPLC. Screening out the high proportion of negative samples to be tested further not only reduces the overall cost it also increases the rate at which samples can be monitored. In addition to testing for toxins in shellfish the Rapid Tests can be used to test for toxicity in samples from plankton nets. *Alexandrium* and *Pseudo-nitzschia* cells were easily extracted into 0.1 M acetic acid without mechanical disruption providing a simple and sensitive field method for phytoplankton monitoring (Rafuse et al., 2002).

The Rapid Tests are essentially self-contained and extracts can be tested without laboratory equipment, allowing their use at shellfish farms, on boats, beaches or camps. However, for use in field conditions the preparation of shellfish extracts is more difficult than in a laboratory. Ineffective extraction could lead to false negatives, especially for samples with toxin concentrations close to the test strip detection limit. Kits are supplied with detailed instructions about making extracts from shellfish or plankton as extraction is a crucial part of the test procedure.

Acknowledgements
The authors thank Dr. Michael Quilliam for the toxin standards used in this study and for his continuing support. Dorothy Easy and Mary Anne Donovan provided technical help.

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Mackintosh F.H., Smith, E.A., 2002. Evaluation of MIST Alert™ Rapid Test Kits For the Detection of Paralytic and Amnesic Shellfish poisoning Toxins in Shellfish. Journal of Shellfish Research. 21, 455-460.

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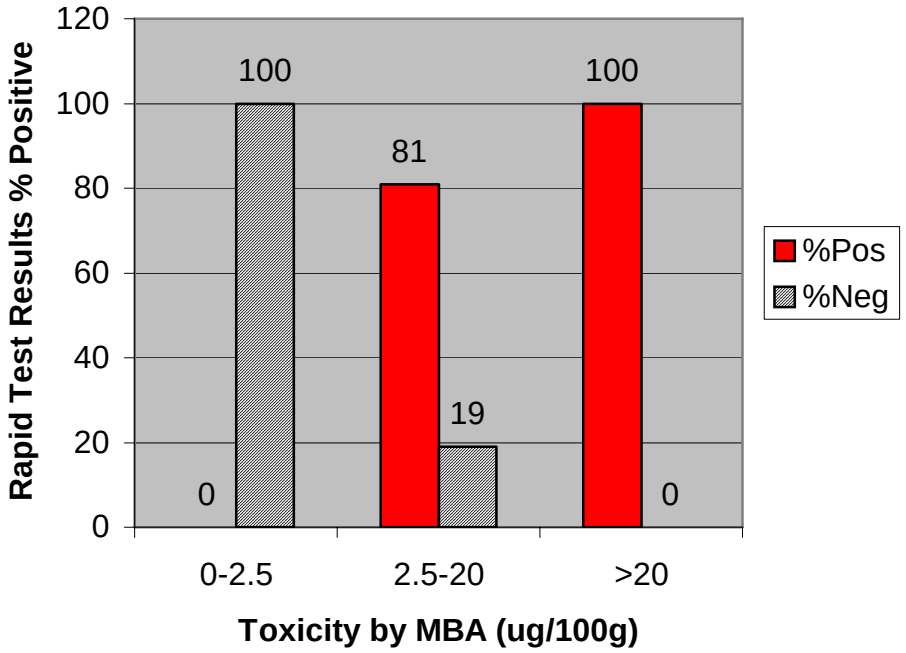
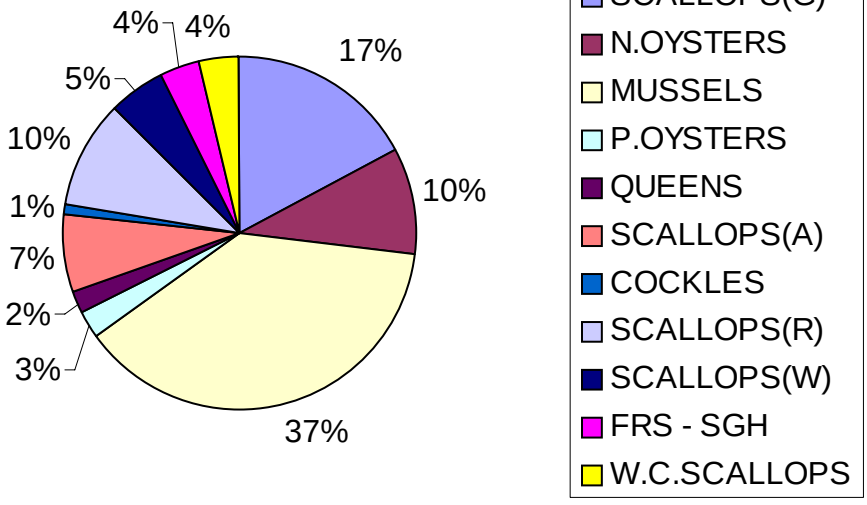
Detection of ASP in Shellfish Tissue
from UK



Tissue Types from UK
(n= 111)



Proposal Subject: Rapid Extraction Method for PSP and ASP

Specific NSSP Guide Reference: Section II. Model Ordinance Chapter III Laboratory @.02 Methods
ISSC Constitution, ByLaws, and Procedures
Procedure XVI, Procedure for Acceptance and Approval of Analytical Methods for the NSSP.

Text of Proposal/ Requested Action

Marine biotoxins affect farmed and wild fish and shellfish, as well as having a deleterious effect on humans. Jellett Rapid Testing has designed and developed rugged tests for the presence of Paralytic Shellfish Poison, Amnesic Shellfish Poison and Diarrhetic Shellfish Poison (under development at the time of this submittal). To facilitate the use of these tests in the field (for aquaculturists, campers, regulatory officials, etc.), Jellett Rapid Testing has developed a "low-tech" rugged alternative to the standard AOAC method designed to extract the toxins in the field as well as the laboratory. The AOAC method requires the sample to be boiled in acid at low pH and the pH adjusted with strong acids. This requires a fully equipped laboratory and significant safety precautions. The JRT Rapid Extraction Method was designed for use in remote areas, with little sophisticated backup support, by average individuals with little training and education. It is faster, less labor-intensive and less expensive than the other available method.

The rapid extraction method requires vinegar and rubbing alcohol to extract the toxins. A simple, rapid, safe method such as this would make rapid tests for marine biotoxins available in remote areas, to fishermen, aquaculturists, and regulatory officials on an instant basis.

The method developed by Jellett Rapid Testing Ltd has been presented to regulatory bodies over the past several years. In cooperation with individuals, governments and those organizations, the analytical method has been refined and improved. The Rapid Extraction Method is being tested in several states and foreign countries. Publications will be forthcoming.

The CONSTITUTION BY-LAWS and PROCEDURES of the INTERSTATE SHELLFISH SANITATION CONFERENCE allows the ISSC, through the Laboratory Methods Review Committee, to accept analytical methods that are sufficiently validated but are not AOAC or APHA methods. This is defined in the Constitution, PROCEDURE XVI. PROCEDURE FOR ACCEPTANCE AND APPROVAL OF ANALYTICAL METHODS FOR THE NSSP. Two possible reasons for considering a method are found in Subdivisions i and ii.

Subdivision i. Meets immediate or continuing need;
Subdivision ii. Improves analytical capability under the NSSP as an alternative to other approved or accepted method(s)

Currently, only the AOAC extraction for PSP and ASP are accepted. The need for a simple safe extraction method has been expressed by regulatory agencies, governmental organizations and industry for many years. The Jellett Rapid Extraction Method is being validated over a wide geographic area to demonstrate its simplicity, reliability, precision and accuracy. As a result of demonstrations of efficacy and the need that has been expressed by industry and state agencies, the Jellett Rapid Extraction Method is presented as an alternative extraction method for PSP and ASP for the NSSP as a Type III or Type IV method.

Please see attached additional information.

Suggested wording:
Section II, Chapter III Laboratory @.02 Methods

C. Biotoxin. Methods for the analyses of shellfish and shellfish harvest waters shall be:

(1) The current AOAC and APHA methods used in bioassay for paralytic shellfish poisoning toxins; and

(2) The current APHA method used in bioassay for *Karemia breve* toxins.

(3) The Jellett Rapid Extraction Method may be used for extracting PSP and ASP toxins from Shellfish by regulatory and industry laboratories.

Public Health Significance:

Currently, only the AOAC extraction for PSP and ASP analyses are accepted. Because of many significant constraints, in practical terms, this means that analyses can be conducted only in laboratories, and then under dangerous conditions. Acceptance of the Jellett Rapid Extraction Method for PSP and ASP would allow harvesters, processors, and regulatory agencies to screen for PSP and ASP with an accepted standardized method that provides valid useable data.

The Jellett Rapid Extraction Method for PSP and ASP was developed over several years in answer to the oft-stated need for a rapid, reliable, rugged, simple and safe sample preparation method. The Jellett Rapid Extraction Method for PSP and ASP is not meant to be a definitive "Standard Method", but rather to provide a supplementary extraction method that can be used in the field as well as in the lab.

Possible applications for The Jellett Rapid Extraction Method for PSP and ASP include:

- as a supplement to analytical methods of screening out negative samples in shellfish regulatory labs;
- as a harvest management tool at aquaculture facilities or in wild shellfish harvest areas (especially near shore areas) to supplement available methods to determine if shellfish are free of PSP or ASP and safe to harvest;
- as a supplement to quality control methods for shellfish processing plants, distributors and wholesalers to ensure incoming shellfish are free of PSP and ASP toxins before processing or further distribution (this test could become part of the plant's HACCP program);
- as a supplement to analytical methods for water classification for biotoxins; and
- as a supplement to analytical methods for broad scale ecological monitoring.

The rationale for using the Jellett Rapid Extraction Method for PSP and ASP is that the method provides a rapid, reliable, rugged, simple, safe and cost-effective extraction method (especially in low-volume laboratories) for PSP and ASP that can supplement accepted tests and substantially reduce the cost of analyses. Used in conjunction with other rapid methods, the Jellett Rapid Extraction Method for PSP and ASP will supplement regulatory agency efforts and help prevent the harvest of contaminated product. Having the ability to conduct tests using an accepted rapid extraction method will allow those processors who choose to use this test to demonstrate that they are truly controlling for PSP and ASP hazards in the harvested shellfish.

The Jellett Rapid Extraction Method for PSP and ASP could contribute to building long-term databases on broader scales than a regulatory lab can afford and, by using an accepted standardized method, will provide consistent results. These databases could be supplemented with industry testing in areas where there is no testing currently. This would extend, augment and strengthen the current food safety system broadening and refining the food safety net by increasing the number of testing sites and generating long term data in more areas.

A simple, rapid, rugged, effective, reliable, safe and cost-effective extraction method, available to all harvesters, regulators, and processors, would increase the monitoring and reduce the chance that shellfish containing ASP toxins above the regulatory limit would be harvested or marketed.

Cost Information (if available): It is difficult to determine exact costs because many government cost models do not consider capitol costs. Both extraction methods are the same through puree step, the chemicals used in both cases are minimal, as is the cost of incidental equipment (blender, pipettes, etc.). However, a comparison of time required using the Rapid Extraction Method (Add rapid liquid; Filter) with the time required using the AOAC Extraction (Add HCL; Boil; Wait; Filter; Pour in tube; Check PH) shows a significant difference. Our experience shows that it takes about 22 minutes for this portion of the AOAC extraction while it takes less than 2 minutes to complete the Jellett Rapid Extraction Method. At a salary of $33 / hour, that is a savings of $11.00 per sample extract.

Action by 2005 Laboratory Methods Review Committee Recommended referral of Proposal 05-111 to the appropriate committee as determined by the Conference Chairman.

Action by 2005 Task Force I Recommended adoption of the Laboratory Methods Review Committee recommendation of Proposal 05-111.

Action by 2005 General Assembly Adopted recommendation of 2005 Task Force I.

Action by USFDA Concurred with Conference action.

Action by 2007 Laboratory Methods Review Committee Recommended no action on Proposal 05-111. Rationale – Alternative extraction method for JRT PSP should be adopted to expand utility of the test; however there are insufficient data for acceptance at this time. The submitter will send data to the Executive Office for Conference approval.

Action by 2007 Task Force I Recommended referral of Proposal 05-111 to an appropriate committee as determined by the Conference Chairman.

Action by 2007 General Assembly Adopted recommendation of 2007 Task Force I.

Action by USFDA December 20, 2007
Concurred with Conference action with the following comments and recommendations for ISSC consideration.

The Conference has made considerable progress in its efforts to recognize new and developing analytical methods for the detection of indicators, pathogens, and marine toxins. Much credit goes to the Laboratory Methods Review Committee and its leadership for ensuring a scientifically defensible process for adopting analytical methods under the NSSP.

At the 2007 meeting numerous analytical methods were proposed for ISSC adoption. However, many of these methods were lacking the validation and associated data needed by the Laboratory Methods Review Committee to make a final determination regarding their efficacy for use in the NSSP. As a result the General Assembly voted "No Action" on analytical method Proposals 05-107, 05-108, 05-109, 05-111, 05-113, and 05-114. It is FDA's understanding that the intent of the "No Action" vote was not to remove these Proposals from ISSC deliberation as "No Action" normally suggests, but rather to maintain them before the Conference pending submission of additional data for further consideration. The Voting Delegates, by requesting the Proposal submitters provide additional data to the Executive Office for methods approval consistent with Procedure XVI, clearly recognized the importance and utility of these methods and intended to maintain them before the Conference for possible adoption following additional data submission. FDA requests that the ISSC Executive Board confirm FDA's understanding of this outcome. FDA fully supports such a Conference action and encourages the Executive Office to pursue submission of additional data as necessary to move forward with acceptance of these methods.

Action by 2009 Laboratory Methods Review Committee

Recommended no action on Proposal 05-111. Rationale: Requested additional information has not been submitted.

Action by 2009 Task Force I

Recommended adoption of Laboratory Methods Review Committee recommendation of Proposal 05-111.

Action by 2009 General Assembly

Referred Proposal 05-111 to the Laboratory Methods Review Committee.

Action by USFDA 02/16/2010

Concurred with Conference action on Proposal 05-111.

Action by 2011 Laboratory Methods Review Committee

Recommended acceptance of the rapid extraction method in Proposal 05-111, specifically 70% isopropanol:5% acetic acid 2.5:1, only for use with the Abraxis shipboard ELISA for PSP as an Emerging Method solely for use in the onboard screening dockside testing protocol in the Northeast region, including George's Bank.

The Laboratory Methods Review Committee further recommended:

1. The data collected during the dockside testing study be submitted to the LMRC in the SLV Method Application Protocol within 6 months of the concurrence by FDA in the Summary of Actions.

2. The validation study conducted by the State of Maine of the Abraxis laboratory ELISA with the extraction method in Proposal 05-111 be submitted to the LMRC in the SLV

Method Application Protocol within 6 months of the concurrence by FDA in the Summary of Actions.

3. No action on the requested language change in Proposal 05-111 for the Model Ordinance Section II, Chapter III Laboratory @.02 Methods.

Section II, Chapter III Laboratory @.02 Methods
C. Biotoxin. Methods for the analyses of shellfish and shellfish harvest waters shall be:
(1) The current AOAC and APHA methods used in bioassay for paralytic shellfish poisoning toxins; and
(2) The current APHA method used in bioassay for *Karenia breve* toxins.
~~**(3) The Jellett Rapid Extraction Method may be used for extracting PSP and ASP toxins from Shellfish by regulatory and industry laboratories.**~~

Action by 2011 Task Force I Recommended adoption of Laboratory Methods Review Committee recommendations on Proposal 05-111.

Action by 2011 General Assembly Adopted recommendation of 2011 Task Force I on Proposal 05-111.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 05-111.

Lab #	CFIA Sample #	CFIA Result HPLC (µg/g)	Jellett Result Approx. (µg/g)
04-01847	1	24.1	16-24
04-02156	2	1.4	0-4
04-01784	3	70.0	72-80
04-01968	4	71.9	72-92
04-01647	5	8.9	12-16
04-02328	6	9.3	6.4-11.2
04-02467	7	4.2	6.0-7.2
04-01646	8	31.2	40-64
04-02351	9	9.4	9.6-12
04-02238	10	4.7	4-5.6
04-01862	11	96.7	60-80
04-02240	12	10.3	12-20
04-01750	13	30.7	24-32
04-02231	14	2.5	0-4
04-01969	15	40.1	64-72

Jellett Rapid Testing Ltd.: NOAA Study - JREM Trial
Sample Record Sheet – Homogenate
State of Alaska - Department of Environmental Conservation

	Collection		Homogenization			Jellett Test						MBA Test					
Sample ID	Date	Species	Field / Site / Lab Name	Date	Size of Sample (mL)	Field / Site / Lab Name	Date	Batch # - Test	Batch # - Buffer	Result (1=Pos, 0=Neg)	Intensity of C Line as % of T	Lab Name	Date	Toxin Standard Used	# of Mice Dead	Result (µg/100g)	# of Mice Sick
20053168-C	3/06/05	Geoduck Viscera	ADEC-EHL	3/14/05	66[2]	ADEC-EHL	3/14/05	40000-13Aug04	40005-05Nov04	1	0%	ADEC-EHL	03/15/05	FDA	3	71	0
20053169-C	3/06/05	Geoduck Viscera	ADEC-EHL	3/14/05	495	ADEC-EHL	3/14/05	40000-13Aug04	40005-05Nov04	1	<10%	ADEC-EHL	03/15/05	FDA	3	39	0
20053170-C	3/06/05		ADEC-EHL	3/14/05	650	ADEC-EHL	3/14/05	40000-13Aug04	40005-05Nov04	1	0%	ADEC-EHL	03/15/05	FDA	3	71	0
20053183-C	3/13/05	Geoduck	ADEC-EHL	3/15/05	416	ADEC-EHL	3/15/05	40000-13Aug04	40005-05Nov04	1	>0%, <25%	ADEC-EHL	03/15/05	FDA	3	70	0
20053184-C	3/13/05	Geoduck	ADEC-EHL	3/15/05	632	ADEC-EHL	3/15/05	40000-13Aug04	40005-05Nov04	1	0%	ADEC-EHL	03/15/05	FDA	3	54	0
20053185-C	3/14/05	Geoduck	ADEC-EHL	3/15/05	561	ADEC-EHL	3/15/05	40000-13Aug04	40005-05Nov04	1	0%	ADEC-EHL	03/15/05	FDA	3	72	0
20053186-C	3/15/05	Geoduck	ADEC-EHL	3/15/05	301	ADEC-EHL	3/15/05	40000-13Aug04	40005-05Nov04	1	0%	ADEC-EHL	03/15/05	FDA	3	90	0
20053137	03/06/05	Oyster	ADEC-EHL	03/08/05	150	ADEC-EHL	03/08/05	40000-13Aug04	40005-05Nov04	INV	C <25% T	ADEC-EHL	03/08/05	FDA	0	NDT	0
20053136	03/06/05	Oyster	ADEC-EHL	03/08/05	500	ADEC-EHL	03/08/05	40000-13Aug04	40005-05Nov04	N/A INV	C <25% T	ADEC-EHL	03/08/05	FDA	0	NDT	0
20053138	03/05/05	Oyster	ADEC-EHL	03/08/05	500	ADEC-EHL	03/09/05	40000-13Aug04	40005-05Nov04	INV	C <25% T	ADEC-EHL	03/08/05	FDA	0	NDT	0
20053142	03/06/05	Oyster	ADEC-EHL	03/09/05	50	ADEC-EHL	03/09/05	40000-13Aug04	40005-05Nov04	INV	C <50% T	ADEC-EHL	03/09/05	FDA	0	NDT	0
20053124-C	3/5/05	Geoduck	ADEC-EHL	3/7/05	495	ADEC-EHL	3/7/05	40000-13Aug04	40005-05Nov04	1	0%	ADEC-EHL	03/07/05	FDA	3	117	0
20053125-C	3/5/05	Geoduck	ADEC-EHL	3/7/05	404	ADEC-EHL	3/7/05	40000-13Aug04	40005-05Nov04	1	75%	ADEC-EHL	03/07/05	FDA	3	58	0
20053006	2/29/05	Oyster	ADEC-EHL	3/3/05	125	ADEC-EHL	3/3/05	40000-13Aug04	40005-05Nov04			ADEC-EHL	3/3/05	FDA	0	NDT	0
20053040-C	03/01/05	Geoduck Viscera	ADEC-EHL	03/02/05	545	ADEC-EHL	03/02/05	40000-13Aug04	40009-06Oct04	1	50%	ADEC-EHL	03/02/05	FDA	3	86	0
20053039-C	03/01/05	Geoduck Viscera	ADEC-EHL	03/02/05	340	ADEC-EHL	03/02/05	40000-13Aug04	40009-06Oct04	1	10%	ADEC-EHL	03/02/05	FDA	3	175	0
20053007-C	02/26/05	Geoduck Viscera	ADEC-EHL	02/28/05	750	ADEC-EHL	03/01/05	40000-13Aug04	40009-06Oct04	1	25%	ADEC-EHL	02/28/05	FDA	3	59	0
20053010-C	02/26/05	Geoduck Viscera	ADEC-EHL	02/28/05	750	ADEC-EHL	03/01/05	40000-13Aug04	40009-06Oct04	1	<25%	ADEC-EHL	02/28/05	FDA	3	65	0
2005301-C	02/27/05	Geoduck Viscera	ADEC-EHL	02/28/05	750	ADEC-EHL	03/01/05	40000-13Aug04	40009-06Oct04	1	0%	ADEC-EHL	02/28/05	FDA	3	151	0

Jellett Rapid Testing Ltd.: NOAA Study
JREM Trial Sample Record Sheet - Homogenate
California - Microbial Disease Lab

	Collection		Homogenization			Jellett Test						MBA Test					
Sample ID	Collection Date	Species	Field / Site / Lab Name	Date	Size of Sample (mL)	Field / Site / Lab Name	Date	Batch # - Test	Batch # - Buffer	Result (1=Pos, 0=Neg)	Intensity of C Line as % of T	Lab Name	Date	Toxin Standard Used	# of Mice Dead	Result µg/100g	# of Mice Sick
05E-00110	02/05/05	LBMU	CA-DHS-EMDS	02/09/05	>130	CA-DHS-EMDS	02/09/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	2/09/05	FDA	0	<36	0
05W-00099	02/01/05	SSMU	CA-DHS-EMDS	02/02/05	>130	CA-DHS-EMDS	02/02/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	02/02/05	FDA	0	<34	0
05E-00096	02/28/05	CBMU	CA-DHS-EMDS	02/02/05	>130	CA-DHS-EMDS	02/02/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	02/02/05	FDA	0	<36	0
05W-00093	02/01/05	SBMU	CA-DHS-EMDS	02/02/05	>130	CA-DHS-EMDS	02/02/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	02/02/05	FDA	0	<36	0
05W-00079	01/25/05	SSMU	CA-DHS-EMDS	01/26/05	>130	CA-DHS-EMDS	01/26/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	1/26/05	FDA	0	<35	0
05W-00076	01/22/05	CBMU	CA-DHS-EMDS	01/26/05	>130	CA-DHS-EMDS	01/26/05	40000-8/13/04	40005-9/7/04	1	50%	CA-DHS-EMDS	01/26/05	FDA	3	39	0
05W-00069	01/24/05	SBMU	CA-DHS-EMDS	01/26/05	>130	CA-DHS-EMDS	01/26/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	01/26/05	FDA	0	<36	3
05W-00059	01/18/05	SSMU	CA-DHS-EMDS	01/19/05	>130	CA-DHS-EMDS	01/19/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	1/19/05	FDA	0	<35	3
05W-00055	01/14/05	CBMU	CA-DHS-EMDS	01/18/005	>130	CA-DHS-EMDS	01/18/05	40000-8/13/04	40005-9/7/04	1	25%	CA-DHS-EMDS	01/18/05	FDA	3	37	
05W-00052	01/17/05	SBMU	CA-DHS-EMDS	01/18/05	>130	CA-DHS-EMDS	01/18/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	01/18/05	FDA	0	<36	0
05W-00025	1/10/05	SBMU	CA-DHS-EMDS	1/12/05	>130	CA-DHS-EMDS	1/12/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	1/12/05	FDA	0	<35	0
05W-00023	1/11/05	SSMU	CA-DHS-EMDS	1/12/05	>130	CA-DHS-EMDS	1/12/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	1/12/05	FDA	0	<36	0
05W-00020	1/7/05	CBMU	CA-DHS-EMDS	01/11/05	>130	CA-DHS-EMDS	01/11/05	40000-8/13/04	40005-9/7/04	1	25%	CA-DHS-EMDS	1/11/05	FDA	3	44	0

Jellett Rapid Testing Ltd.: NOAA Study
JREM Trial Sample Record Sheet - Homogenate
California - Microbial Disease Lab (CONTINUED)

	Collection		Homogenization			Jellett Test						MBA Test					
Sample ID	**Collection Date**	**Species**	**Field / Site / Lab Name**	**Date**	**Size of Sample (mL)**	**Field / Site / Lab Name**	**Date**	**Batch # - Test**	**Batch # - Buffer**	**Result (1=Pos, 0=Neg)**	**Intensity of C Line as % of T**	**Lab Name**	**Date**	**Toxin Standard Used**	**# of Mice Dead**	**Result µg/100g**	**# of Mice Sick**
05W-00011	1/3/05	SBMU	CA-DHS-EMDS	1/5/05	>130	CA-DHS-EMDS	1/5/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	1/5/05	FDA	0	<34	0
05W-00007	1/4/05	SSMU	CA-DHS-EMDS	1/5/05	>130	CA-DHS-EMDS	1/5/05	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	1/5/05	FDA	0	<34	0
05W-00002	12/30/04	CBMU	CA-DHS-EMDS	1/04/05	>130	CA-DHS-EMDS	1/04/05	40000-8/13/04	40005-9/7/04	0	75%	CA-DHS-EMDS	1/04/05	FDA	2	36	1
04W-01458	12/28/04	SSMU	CA-DHS-EMDS	12/29/04	>130	CA-DHS-EMDS	12/29/04	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	12/29/04	FDA	0	<36	0
04W-01454	12/27/04	SBMU	CA-DHS-EMDS	12/29/04	>130	CA-DHS-EMDS	12/29/04	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	12/29/04	FDA	0	<36	0
04W-01457	12/24/04	CBMU	CA-DHS-EMDS	12/28/04	>130	CA-DHS-EMDS	12/28/04	40000-8/13/04	40005-9/7/04	1	<25%	CA-DHS-EMDS	12/28/04	FDA	3	42	0
04W-1446	12/21/04	SSMU	CA-DHS-EMDS	12/22/04	>130	CA-DHS-EMDS	12/22/04	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	12/22/04	FDA	0	<34	0
04W-01436	12/20/04	SBMU	CA-DHS-EMDS	12/21/04	>130	CA-DHS-EMDS	12/21/04	40000-8/13/04	40005-9/7/04	0	75%	CA-DHS-EMDS	12/21/04	FDA	0	<34	3
04W-01399	12/13/04	SBMU	CA-DHS-EMDS	12/14/04	>130	CA-DHS-EMDS	12/15/04	40000-8/13/04	40005-9/7/04	1	50%	CA-DHS-EMDS	12/15/04	FDA	2	35	0
04W-01421	12/11/04	CBMU	CA-DHS-EMDS	12/15/04	>130	CA-DHS-EMDS	12/15/04	40000-8/13/04	40005-9/7/04	1	0%	CA-DHS-EMDS	12/15/04	FDA	3	48	0
04W-01424	12/14/04	SSMU	CA-DHS-EMDS	12/15/04	>130	CA-DHS-EMDS	12/15/04	40000-8/13/04	40005-9/7/04	0	100%	CA-DHS-EMDS	12/15/04	FDA	0	<35	0

Proposal Subject:	Thermazyme™ ACP Test
Specific NSSP Guide Reference:	NSSP Section IV Guidance Documents Chapter II. Growing Areas .10 Approved Laboratory Tests
Text of Proposal/ Requested Action	Advanced Instruments, Inc. request ISSC adoption of this method for use in the National Shellfish Sanitation Program
Public Health Significance:	Thermazyme™ ACP Test will provide the basis for determining if shellfish have been thermally processed. This test will allow decisions to be based on a rapid, quantitative method rather than sensory related methods.
Cost Information (if available):	Not available
Action by 2005 Laboratory Methods Review Committee	Recommended the Conference direct the ISSC Executive Office to continue to investigate the issue of standards and pursue the development of standards and report back to the Laboratory Methods Committee with progress on the issue in six (6) months.
Action by 2005 Task Force I	Recommended adoption of the Laboratory Methods Review Committee recommendation for Proposal 05-115.
Action by 2005 General Assembly	Adopted recommendation of 2005 Task Force I.
Action by USFDA	Concurred with Conference action.
Action by 2007 Laboratory Methods Review Committee	Recommended referral of Proposal 05-115 to the Executive Board for consideration for interim approval. Insufficient data at this time to approve this method under Procedure XVI. Need AP curves at 145 for 15 seconds for each type of shellfish.
Action by 2007 Task Force I	Recommended adoption of the Laboratory Methods Review Committee recommendation on Proposal 05-115.
Action by 2007 General Assembly	Adopted recommendation of 2007 Task Force I.
Action by USFDA	December 20, 2007 Concurred with Conference action.
Action by 2009 Laboratory Methods Review Committee	Recommended referral of Proposal 05-115 to the appropriate Committee as determined by the Conference Chairman to review new data as it becomes available.
Action by 2009 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 05-115.

Action by 2009 General Assembly	Adopted recommendation of 2009 Task Force I on Proposal 05-115.
Action by USFDA 02/16/2010	Concurred with Conference action on Proposal 05-115.
Action by 2011 Laboratory Methods Review Committee	Recommended referral of Proposal 05-115 to the appropriate committee as determined by the Conference Chairman to continue the validation of the Thermazyme ACP Test for possible use in the NSSP. LMRC further Recommended the information requested by the testing lab and Advanced Instruments for validation be submitted within 6 months to be considered as an emerging method.
Action by 2011 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 05-115.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 05-115.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 05-115.

ADVANCED INSTRUMENTS, INC.

RECEIVED JUN 2 8 2004

June 25, 2004

Ken Moore
Executive Director
Interstate Shellfish Sanitation Conference
209-2 Dawson Drive
Columbia, SC 29223

Dear Mr. Moore:

Due to the advice of the USFDA Office of Seafood, I am writing this letter to request a review and approval of the Thermazyme™ ACP Test for use on thermally processed (cooked versus raw) shellfish products in order to make decisions based on a rapid, quantitative method rather than sensory related methods.

I have enclosed some literature and materials to demonstrate how the Fluorophos® ALP Test and Thermazyme ACP Test have brought value to the dairy and meat processing industries as an assessment tool for determining lethality of the kill step and finished product analysis.

Catherine Cutter, Ph.D documents the current situation in the seafood industry and has scientifically demonstrated that the Thermazyme ACP Test could also be utilized to advance the cause of food safety, thereby protecting consumers by minimizing the potential of under processed products making it into distribution channels.

Please have this method reviewed and approved for its use by seafood processors and agencies interested in maintaining the highest level of public safety.

I will be out of the office from June 28-July 6th. For assistance you may contact Eileen Garry, R&D Lab Manager, Advanced /Instrument, Inc. at 781-320-9000 X2118 or email eileeng@aicompanies.com or Gary Wolf, Regional Shellfish Specialist, FDA Office of Seafood, Vorhees, NJ, at 856-783-1420 X13 or Email - gwolf@ora.fda.gov.

I look forward to speaking with you about this exciting opportunity for the industry and thank you for your attention to this important development.

Sincerely,

Kenneth F. Micciche
Director of Marketing
Advanced Instruments, Inc.
Office – 781-471-2145
Facsimile 781-320-8181
Cell 781-354-9739

RECEIVED JUN 2 8 2004

LEAST COST FORMULATIONS, LTD.
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TECHNICAL REPORT

NUMBER: TR203 DATE: 04 May 24

TITLE: Performance characteristics of the ThermaZyme® acid phosphatase ("ACP") measurement system on seafood.

AUTHOR: R. A. LaBudde

ABSTRACT: Data from a recent study of the use of the ThermaZyme® acid phosphatase measurement system on seafood was analyzed to assess relevant performance characteristics such as accuracy and precision, false positive and false negative error rates and other parameters. Although the data in the study were limited, some quantitative assessment of these parameters was possible.

KEYWORDS: 1) THERMAZYME 2) ACP 3) EPT

REL.DOC.:

REVISED: 04 May 28

INTRODUCTION

The use of heat-labile enzymes in the determination of cook endpoint temperatures has a long history in the food industry [1-13]. Heat lethality in bacteria is believed to be due primarily to denaturation of cellular enzymes, so verification of destruction of such enzymes is highly correlated to bacterial destruction.

Catalase (meat), alkaline phosphatase (milk) and acid phosphatase (various foods) have been used as surrogates to verify post-process that adequate pasteurization has taken place [2-12].

The ThermaZyme® system, distributed by Advanced Instruments, is based on the fluorometric measurement of acid phosphatase ("ACP") enzyme. Several validation studies have been published for the system involving a variety of food products.

Recently, a study by Cutter and Miller [1] has investigated ACP for endpoint temperature verification in seafood.

BACKGROUND ASSUMPTIONS RELATED TO ACP IN SEAFOOD

The performance characteristics and inferences with respect to ACP in seafood are based entirely on the work done by Cutter and Miller [1]. In particular, the population of inference is limited to those sources of supply locally available to these authors. However, in recommended use, the method may be calibrated using samples of control raw material and cook EPT determination to establish a standard curve for the relevant population of inference.

PERFORMANCE CHARACTERISTICS IN SEAFOOD

The ThermaZyme system may be used to verify endpoint temperatures in two different ways:

1. INFERENTIAL: Was the food processed to a specific minimum endpoint temperature ("EPT") or higher?
2. ESTIMATION: What was the highest equivalent endpoint temperature to which the food was exposed?

A. ACCURACY:

The ThermaZyme Test System can detect as low as 0.1 U/kg of sample, based on a 1:3 initial dilution.

Based on the Cutter and Miller data [1], estimation of endpoint temperature is subject to the following precisions, based on each test comprising the average of 5 replications:

PRECISION OF ENDPOINT TEMPERATURE			
Seafood	*Range*	*Standard error of fit*	*95% Confidence Interval of EPT*
Clams	130-165 F	9.1 F	+/- 8.6 – 14.9 F
Lobster	140-165 F	5.4 F	+/- 6.1 – 13.9 F
Oysters	140-175 F	3.8 F	+/- 3.4 – 5.9 F
Shrimp	140-165 F	5.4 F	+/- 6.1 – 13.9 F

B. SPECIFICITY:

For the inference that raw seafood has been cooked to a specified minimum EPT:

RAW SEAFOOD COMPARED TO MINIMUM ENDPOINT TEMPERATURE		
Seafood	*Minimum EPT*	*False Positive Rate*
Clams	130 F	0.0064%
Lobster	140 F	0.0987%
Oysters	150 F	1.7385%
Shrimp	140 F	0.3711%

C. PRECISION:

Based on the Cutter and Miller data [1], estimation of endpoint temperature is subject to the following precisions, based on each test comprising the average of 5 replications:

PRECISION OF ENDPOINT TEMPERATURE			
Seafood	*Range*	*Standard error of fit*	*95% Confidence Interval of EPT*
Clams	130-165 F	9.1 F	+/- 8.6 – 14.9 F
Lobster	140-165 F	5.4 F	+/- 6.1 – 13.9 F
Oysters	140-175 F	3.8 F	+/- 3.4 – 5.9 F
Shrimp	140-165 F	5.4 F	+/- 6.1 – 13.9 F

D. SENSITIVITY:

The ThermaZyme Test System can detect as low as 0.1 U/kg of sample, based on a 1:3 initial dilution.

E. SELECTIVITY:

The test is specific for the ACP enzyme involved and has no interferences from other compounds.

F. ASSAY INTERVAL:

Each test involves comminution of the bulk sample, possible draining, weighing of a 0.8 g specimen, dilution with standard reagents, homogenization and measurement in the fluorometer. Total time expended per sample is less than 10 minutes for one replicate and an additional 5 minutes for each further replicate.

G. ASSAY COST:

Reagent costs per replicate are approximately $3.00 with approximately 1/6 hr of analyst time.

H. COMPARABILITY:

Alternative methods of verifying EPT are limited. The most obvious being Aerobic Plate Count ("APC") microbial determination. In this case, the analysis cost is approximately $1.00-$2.00 in supplies and 1/6 hr of analyst time per replicate. For viral determinations, the cost would be significantly higher ($30-$100).

I. OTHER STUDIES:

See references [2-13] for studies based on acid or alkaline phosphatase as a means of cook endpoint temperature determination in various meat and dairy products.

J. REGULATORY APPROVALS:

1. AOAC First Action, 1991.
2. AOAC Final Action, 1995. Method 979.13.
3. International Dairy Federation, 1992.
4. Interstate Milk Shippers, 1993.
5. ISO/DIS 11816-2, 2001.
6. FDA, 1995. (Cheese)
7. NCIMS, 2001. (Cream)

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1. Cutter CN and Miller BJ. 2003. Use of an acid phosphatase assay to detect deviations in thermal processing of seafood. J Assoc Food and Drug Officials 67(4):1-14.
2. Davis CE. 1998. Fluorometric determination of acid phosphatase in cooked, boneless, nonbreaded broiler breast and thigh meat. J AOAC Inter 81(4):887-906.
3. Davis CE and Townsend WE. 1994. Rapid fluorometric analysis of acid phosphatase activity in cooked poultry meat. J Food Prot 57:1094-1097.
4. Lyon BG et al. 2001. Acid phosphatase activity and color changes in consumer-style griddle-cooked ground beef patties. J Food Prot 64:1199-1205.
5. Wang SF et al. 1996. Proteins as potential endpoint temperature indicators for ground beef patties. J Food Sci 61(1):5-7.
6. Wilson CE et al. 2002. Influence of Listeria spp. contamination on end-point temperature determination in broiler breast patties by a fluorometric acid phosphatase assay.
7. Cohen EH. 1969. Determination of acid phosphatase activity in cooked hams as an indicator of temperature attained during cooking. Food Tech 23:101-104.
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10. Rocco RM. 1990. Fluorometric analysis of alkaline phosphatase in fluid dairy products. J Food Prot 53:588-591.
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12. Anonymous. 1986. Determination of internal cooking temperature (acid phosphatase activity). Revised Basic Chemistry Laboratory Guidebook (rev. March 1986), USDA-FSIS No. 3.018:3-49.
13. Jones DR et al. 2002. Variations in levels of acid phosphatase present in chicken whole leg meat. Poultry Sci 81(10):1567-1570.

Proposal Subject: Correction of the wording for the action level for NSP toxins and the incorporation of action levels for AZP and DSP toxins in shellfish in the Guide.

Specific NSSP Guide Reference: Section II. Model Ordinance Chapter IV. Shellstock Growing Areas
@.04 Marine Biotoxin Control C. (1)

Section IV. Guidance Documents Chapter II. Growing Areas
.04 Action Levels, Tolerances and Guidance Levels for Poisonous or Deleterious Substances in Seafood

Text of Proposal/ Requested Action In Section II Model Ordinance, Chapter IV. Shellstock Growing Areas @.04 Marine Biotoxin Control C. (1), correct the wording for NSP toxins and add the action levels for azaspiracids (AZP) and DSP toxins, as follows:

C. Closed Status of Growing Areas.

(1) A growing area, or portion(s) thereof as provided in §A.(4), shall be placed in the closed status for the taking of shellstock when the Authority determines that the number of toxin-forming organisms in the growing waters and/or the level of biotoxin present in shellfish meats is sufficient to cause a health risk. The closed status shall be established based on the following criteria:

PSP - cells/L n/a; 80 µg/100 grams
NSP - 5,000 cells/L or 20 MU/**100 grams** (~~approximate as 80 µg/100 g~~**0.8 mg brevetoxin-2 equivalents/kg)**
AZP - cells/L n/a; 0.16 mg AZA-1 equivalents/kg (0.16 ppm)
DSP - cells/L n/a; 0.16 mg OA equivalents/kg (0.16 ppm)
ASP - cells/L n/a; 2 mg/100 grams (20 ppm)

(a) The concentration of paralytic shellfish poison (PSP) equals or exceeds 80 micrograms per 100 grams of edible portion of raw shellfish; or

(b) For neurotoxic shellfish poisoning (NSP), the harvesting of shellstock shall not be allowed when:
(i) The concentration of NSP equals or exceeds 20 mouse units per 100 grams of edible portion of raw shellfish; or
(ii) The cell counts for *Karenia brevis* organisms in the water column exceed 5,000 per liter; or

(c) For domoic acid, the toxin concentration shall not be equal to or exceed 20 ppm in the edible portion of raw shellfish.

(d) For azaspiracid shellfish poisoning (AZP), the concentration of azaspiracids shall not be equal to or exceed 0.16 mg/kg (AZA-1 equiv.) in the edible portion of raw shellfish.

(e) For diarrhetic shellfish poisoning (DSP), the concentration of DSP toxins shall not be equal to or exceed 0.16 mg/kg (OA equiv.) in the edible portion of raw shellfish.

And under the Natural Toxins section of Table 1 of the Guidance Documents: Chapter II-Growing Areas; .04 Action Levels, Tolerances and Guidance Levels for Poisonous or Deleterious Substances in Seafood, correct and insert the following:

Substance	Level	Food Commodity[a]	Reference
Neurotoxic Shellfish Poison**ing** (NSP) **toxins**	20 MU/**100g**	Clams, mussels, oysters, fresh frozen or canned	NSSP MO
Azaspiracid Shellfish Poisoning (AZP) toxins	**0.16 mg/kg**	**Clams, mussels, oysters, fresh frozen or canned**	**NSSP MO**
Diarrhetic Shellfish Poisoning (DSP) toxins	**0.16 mg/kg**	**Clams, mussels, oysters, fresh frozen or canned**	**NSSP MO**

Public Health Significance:

NSP Toxins

Neurotoxic shellfish poisoning (NSP) is caused by consumption of shellfish contaminated with brevetoxins. Brevetoxins are a group of lipophilic neurotoxins produced by the marine dinoflagellate *Karenia brevis* and other algal species (e.g., *Chattonella* spp.). Brevetoxins are accumulated and extensively metabolized in filter-feeding molluscan shellfish. Toxicity of shellfish has been historically assessed by mouse bioassay, while efforts are underway to validate alternative methods of analysis (e.g., LC-MS, immunoassay). Shellfish exhibiting any detectable level of toxicity by mouse bioassay are considered potentially unsafe for human consumption. In practice, a value of 20 MU/100 g shellfish tissue has been considered the regulatory limit by the States. Expressed in brevetoxin-2 (PbTx-2) equivalents, this level is 0.8 mg/kg in shellfish tissue. Method alternative to mouse bioassay must provide an equivalent level of public health protection.

The requested action is editorial corrections to the Guide with respect to the current action level.

AZP Toxins

Azaspiracids (AZA) are a group of lipophilic marine algal toxins that accumulate in various shellfish species (Twiner et al., 2008). Consumption of AZA-contaminated shellfish causes the acute illness azaspiracid shellfish poisoning (AZP). AZP is characterized by severe gastrointestinal disturbances; symptoms include nausea, vomiting, diarrhea, abdominal pain and cramps. AZA were first discovered in 1995 following an outbreak linked to consumption of Irish mussels. Since then, several documented outbreaks of AZP have been reported in Europe, and AZA have been isolated from shellfish along the European Atlantic coast from Norway to Portugal, and in Morocco. In 2008, the first recognized cases of AZP in the U.S. were reported, and linked to consumption of imported mussels from Ireland (Klontz et al., 2009). The finding of AZA in the imported product highlights the concern for the consumer safety of molluscan shellfish marketed internationally.

The first risk assessment for AZA was conducted by the Food Safety Authority of Ireland (FSAI) in 2001. In 2002, the European Commission set the regulatory limit for AZA (AZA-1, -2, and -3) at 0.16 mg/kg, based on the FSAI data and the limit believed to be detectable by mouse bioassay (EC, 2002). This regulatory limit was strengthened by a second risk assessment conducted by the FSAI (FSAI, 2006). The latter incorporated new data with respect to tissue distribution of AZA in mussels, ratios of different analogues, and the effects of cooking. The calculated median acute reference

dose (ARfD, 0.63 □g/kg b.w.) was comparable to the intake value for a 60 kg individual consuming 250 g mussels contaminated with AZA at the 0.16 mg/kg regulatory limit.

EC regulation allows for the use of alternative methods (e.g., LC-MS, immunoassay) to the reference test (mouse bioassay) for AZA in shellfish (EC,2005). These methods must be capable of detecting the AZA analogues AZA-1, -2, and -3. And they must provide an equivalent level of public health protection to the biological method. The EU-harmonized mouse bioassay and LC-MS methods were recently demonstrated equivalent in their effectiveness in implementation of this regulatory limit (Hess et al., 2009).

The FSAI risk assessment did recognize the uncertainties inherent in its outcome, particularly relating to limitations in the available epidemiological data. Moreover, the toxicity of AZA analogues, and their distribution and metabolism in various shellfish species, have not been well characterized. Chronic and low dose effects of AZA are unknown. Refinement of the risk assessment and revision of regulatory limit may be necessary when additional toxicological and epidemiological data become available.

The requested action is adoption of a regulatory limit for azaspiracids (AZA) of 0.16 mg/kg in molluscan shellfish, in accordance with that set by the European Commission (EC, 2002). By using LC-MS, this limit is based on the sum of the individual azaspiracid toxin analogues AZA-1, -2, and -3, expressed in AZA-1 equivalents. AZA-1 is the only certified analytical standard presently available. AZA-1 equivalents of AZA-2 and -3 are calculated by weighting their relative response factor (RRF)-corrected concentrations with their toxic equivalence factors (TEFs). TEF multipliers derived from initial studies on mice are 1, 1.8, and 1.4 for AZA-1, -2, and -3, respectively (Ofuji et al., 1999).

DSP Toxins

Diarrhetic shellfish poisoning (DSP) is caused by consumption of molluscan shellfish contaminated with toxins of the okadaic acid (OA) group, the origin of which is principally marine dinoflagellates (e.g., *Dinophysis, Prorocentrum* spp.) DSP is characterized by acute gastrointestinal disturbance (e.g., diarrhea, nausea, vomiting, abdominal pain). Toxins responsible are primarily okadaic acid (OA) and the related dinophysistoxins (DTXs) and their acyl esters. Pectenotoxins (PTX) and yessotoxins (YTX) may co-occur, the former of similar toxic potency.

DSP outbreaks were first reported in 1976 in Japan, and in the 1980s in Europe. The first documented outbreak in N. America occurred in 1990, in eastern Canada (Qulliam et al., 1993). There have been no reported cases of DSP to date in the U.S. However, in 2008, toxin-producing *Dinophysis*, and DSP toxins in shellfish above the proposed action levels, were recorded for the first time in the Gulf of Mexico (Deeds, pers. comm.). *Dinophysis* has been found along the east and west coast of the U.S. Since DSP toxin-producing organisms occur throughout the world, DSP toxins in molluscan shellfish are a significant public health concern.

DSP toxins in shellfish have been assessed traditionally by mouse bioassay, and more recently by instrumental methods (LC-FTD, LC-MS), immunoassay, and pharmacology-based assays (protein phosphatase assay). Current EU regulatory limit is 0.16 mg OA equivalents/kg shellfish meat (EC, 2002, 2005). This level represents the sum of that of OA, DTXs, and PTXs. Methods alternative to mouse bioassay incorporate a base hydrolysis step for conversion of DTX acyl esters to free acid forms.

The requested action is adoption of a regulatory limit for DSP toxins of 0.16 mg/kg (OA equivalents) in molluscan shellfish. This limit is based on the sum of OA, DTXs (including acyl esters), and PTXs. Revision of regulatory limit may be necessary when additional toxicological and epidemiological data become available.

References

EC, 2002. Commission decision 2002/225/EC of 15 March 2002 laying down detailed rules for the implementation of Council Directive 91/492/EEC as regards the maximum levels and the methods of analysis of certain marine biotoxins in bivalve mollusks, echinoderms, tunicates and marine gastropods. Off. J. Eur. Comm. L75:62-64.

EC, 2005. Commission Regulation (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the organisation of official controls under Regulation (EC) No 854/2004 of the European Parliament and of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) No 854/2004. Off. J. Eur. Union. L338:27-59.

FSAI. 2006. Risk assessment of azaspiracids (AZAs) in shellfish. August 2006. A Report of the Scientific Committee of the Food Safety Authority of Ireland (FSAI), Dublin, Ireland.

Hess, P., Butter, T., Peterson, A., Silke, J., McMahon, T. 2009. Performance of the EU-harmonized mouse bioassay for lipophilic toxins for the detection of azaspiracids in naturally contaminated mussel (*Mytilus edulis*) hepatopancreas tissue homogenates characterized by liquid chromatography coupled to tandem mass spectrometry. Toxicon 53:713-722.

Klontz, K.C., Abraham, A., Plakas, S.M., Dickey, R.W. 2009. Mussel-associated azaspiracid intoxication in the United States. Ann. Int. Med. 150:361.

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Twiner, M.J., Rehmann, N., Hess, P., Doucette, G.J. 2008. Azaspiracid shellfish poisoning: a review on the chemistry, ecology, and toxicology with an emphasis on human health impacts. Mar. Drugs 6:39–72.

Cost Information (if available):

Action by 2009 Task Force I	Recommended referral of Proposal 09-101 to an appropriate committee as determined by the Conference Chairman. The Committee should be directed to gather more information on the standards, methods and costs.
Action by 2009 General Assembly	Adopted recommendation of 2009 Task Force I on Proposal 09-101.
Action by USFDA 02/16/2010	Concurred with Conference action on Proposal 09-101.
Action by 2011 Biotoxin Committee	Recommended adoption of Proposal 09-101 as submitted. The Committee also recommended that the last sentence of the first paragraph in the DSP Toxins Public Health Significance section was no longer appropriate and should be deleted.
Action by 2011 Task Force I	Recommended adoption of Biotoxin Committee recommendation on Proposal 09-101.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 09-101.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 09-101.

Proposal Subject:	Alternative analytical method for *Vibrio vulnificus, Vibrio cholerae, Vibrio parahaemolyticus*
Specific NSSP Guide Reference:	Section IV. Guidance Documents Chapter II Growing Areas .10 Approved National Shellfish Sanitation Program Laboratory Tests: Microbiological and Biotoxin Analytical Methods. (5) Interim Approval by ISSC Executive Board August 2007
Text of Proposal/ Requested Action	Text of proposal: See attached proposal Requested actions: Accept the adoption of DuPont Qualicon BAX ® Real Time *Vibrio* Test Kit as an alternative analytical protocol to determine the levels of *Vibrio vulnificus, V. cholerae, V.parahaemolyticus*
Public Health Significance:	Proposed method will greatly improve the speed of analysis to help the industry to increase the amount of PHP products in the market. For details see attached proposal
Cost Information (if available):	See attached proposal.
Action by 2009 Laboratory Methods Review Committee	Recommended referral of Proposal 09-102 to appropriate committee as determined by Conference Chairman. Rationale: Additional data under development.
Action by 2009 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 09-102.
Action by 2009 General Assembly	Adopted recommendation of 2009 Task Force I on Proposal 09-102.
Action by USFDA 02/16/2010	Concurred with Conference action on Proposal 09-102.
Action by 2011 Laboratory Methods Review Committee	Recommended no action on Proposal 09-102. Rationale – Proposal 09-102 has been withdrawn by the submitter.
Action by 2011 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 09-102.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 09-102.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 09-102.

Proposed Specific Research Need/Problem to be Addressed:

Improve the speed of analysis to help the industry to increase the amount of PHP products in the market.

How will addressing this research support/improve the mission/role of the ISSC/NSSP/Industry? Support need with literature citations as appropriate.

See attached description

Relative Priority Rank in Terms of Resolving Research Need:

Immediate	☒	**Important**	☐
Required	☐	**Other**	☐
Valuable	☐		

Estimated Cost:

Proposed Sources of Funding/Support:

Time Frame Anticipated: 2009-2010

ISSC Method Application and Single Lab Validation Checklist For Acceptance of a Method for Use in the NSSP

The purpose of single laboratory validation in the National Shellfish Sanitation Program (NSSP) is to ensure that the analytical method under consideration for adoption by the NSSP is fit for its intended use in the Program. A Checklist has been developed which explores and articulates the need for the method in the NSSP; provides an itemized list of method documentation requirements; and, sets forth the performance characteristics to be tested as part of the overall process of single laboratory validation. For ease in application, the performance characteristics listed under validation criteria on the Checklist have been defined and accompany the Checklist as part of the process of single laboratory validation. Further a generic protocol has been developed that provides the basic framework for integrating the requirements for the single laboratory validation of all analytical methods intended for adoption by the NSSP. Methods submitted to the Interstate Shellfish Sanitation Conference (ISSC) Laboratory Methods Review (LMR) Committee for acceptance will require, at a minimum, six (6) months for review from the date of submission.

Name of the New Method	**QPCR-MPN Assay using DuPont Qualicon BAX ® Real Time *Vibrio* Test Kit for Rapid Detection of *Vibrio* speices in seafood**
Name of the Method Developer	Anita Wright et. al.
Developer Contact Information	Anita Wright 461 AFPL bldg. Newell Dr. Gainesville, FL 32611 352-392-1991 ext. 311

Checklist	Y/N	Submitter Comments
A. Need for the New Method		
1. Clearly define the need for which the method has been developed.	Y	An alternative method to confirm vibrio bacteria in shellfish
2. What is the intended purpose of the method?	Y	Replace confirmation step in MPN determination of Vibrios in shellfish
3. Is there an acknowledged need for this method in the NSSP?	Y	End users are requiring faster more economical alternatives to the current approved method
4. What type of method? i.e. chemical, molecular, culture, etc.	Y	Quantitative PCR
B. Method Documentation		
1. Method documentation includes the following information:		
Method Title	Y	
Method Scope	Y	
References	Y	
Principle	Y	
Any Proprietary Aspects	Y	
Equipment Required	Y	
Reagents Required	Y	
Sample Collection, Preservation and Storage Requirements	Y	
Safety Requirements	Y	
Clear and Easy to Follow Step-by-Step Procedure	Y	
Quality Control Steps Specific for this Method	Y	
C. Validation Criteria		
1. Accuracy / Trueness	Y	
2. Measurement Uncertainty	Y	
3. Precision Characteristics (repeatability and reproducibility)	Y	
4. Recovery	n/a	
5. Specificity	Y	
6. Working and Linear Ranges	Y	
7. Limit of Detection	Y	
8. Limit of Quantitation / Sensitivity	Y	
9. Ruggedness	Y	
10. Matrix Effects	Y	

11. Comparability (if intended as a substitute for an established method accepted by the NSSP)	Y	
D. Other Information		
1. Cost of the Method	Y	
2. Special Technical Skills Required to Perform the Method	Y	
3. Special Equipment Required and Associated Cost	Y	
4. Abbreviations and Acronyms Defined	N/A	
5. Details of Turn Around Times (time involved to complete the method)	Y	
6. Provide Brief Overview of the Quality Systems Used in the Lab	Y	
Submitters Signature	Date:	
Submission of Validation Data and Draft Method to Committee	Date:	
Reviewing Members	Date:	
Accepted	Date:	
Recommendations for Further Work	Date:	
Comments: See attached application document.		

DEFINITIONS

1. **Accuracy/Trueness** - Closeness of agreement between a test result and the accepted reference value.
2. **Analyte/measurand** - The specific organism or chemical substance sought or determined in a sample.
3. **Blank** - Sample material containing no detectable level of the analyte or measurand of interest that is subjected to the analytical process and monitors contamination during analysis.
4. **Comparability** – The acceptability of a new or modified method as a substitute for an established method in the NSSP. Comparability must be demonstrated for each substrate or tissue type by season and geographic area if applicable.
5. **Fit for purpose** – The analytical method is appropriate to the purpose for which the results are likely to be used.
6. **HORRAT value** – HORRAT values give a measure of the acceptability of the precision characteristics of a method.[4]
7. **Limit of Detection** – the minimum concentration at which the analyte or measurand can be identified. Limit of detection is matrix and analyte/measurand dependent.[4]
8. **Limit of Quantitation/Sensitivity** – the minimum concentration of the analyte or measurand that can be quantified with an acceptable level of precision and accuracy under the conditions of the test.
9. **Linear Range** – the range within the working range where the results are proportional to the concentration of the analyte or measurand present in the sample.
10. **Measurement Uncertainty** – A single parameter (usually a standard deviation or confidence interval) expressing the possible range of values around the measured result within which the true value is expected to be with a stated degree of probability. It takes into account all recognized effects operating on the result including: overall precision of the complete method, the method and laboratory bias and matrix effects.
11. **Matrix** – The component or substrate of a test sample.
12. **Method Validation** – The process of verifying that a method is fit for purpose.[1]
13. **Precision** – the closeness of agreement between independent test results obtained under stipulated conditions.[1, 2] There are two components of precision:
    a. **Repeatability** – the measure of agreement of replicate tests carried out on the same sample in the same laboratory by the same analyst within short intervals of time.
    b. **Reproducibility** – the measure of agreement between tests carried out in different laboratories. In single laboratory validation studies reproducibility is the closeness of agreement between results obtained with the same method on replicate analytical portions with different analysts or with the same analyst on different days.
14. **Quality System** - The laboratory's quality system is the process by which the laboratory conducts its activities so as to provide data of known and documented quality with which to demonstrate regulatory compliance and for other decision–making purposes. This system includes a process by which appropriate analytical methods are selected, their capability is evaluated, and their performance is documented. The quality system shall be documented in the laboratory's quality manual.
15. **Recovery** – The fraction or percentage of an analyte or measure and recovered following sample analysis.
16. **Ruggedness** – the ability of a particular method to withstand relatively minor changes in analytical technique, reagents, or environmental factors likely to arise in different test environments.[4]
17. **Specificity** – the ability of a method to measure only what it is intended to measure.[1]
18. **Working Range** – the range of analyte or measure and concentration over which the method is applied.

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Title: QPCR-MPN Assay using DuPont Qualicon BAX ® Real Time *Vibrio* Test Kit for Rapid Detection of *Vibrio* species in seafood

JUSTIFICATION FOR NEW METHOD

This protocol is submitted for approval to the Laboratory Methods Review Committee. This proposal was prepared to support the use of a new molecular detection method: **DuPont Qualicon BAX ® Real Time *Vibrio* Test Kit for** rapid detection of *Vibrio cholerae, V. parahaemolyticus, and V. vulnificus* It will be used in conjunction with current Vibrio MPN assay and will substitute for the use of DNA probe colony hybridization for confirmation of the presence of *Vibrio* species (8). Method was developed by collaborative efforts of Dr. Anita Wright, Dr. Steve Otwell, Victor Garrido, Charlene Burke, and Melissa Evans, University of Florida, Gainesville, Florida and DuPont Qualicon Laboratories. The QPCR method was recently approved for American Organization of Analytical Chemists (AOAC) and has been accepted for publication by the Journal of AOAAC: Morgan Wallace, Anita Wright, Tim Dambaugh, Monica Kingsley, Chris Malota, Bridget Andaloro, Dawn Fallon, Daniel Delduco, George Tice and, DuPont Qualicon BAX ® Real Time *Vibrio* Test Kit for the Detection of *Vibrio cholera, parahaemolyticus and vulnificus* from Tuna, Shrimp and Oysters, *AOAC Performance Tested Methods* (15)

The QPCR-MPN method described herein provided increased assay sensitivity and reduced both time and labor costs. Detection of *Vibrio* species was achieved at levels < 30 CFU/g as required for validation protocols (2, 10, 16). For these reasons we propose acceptance of the application of QPCR-MPN for improved assessment of validation and verification protocols related to oyster post harvest processing. The oyster industry's livelihood will be determined by their ability to adapt to FDA demands, and evolving technological breakthroughs. Until this demand has abated, the industry and the scientific community will continue to work in conjunction to learn more and thus protect the public from Vibrio disease.

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Date of Submission
Proposal submission date is June 20, 2009.

Purpose and Intended Use of the Method. *Vibrio* species are responsible for 75% of seafoodborne bacterial infections and 95% of related fatalities (7). *V. vulnificus* the leading cause of death in the US related to seafood consumption and is predominantly associated with consumption uncooked Gulf Coast oysters. *V. parahaemolyticus* is the most common source of outbreaks of infectious disease related to seafood, and *V. cholerae* contamination threatens the safety of imported seafood products. The proposed method will benefit the seafood industry and the consumer by providing improved, faster, and more accruate deteiction of these pathogens in oysters and other seafood products. This method is being proposed for use in screening potential contamination of seafood products and for validation of Post Harvest Processing (PHP) protocols, as well as for future applications to assure the public of a safer product.

Need for the New Method in the NSSP

QPCR-MPN assay described herein is proposed as an alternative to the standard MPN assay for enumeration of *Vibrio* species using most probable number (MPN) end-point titration of replicate samples in enrichment broth cultures (4, 17). The current standard protocols described in the FDA Bacteriological Analytical Manual (BAM) use growth in enrichment broth, followed by isolation of typical colonies on selective agar medium with subsequent confirmation of each species by DNA probe (16), PCR, or biochemical profiling (8). This method is laborious cost prohibitive, labor intensive, and time consuming (6, 8). Enumeration of multiple *Vibrio* species requires isolation on different selective agars followed by separate confirmation tests that are different for each species. Furthermore, users of this protocol have expresssed difficulty with DNA probe product reliability and plating problems related to "spreading" colonies that interfer with the assay. Total amount of time to perform the traditional MPN method with DNA colony blot hybridization as a confirmatory method is at least 4 days, with numerous steps; additionally, technician requires a great deal of experience in performing this assay for successful quantification to be possible. QPCR-MPN method reduces working time half and offers greater sensitivity for detection of *V. vulnificus*; with detection of 1 bacterium per gram post enrichment in alkaline peptone water (APW) overnight (1, 4, 9, 10, 11, 17).

Although PHP methods are currently employed on < 10% of all domestic raw oyster sales in the United States, the industry continues to examine and employ new technologies and take initiative on expanding acceptance and knowledge regarding these treated oyster products (5). The industry is investing money and resources to ensure a market acceptance by educated oyster public, in addition to mitigating risk potential for the at risk consumers of fresh oysters. ISSC mandated that 25% of oysters havested from the Gulf of Mexico receive some type of validated post havrest processing. Thus, there is an urgent need for improved and more rapid validation methods.

The University of Florida has partnered with several dealers who are using ISSC methods for validation of oyster PHP. Work supporting this proposal was perfomred in 2007-2009 working with mild heat treatment (Panama City), nitrogen freezing (Leavin's seafood) and blast freezing (Buddy Ward's Seafood). Throughout the validation, samples were randomly selected for side-by-side comparisons of standard MPN described by the FDA BAM (8) to MPN using the DuPont Bax QPCR for MPN species-specific identification. Test results support the application of QPCR-MPN for improved assessment of validation and verification protocols related to oyster PHP, which was described in a publication by Wright et al., 2007.

Method Limitations and Potential Indications of Cases Where the Method May Not Be Applicable to Specific Matrix Types

This method is specific to applications testing growth of *V. cholerae, V. parahaemolyticus, and V. vulnificus* in MPN enrichment of oyster homogenates. This QPCR method does not claim to differentiate between pathogenic and nonpathogenic *Vibrio* species. Method was found to be appropriate for up to 1g of oyster tissues. QPCR-MPN provided more sensitive detection than standard MPN, as enriched samples that were PCR positive but negative on selective media were falsely negative on mCPC, as indicated by agreement of positive mCPC and QPCR results in more diluted inocula of the same sample (16). The result is an increase in sensitivity and a reduction in time and labor costs while still permitting detection of *Vibrios* at levels < 30 CFU/g as required for validation protocols (2, 10, 16). For these reasons we propose acceptance of the application of QPCR-MPN for improved assessment of validation and verification protocols related to oyster post harvest processing.

METHOD DOCUMENTATION

Method Title
QPCR-MPN Assay using DuPont Qualicon BAX ® Real Time *Vibrio* Test Kit

Method Scope
This method is designed for MPN analysis of validation trials for oyster PHP and for detection of *Vibrio* species in seafood and monitoring shellfish harvesting waters.

Principle
QPCR-MPN will be substituted as an alternative to the officially recognized NSSP method for MPN analysis of validation trials for oyster PHP (3). Specifically QPCR will be substituted for microbiological/DNA probe confirmation of *V. cholerae, V. parahaemolyticus, and V. vulnificus* following growth in MPN enrichment. Since the FDA and the ISSC have mandated postharvest processing (PHP) of oysters harvested from Gulf Coast states in order to reduce *V. vulnificus* infections validation and verification are necessary in order to ensure that the process will substantially reduce numbers of *V. vulnificus* bacteria to levels to below the predicted threshold for disease. QPCR-MPN is a rapid and reliable method to accomplish agency mandates and industry goals. Validation criteria was recently expanded to include reduction of *V. parahaemolyticus* in PHP oysters. Application to evaluation of other seafood products is also anticipated, especially imported products that may be a greater risk for *V. cholerae* contamination

Proprietary Aspects
Ingredients in **DuPont Qualicon BAX ® Real Time *Vibrio* Test Kit** are proprietary information.

Equipment
Applied Biosystems Inc real-time thermocycler 7500S

Reagents
- **DuPont Qualicon BAX ® Real Time *Vibrio* Test Kit**
- SYBR green I (Invitrogen)
- Autoclaved molecular grade water

Media (Media are specified in FDA BAM, reference 8)
- Modified colistin polymyxin cellobiose (mCPC) agar
- T1N1 agar
- Alkaline peptone water (APW) enrichment broth
- Phosphate buffered saline (PBS)

Matrix or Matrices of Interest
The validation of post harvest processing for raw gulf coast oysters is performed on oyster homogenate. Thus the matrix is dilutions of oyster homogenate, consisting of oyster meats and PBS.

Sample Collection, Preservation, Preparation, Storage, Cleanup, Test Procedures:
Sample collection will follow procedures described by NSSP for validation of oyster PHP.
Preservation, preparation, storage, cleanup and test procedures follow manufacture's recommendations

Cost of the Method
The cost of the **DuPont Qualicon BAX ® Real Time *Vibrio* Test Kit** platform costs approximately $9 per PCR reaction.

Special Technical Skills Required to Perform the Method
Only basic laboratory skills are required.

Special Equipment Required and Associated Cost

Equipment	Approximate Cost
Dupont Bax thermocycler	$45,000 + accessories
Incubator	$3,000 - $6,000
Centrifuge	$2,000
Heat block	$500

Abbreviations and Acronyms

- PHP –post harvest processing
- DNA- deoxyribonucleic acid
- QPCR- quantitative polymerase chain reaction
- APW- alkaline peptone water
- PBS- phosphate buffered saline
- MPN- most probable number

Test Procedures and Quality Control

MEDIA: Dehydrated media is commercially dehydrated. Media must be sterilized according to manufacturer's instructions. Prepared culture media, dehydrated media and media components must be stored in a cool, clean, dry space unless refrigeration is required as per manufacturer instruction. Stored media is labeled with batch number, expiration date and sterilization date. Storage of prepared culture media at room temperature does not exceed 7 days. Refrigerated storage of prepared media with loose fitting closures does not exceed 1 month; screw-cap closures do not exceed 3 months. All prepared media stored under refrigeration are held at room temperature overnight prior to use. To determine the pH of prepared media, a pH meter with a standard accuracy of 0.1 units is used. The pH meter is calibrated with each use and a minimum of two standard buffer solutions (ph 4, 7 and 10) are used to calibrate the pH meter. Standard buffer solutions are used once and discarded.

COLD STORAGE: Refrigerator temperature must be monitored daily; temperature is maintained between 0˚C to 4˚C. Freezer temperature must be monitored at least once daily, freezer temperatures is maintained at -20˚C (DNA storage) and –80˚C (strain storage).

INCUBATOR: Temperature of incubators must be maintained at 30˚C (+/-0.5), 37˚C (+/-0.5), and 40˚C (+/-0.5). Thermometers must be graduated no greater than 0.5˚C increments. Temperatures are taken twice daily.

SUPPLIES: Utensils and containers made of clean borosilicate glass, stainless steel or other non-corroding material. Culture tubes made of a suitable size to accommodate the volume for broth and samples. Sample containers made of glass or other inert material. Dilution bottles and tubes are made of plastic and closed with attached snap-lock lids. Graduations are indelibly marked on dilution bottles and tubes or an acceptable alternative method is used to ensure appropriate volumes. Reusable sample containers must be capable of being properly washed and sterilized. Hardwood applicator transfer sticks, utilized for streaking and picking positive colonies, and Whatman # 3 and #541 filter papers, utilized in colony blot hybridization, are sterilized prior to use and stored in sterile, airtight containers. Pipettes used to inoculate the sample deliver accurate aliquots, have unbroken tips and are appropriately graduated. Pipettes larger than 10ml are not used to deliver 1ml; nor, are pipettes larger than 1ml used to deliver 0.1ml. Reagents for DNA extraction and PCR reaction are included in **DuPont Qualicon BAX ® Real Time *Vibrio* Test Kit**

MAINTENANCE: Routine autoclave maintenance must be performed and serviced annually or as needed by a qualified technician and records maintained. Autoclave provides a sterilizing temperature of 121˚C (tolerance 121 +/- 2˚C) as determined daily. Spore suspensions or strips must be used monthly to evaluate the effectiveness of the autoclave sterilization process, with results recorded. Heat sensitive tape must be used with each autoclave

batch. Autoclave sterilization records including length of sterilization, total heat exposure time and chamber temperature must be maintained in an autoclave log.

SHELLSTOCK SAMPLES: A representative sample of shellstock is collected. Shellstock is collected in clean, waterproof, puncture resistant containers. Shellstock labeled with collector's name, type of shellstock, the source, the harvest area, time, date and place of collection. Shellstock are maintained in dry storage between 0 and 10˚C until examined. Examination of the sample is initiated as soon as possible after collection, and does not exceed 24 hours after collection. Shucking knives, scrub brushes and blender jars are sterilized for 35 minutes prior to use. Blades of shucking knives free from debris corrosion. Prior to scrubbing and rinsing debris off shellstock, the hands of the technician are thoroughly washed with soap and water. Shellstock are scrubbed with a stiff, sterile brush and rinsed under water of drinking water quality. Shellstock are allowed to drain in a clean container or on clean towels prior to opening. Prior to opening, the technician washes hands and rinses with 70% alcohol. Shellstock are not shucked directly through the hinge.

FDA-MPN PREPARATION AND METHOD: Contents of shellstock are shucked into a sterile, tared blender jar. At least 12 animals (100 g of meat) are used for analysis. The sample is weighted to the nearest 0.1 gram and an equal amount by weight of sterile PBS diluent is added. Samples are blended at high speed for 90 seconds. Immediately after blending, the homogenized sample is diluted in a multiple dilution series with 3 replicas and inoculated into tubes of APW presumptive media for MPN analysis. Positive and negative controls cultures accompany samples throughout the procedure. Inoculated media are incubated at 37 +/- 0.5˚C. Presumptive tubes are read at 24+/- 2 hours of incubation and transferred if positive. Transfers are made to mCPC plates by sterile hardwood applicator sticks from presumptive positive APW tubes and confirmed by DNA probe.

QPCR-MPN PREPARATION: Prior to DNA extraction and preparing Cepheid© unit for QPCR, all micro-centrifuge tubes and pipette tips are sterilized for 35 minutes. The technician's hands are washed with soap and water. Gloves are worn and rinsed with 70% alcohol. All Pipetteman and Eppendorf pipettes are calibrated semi-annually and prior to use are wiped down with 70% alcohol. All working areas, centrifuge racks, and equipment are wiped down with 70% alcohol. Proper sterile technique is observed throughout the procedure to ensure contamination free samples. 1ml of sample from each positive MPN tube is used for the boil extraction procedure (appendix 1) to extract DNA to be used as template for Sybr green 1 QPCR-MPN assay as described in appendix 2. Cepheid© thermocycler cycle threshold is set at 30 and factory default is utilized for melt curve analysis regarding peak height.

VALIDATION CRITERIA

Ruggedness of Assay

DuPont Qualicon BAX ® Real Time *Vibrio* Test Kit for detection of *V. vulnificus, V. parahaemolyticus* and *V. cholerae* was recently accepted for AOAC approval (15). Proposed method will extend applications to MPN analysis of oyster PHP. Validity of MPN assay for detection of *V. vulnificus* has been previously established by ISSC and FDA. The ruggedness of reagents used for PCR is determined by manufacturer and meets specifications. Method uses a bead format that incorporates all reagents on bead to eliminate common pipetting and cross-contamination errors.

Data Comparability and Statistical Analysis

Quantitative PCR was previously applied to most probable number (QPCR-MPN) for validation of PHP and single specie detection of *V. vulnificus in* oysters (17). Published results by Wright et al., 2007 showed that immediately following inoculation of APW (pre-enrichment with either 0.1 or 0.01 g oyster homogenate detection *V. vulnificus* was 100 to 1000 fold more sensitive by QPCR than by growth on selective agar. Following O.N. growth in enrichment, both assays were equally as sensitive. For PHP oysters received nitrogen immersion, side by side comparison of standard MPN vs. QPCR-MPN showed excellent correlation (R^2=0.97 by Pearson's correlation co-efficient) and no significant differences between the two assays (Table 2). Results were comparable for untreated oysters and for PHP oysters at both 1 and 7 days post treatment. In this study results

were also examined side by side for both Nitrogen Immersion and Nitrogen Tunnel PHP treatments and statistical comparison of this data, utilizing both JMP from SAS and Minitab, both one way ANOVA and Tukeys post hoc tests show no significant differences ($p < 0.05$) between detection methods.

The AOAC evaluation of the DuPont Bax Vibrio QPCR test kit described application of the assay on five food types; raw shrimp, cooked shrimp, oysters, raw ahi tuna, and raw scallops (See attached draft of publication in appendix). Results supported the applicability of the BAX ® system for detecting *Vibrio* in foods. Samples were analyzed using the BAX ® system method and the FDA-BAM methods for detecting *Vibrio.* One food type, ahi tuna, was tested by an external independent laboratory (the State of Texas Department of Public Health, Consumer Microbiology Division) as a shared matrix. Results were in nearly complete concordance with only two cases where the test kit yielded a result that could not be confirmed by culture. Inclusivity and exclusivity of the assay was determined with all tested isolates (n = 126 target *Vibrio* strains and n = 55 non-*Vibrio* and non-target *Vibrio* species strains) demonstrating expected results and an assessment of test kit stability, lot to lot variability, and assay ruggedness was also performed demonstrating robustness of the assay.

During 2007 summer PHP validation trials were conducted by The University of Florida Aquatic Food Products group in a partnership with the oyster industry in Apalachicola FL. Side by side field trials compared the FDA-MPN to the QPCR-MPN assay are described below (Table 1). Side-by-side sample comparisons of the two assays support application of QPCR technology for validation oyster processing protocols. Samples (n=3), consisting of 12 oysters each, were obtained from untreated oysters (25IS, 29IS); temperature abused (26 TA, 30TA) by incubation O.N. at room temp; PHP heat treated oysters (65.5 for 5 min) after 7 days storage at -20C (26HSD7, 30HSD7); or Blast frozen oyster (-50C) after 42 days storage (26BLD42). The mean MPN/g for the two assay were nearly identical with R^2=0.99.

Table 1: Comparison of MPN Protocols

OYSTER LOT:	Log MPN/g	
	FDA MPN	BAX-QPCR MPN
25IS25,	2.0±0.56	2.0±0.62
29IS	2.0±0.6	2.0±1.03
26TA	4.0±0.64	4.0±0.40
30TA	6.0±0.11	6.0±0.22
26HSD7	<3.0	<3.0
30HSD7	1.0±0.66	1.1±0.58
26BLD42	2.0±0.43	2.1±0.51

Limit of Quantitation and Specificity

The attached AOAC draft manuscript details the limits of quantitation and specificity.
Inclusivity testing (n=50 strains) was performed at ~10^5 cfu/ml, while exclusivity testing (n= 50 strains) was performed at ~10^8 cfu/ml from broth cultures. Additional strains were tested by Wright Lab (see attached Table 2, 3, 4 in appendix)

For AOAC approval for spiked foods, Vibrio strains were inoculated to yield fractional positive results for plus/minus screening, or at levels informative of method performance for MPN-based approaches. Samples were tested with the FDA-BAM culture-based method and by PCR using the BAX® system. Ahi tuna was spiked at three levels with Vc and tested for presence or absence of target in sets of twenty 25g sub-samples and five unspiked sub-samples, with PCR testing from the BAM enrichments. Similarly, scallops were spiked with Vv at a level giving fractional results for the (how many samples?) 1g samples, and each MPN tube was tested by the BAM method and PCR as were five 25g samples enriched in a comparable manner. Naturally occurring low-level Vc in raw shrimp was also tested using twenty 25g samples with both the BAM method and PCR testing from the same enrichments. All inclusivity/exclusivity testing demonstrated expected results. For effectiveness testing, comparing PCR and culture, results for the spiked ahi tuna (36 positive of 65 samples tested) and shrimp (5 positive of 20 samples tested) were identical with no false negative or false positive results by PCR. Scallop data gave identical MPN results for test and reference methods and 25g enrichments were all positive by PCR.

Additional seeding studies conducted by Wright lab utilized known concentrations of *Vibrio* species to spike APW with or without oyster homogenates. Samples were assayed by QPCR immediately without growth using various combinations of high (10^6), mediun (10^4), low (10^2) concentrations of the three species. All samples were positive for all species with the exception of samples with High Vp and low or medium concentrations of Vv. In these cases, Vv was not detected. However, samples where growth was permitted (O.N. incubation at 37C), all species were detected in all samples.

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APPENDIX 1

Table 2 QPCR analysis for *V. cholerae* strains

***V. cholerae* Strains**	**Clinical (C) vs. Environmental (E): Description**	***Vjp***	***Vv***	***Vc***	**CT**	**CFU/ml**
1. Vc598	**C:** classical Inaba O1	-	-	+	23.7	>1.0E+09
2. Vc NRT 36S	C: non O1, O139, NAG-ST (Japan)	-	-	+	23.1	>1.0E+09
3. Vc JVY212		-	-	+	25.7	5.50E+08
4. Vc JVB52		-	-	+	27.2	1.60E+08
5. Vc 5439/62		-	-	+	28.2	7.00E+07
6. Vc 569B	**C:** O1, classical, inaba (India)	-	-	+	24.6	>1.0E+09
7. Vc S171		-	-	+	24.7	>1.0E+09
8. Vc NAG12		-	-	+	25.5	6.50E+08
9. Vc ATCC25874		-	-	+	22.4	>1.0E+09
10. Vc 8		-	-	+	24.8	>1.0E+09
11. Vc B1307 Dacca		-	-	+	24.8	>1.0E+09
12. Vc A5		-	-	+	26.3	3.50E+08
13. Vc I10		-	-	+	25.2	8.20E+08
14. Vc 646	**C:** O1, Ogawa,	-	-	+	24	>1.0E+09
15. Vc 395	**C:** Classical Ogawa O1 (India)	-	-	+	25.6	6.10E+08
16. Vc 6337		-	-	+	35.4	1.90E+05
17. Vc T2001		-	-	+	34.7	3.40E+05
18. Vc T5957		-	-	+	34.7	3.60E+05
19. Vc 2076-79	C: non O1, O139, NAG-ST (oysters, US)	-	-	+	33.7	7.60E+05
20. Vc BA312		-	-	+	34.4	4.30E+05
21. Vc 569B	**C: O1 classical Inaba (India)**	-	-	+	33.9	6.60E+05
22. Vc AI1837		-	-	+	34	6.20E+05
23. Vc Arg-3	**E:**	-	-	+	34.4	4.40E+05
24. Vc C6706	**C: O1, el tor, Inaba (Peru)**	-	-	+	33.9	6.50E+05
25. Vc CA385	**C: O1**	-	-	+	34.6	3.80E+05
26. Vc CO603		-	-	+	34.2	5.00E+05
27. Vc CO845		-	-	+	33.8	7.10E+05
28. Vc N16961	**C: 01 eltor Inaba**	-	-	+	33.9	6.40E+05
29. Vc NG288/36	**C: O139 (Thailand)**	-	-	+	33.7	7.50E+05
30. Vc NRT36S		-	-	+	36.6	7.20E+04
31. Vc PS15		-	-	+	36.2	1.00E+05
32. Vc V5C		-	-	+	34.3	5.00E+05
33. Vc ATCC 25873		-	-	+	0	0
34. Vc SO47W		-	-	+	33.9	6.40E+05
35. Vc RB1		-	-	+	34.9	3.00E+05
36. Vc J31W		-	-	+	39.3	<1.0E+04
37. Vc T96W		-	-	+	36.6	7.00E+04
38. Vc 6358		-	-	+	0	0
39. Vc 5057		-	-	+	34.5	4.20E+05
40. Vc 7261		-	-	+	34	6.40E+05
41. Vc 7165		-	-	+	33.2	1.20E+06
42. Vc 9115		-	-	+	0	0
43. Vc 5145		-	-	+	34.3	4.80E+05
Blank		-	-	-	0	0

Table 3 QPCR analysis for *V. parahaemolyticus* strains

V. parahaemolyticus Strains	Clinical (C) vs. Environmental (E): Description	*VP*	*Vv*	*Vc*	CT	CFU/ml
1. Vp TX2103		+	-	-	25.6	3.50E+08
2. Vp TX3547		+	-	-	26	2.60E+08
3. Vp DAL1094		+	-	-	26.2	2.30E+08
4. Vp 17802		+	-	-	24.6	6.80E+08
5. Vp 43996		+	-	-	25.5	3.70E+08
6. Vp 10290		+	-	-	25.5	3.70E+08
7. Vp Y 9398		+	-	-	23.7	>1.0E+09
8. Vp 5E-3		+	-	-	34.1	1.40E+06
9. Vp 205-757		+	-	-	33.4	2.30E+06
10. Vp AQ3810		+	-	-	33.5	2.10E+06
11. Vp AQ4235		+	-	-	33.1	2.80E+06
12. Vp EDL896		+	-	-	33.4	2.30E+06
13. Vp VP2		+	-	-	33.5	2.20E+06
14. Vp VP250		+	-	-	33	3.00E+06
15. Vp VP331		+	-	-	33.2	2.70E+06
16. Vp VP356		+	-	-	33.3	2.40E+06
17. Vp VP381		+	-	-	33.4	2.30E+06
18. Vp VP53		+	-	-	33.2	2.50E+06
19. Vp VP81		+	-	-	31.7	6.70E+06
20. Vp WP-1		+	-	-	34.2	1.40E+06
21. Vp S162-71		+	-	-	33.5	2.10E+06
22. Vp 3D-38		+	-	-	33.5	2.10E+06
23. Vp EDL 1044		+	-	-	33.1	2.80E+06
24. Vp SN36		+	-	-	33.8	1.80E+06
25. Vp 14d13		+	-	-	33.4	2.30E+06
26. Vp AAG9574		+	-	-	33.3	2.50E+06
27. Vp P125		+	-	-	33.5	2.20E+06
28. Vp HMG38		+	-	-	33.2	2.60E+06
29. Vp P29		+	-	-	34.1	1.40E+06
30. Vp VV104		+	-	-	33.7	1.90E+06
31. Vp VV27-1		+	-	-	33.5	2.10E+06
32. Vp VV27-2		+	-	-	33.9	1.60E+06
33. Vp A602		+	-	-	32.9	3.20E+06
34. Vp FC1011		+	-	-	34.7	9.70E+05
35. Vp B10576		+	-	-	33.4	2.30E+06
36. Vp EDL1041		+	-	-	34	1.60E+06
Blank		-	-	-	0	0

Table 4 QPCR analysis for *V. vulnificus* strains:

vulnificus rains	Clinical (C) vs. Environmental (E): Description	*Vjp*	*Vv*	*Vc*	CT	cfu/ml
Vv MO6-24/O	C	-	+	-	24.3	5.20E+08
Vv FLA 109	C	-	+	-	27	5.90E+07
Vv FLA141	C	-	+	-	27.3	4.80E+07
Vv FLA126	C	-	+	-	28.5	1.90E+07
Vv FLA134	E: oyster	-	+	-	26.5	8.90E+07
Vv FLA129	C	-	+	-	26.8	6.80E+07
Vv FLA127	E: oyster	-	+	-	26.5	9.10E+07
Vv FLA135	E: oyster	-	+	-	26.8	7.00E+07
Vv FLA 115	E: oyster	-	+	-	27.1	5.60E+07
Vv FLA 149	C	-	+	-	28.5	1.90E+07
Vv B3-313/98	E: fish	-	+	-	27.3	4.70E+07
Vv FLA121	E: oyster	-	+	-	26.9	6.20E+07
Vv FLA137	E: oyster	-	+	-	26.4	9.40E+07
Vv B3-302/99	E: fish	-	+	-	26.7	7.60E+07
Vv FLA119	E: oyster	-	+	-	26.7	7.30E+07
Vv FLA116	C	-	+	-	26.5	8.80E+07
Vv FLA102	C	-	+	-	26.7	7.60E+07
Vv B2-2	E: fish	-	??	-	0	0
Vv FLA108	C	-	+	-	26	1.30E+08
ank		-	-	-	0	0

APPENDIX 2: Draft manuscript for AOAC approval:

DuPont Qualicon BAX ® Real Time *Vibrio* Test Kit for the Detection of *Vibrio cholera, parahaemolyticus and vulnificus* from Tuna, Shrimp and Oysters

AOAC Performance Tested Methodsm YYMMXX

ABSTRACT
An evaluation was conducted on five food types; raw shrimp, cooked shrimp, oysters, raw ahi tuna, and raw scallops to demonstrate the applicability of the BAX ® system for detecting *Vibrio* in foods. Samples were analyzed using the BAX ® system method and the FDA-BAM methods for detecting *Vibrio.* One food type, ahi tuna, was tested by an external independent laboratory (the State of Texas Department of Public Health, Consumer Microbiology Division) as a shared matrix. Results were in nearly complete concordance with only two cases where the test kit yielded a result that could not be confirmed by culture. Inclusivity and exclusivity of the assay was determined with all tested isolates (n = 126 target *Vibrio* strains and n = 55 non-*Vibrio* and non-target *Vibrio* species strains) demonstrating expected results and an assessment of test kit stability, lot to lot variability, and assay ruggedness was also performed demonstrating robustness of the assay.

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Scope of method
1.1 Target organisms – Vibrio cholera, parahaemolyticus, and vulnificus. A wide range of *Vibrio* and non-*Vibrio* strains was used for inclusivity/exclusivity testing.
1.2 Matrices – Specific foods tested included shrimp, oysters, tuna, and scallops.
1.3 Performance claims – Sensitivity and specificity equivalent to the official FDA-BAM culture-based method.

Definitions

- From the AOAC International Official Methods of Analysis Program Manual Appendix X [1]: Sensitivity rate (p+) for a food type and inoculation level - The probability that the method, alternative or reference, will classify a test sample as positive, given that a test sample is a known positive. A known positive refers to the confirmation of innoculated analyte.

Sensitivity rate is defined as: Total number of confirmed positive test portions by the method divided by total number of confirmed positive test portions by both the alternative and reference methods.
Specificity rate (p-) for a food type and inoculation level - The probability that the method will classify the test sample as negative, given that the test sample is a known negative. A known negative refers to a confirmed negative test portion.
Specificity rate is defined as: Total number of analyzed negative test portions by the method divided by total number of confirmed negative test portions by both the alternative and reference methods. For microbiological

methods involving a confirmation step, a presumptive positive result is taken through the cultural procedure and confirmed to be a positive or determined to be a negative. In other words, the confirmation procedure allows the sample to be reclassified as a known positive or a known negative. As such, the specificity rate of results after confirmation is always 100%.
False negative rate (pf-) for a food type and inoculation level - The probability that a test sample is a known positive, given that the test sample has been classified as negative by the method. pf- is the number of misclassified known positives divided by the total number of positive test samples (misclassified positives plus the number of correctly classified known positives) obtained with the method. Incidence of false negatives equals 100 minus the sensitivity rate.
False positive rate (pf+) for a food type and inoculation level - The probability that a test sample is a known negative, given that the test sample has been classified as positive by the method. pf+ is the number of misclassified known negatives divided by the total test samples (misclassified positives plus the number of correctly classified known negatives) obtained with the method.
Incidence of false positives equals 100 minus the specificity rate.

Principle

The BAX® system uses the Polymerase Chain Reaction (PCR) to amplify specific DNA fragments, which are stable and unaffected by growth conditions [2]. Each fragment is a genetic sequence that is unique to the targeted organism, thus providing a highly reliable indicator that the organism is present. The BAX® system simplifies the PCR process by combining the requisite PCR reagents into a stable, dry, manufactured tablet already packaged inside the PCR tubes. After hydrating these tablets with prepared samples, the tubes remain sealed to reduce the potential for contamination.

In a typical PCR application, sample DNA is combined with DNA polymerase, nucleotides and primers that are specific for a given nucleotide sequence. The mixture then undergoes a series of timed heating and cooling cycles. Heating denatures the DNA, separating it into single strands. As the mixture cools, the primers recognize and anneal (bind) to the targeted DNA sequence. DNA polymerase then uses nucleotides to extend the primers, thus creating two copies of the targeted fragment (amplification). Repeating cycles of denaturing, annealing and extending produces an exponential increase in the number of target DNA fragments, creating millions of copies in a very short time. If the target sequence is not present, no detectable amplification takes place [2]. Inhibitors to PCR are present in some food matrices. In particular, phenolic compounds found in some spices and other plant-based materials such as high purity cocoa can cause the PCR reaction to shut down. Because of this, each BAX reagent tablet is formulated with a low level control DNA molecule and associated primers. This Internal Positive Control (INPC) must be shown to amplify in the absence of specific pathogen target amplification product for the BAX ® instrument to report a negative result. In the absence of any target or INPC associated product, the instrument reports an indeterminate result.

The BAX® system PCR tablets used in real-time assays also contain multiple dye-labeled probes. Intact probes are short oligonucleotides with quencher dye at one end that absorbs the signal from fluorescent reporter dye at the opposite end. During PCR cooling cycles, probes bind to a specific area within the targeted fragment. During extension, DNA polymerase encounters the probe in its path and breaks the probe apart. This releases the reporter dye, resulting in increased fluorescent signal [3]. In multiplex reactions such as in this test kit, each species specific probe is labeled with a different fluorescent reporter dye, allowing independent detection of the presence or absence of each target. The BAX® system Q7 instrument uses multiple filters to measure specific signal resulting from the presence of each target at the end of each cycle and report results for the presence or absence of *Vibrio cholera*, *vulnificus*, or *parahaemolyticus* in less than 90 minutes.

General information

Vibrio is a gram-negative genera consisting of 65 known species [4]. It can cause seafood and water-borne illnesses and infections in humans. It is most commonly found in marine and freshwater environments and is

transmitted to humans mainly through the consumption of raw or undercooked shellfish, particularly oysters, or through contaminated drinking water [5].

The risk of *Vibrio*-caused illness is increased following a natural disaster leading to disruption of water and sanitation systems or massive displacement of a population to inadequate and overcrowded temporary housing. Such an effect was seen in the aftermath of Hurricane Katrina in 2005, where surveillance identified 22 new cases of *Vibrio* illness, including five deaths [5].

The three species of Vibrio that cause the majority of human illness and infection are *Vibrio cholera*, *parahaemolyticus*, and *vulnificus* [6].

Cholera is a major disease that occurs when *Vibrio cholera* colonizes the small intestine and releases enterotoxin(s) leading to a secretory diarrhea that without supportive oral rehydration and replacement of salts can prove fatal. The disease is currently endemic in many countries in South Asia, Africa and the Americas and remains a global threat to public health [6].

Vibrio parahaemolyticus is an invasive organism that primarily affects the colon. It is estimated that up to 4500 cases of *Vibrio parahaemolyticus* infection occur annually in the United States [7]. These illnesses are mainly due to the consumption of undercooked oysters and other seafood.

Vibrio vulnificus is an emerging human pathogen that can cause illnesses such as gastroenteritis and can cause wound infections that can progress to septicemia. Though the total number of cases of *V. vulnificus* infection is small, it is highly pathogenic in certain populations, and thus is responsible for an estimated 1% of all foodborne deaths in the United States [8].

Test Kits Information

5.1 *Test kit name* – BAX® System Real-Time PCR Assay for Screening *Vibrio cholerae*, *parahaemolyticus*, *vulnificus*

5.2 *Test kits catalog numbers* – D12863877

5.3 Ordering information –

5.3.1 DuPont Qualicon, Experimental Station, Bldg. 400, P.O. Box 80400, Rt. 141 & Henry Clay Road, Wilmington, DE 19880-0400, USA, Phone 800-863-6842 or 302-695-5300, Fax 302-695-5301, Internet www.qualicon.com

5.3.2 DuPont Qualicon Europe, Ltd Wedgwood Way, Stevenage Herts SG1 4QN, UK

5.3.3 DuPont Qualicon, Asia/Pacific DuPont Company (Singapore) Pte, Ltd. 1 Harbour Front Place #11-01, Harbour Front Tower One, Singapore 098633

5.4 Test kit components –

5.4.1 PCR tubes with tablets (twelve 8-tube strips, each tube containing 1 PCR tablet)

5.4.2 Flat optical caps for PCR tubes (twelve 8-cap strips)

5.4.3 Lysis buffer (two 12-ml bottles)

5.4.4 Protease (one 400-µl vial)

5.4.5 Package insert (1)

Additional reagents

Protease reagent – Using test kit reagents, pipette 150 µL of protease into one 12-mL bottle of lysis buffer. Label bottle with the date prepared. Reagent will remain stable for up to two weeks if stored at 2-8ºC.

Apparatus

7.1 Incubators – Static incubators at 35 ± 2ºC, 39-40ºC, and a heated water bath capable of maintaining a temperature of 41± 0.2ºC.

7.2 Stomacher, Blender, and Scissors – For sample preparation. Seward model 400 or equivalent stomacher, Blender with blending jars, and autoclavable scissors.

7.3 BAX® system Q7 apparatus (all components listed in this section are included with the *BAX®* Q7 System Start Up package. Components 7.3.3 – Cluster tubes with caps, and 7.3.6 – Pipette tips; after the initial boxes included with the start-up package are used; must be purchased by the test kit user).

7.3.1 BAX® System cycler/detector with computer workstation

7.3.2 BAX® System application software

7.3.3 Cluster tubes with caps and racks for lysis

7.3.4 Capping/de-capping tools – for removing and sealing cluster tube caps and PCR tube caps without jarring the contents

7.3.5 Heating blocks with inserts and thermometers – for maintaining lysis tubes at 37ºC ± 1ºC, 55ºC ± 1ºC and 95ºC ± 1ºC

7.3.6 Pipettes – for transferring reagents; two adjustable mechanical pipettes covering 20-200 µl and 5-50 µl; one repeating pipette; and one multi-channel pipette covering 8 channels and 5-50 µl. Pipettes should be calibrated to deliver required volumes within 10%.

7.3.7 Pipette tips with barriers: 0.5-250 µl, 0.5-100 µl extended barrier; 2.5 ml and 5 ml repeater pipette tips

7.3.8 Cooling block assemblies – for keeping lysate tubes and PCR tubes chilled at 2-8ºC during sample preparation

7.3.9 PCR tube holders – for transferring a rack of tubes from the cooling block to the cycler/detector

7.3.10 Printer

Standard Reference Materials

8.1 DuPont Qualicon culture collection (DD) - proprietary

8.2 *American Type Culture Collection* (ATCC) - *American Type Culture Collection* (ATCC) - www.atcc.org, American Type Culture Collection (ATCC), P.O. Box 1549, Manassas, VA 20108, USA.

Standard solutions, consumables, and media

Media - where applicable FDA-BAM designations listed in parentheses.

Alkaline peptone water (APW) (M10)
AKI medium (M7)
Arginine glucose slants (AGS) (M16)
Blood agar (5% sheep red blood cells) (M20)
Casamino acids yeast extract (CAYE) broth (M34)
modified Cellobiose polymyxin colistin (mCPC) agar (M98)
Cellobiose colistin (CC) agar (M189)
Motility test medium-1% NaCl (M103)
Oxidase reagent (1% N,N,N,N'-tetramethyl-p-phenylenediamine.2HCl in dH2O) (R54)
Phosphate buffered saline (PBS) (R59)
Polymyxin B disks, 50 U (Difco or equivalent) (R64)
Saline soln - 0.85% in dH2O (R63)
2% NaCl soln (R71)
Sodium desoxycholate - 0.5% in sterile dH2O (R91)
Thiosulfate citrate bile salts sucrose (TCBS) agar (M147)
T_1N_1 and T_1N_3 agars (1% tryptone and either 1% or 3% NaCl) (M163)
T_1N_0, T_1N_3, T_1N_6, T_1N_8, T_1N_{10} broths (M161)
Tryptic soy agar-magnesium sulfate- 3% NaCl (TSAMS) (32) Trypticase (or tryptic) soy broth (TSB), agar (TSA)(M152) (with added NaCl, 2%)
TSB-1% NaCl-24% glycerol
Urea broth (M171) (or Christensen's urea agar (M4+0) with added NaCl (2%) (R71)
Vibrio parahaemolyticus sucrose agar (VPSA) (M191)
Vibrio vulnificus agar (VVA) (M190)
Chromagar *Vibrio* (DRG International Mountainside, NJ Product number VB912)
API 20E diagnostic strips and reagents (BioMerieux, Hazelwood, Mo.)

All microbiological media was prepared by autoclaving at 121°C at 15 psi for 15 min if preparing $\leq$ 4 L of media and 20 min if preparing > 4 L of media.

Safety Precautions

10.1 *Kits* – The reagents used in the BAX® system should pose no hazards when used as directed. Dispose of lysate, PCR mixture and other waste according to your site practices.

10.2 *Cycler/detector* – Only qualified laboratory personnel should operate the cycler/detector. Do not attempt to repair the instrument. Live power may still be available inside the unit even when a fuse has blown or been removed. Refer to the *User Guide* for maintenance procedures when cleaning the unit or changing a fuse. The heating block can become hot enough during normal operation to cause burns or cause liquids to boil. Wear safety glasses or other eye protection at all times during operation.

10.3 Enrichment Broths- All enrichment broths whether testing positive or negative for this assays targets, may contain enriched pathogens and should be autoclaved following any culture-based confirmatory steps.

General Preparation / Sample preparation and recovery

11.1 *Selection of strains for testing*- Strains were taken from the DuPont/Qualicon culture collection (samples tested by Qualicon) (see Table 2), collaborators' culture collections (the University of Florida and the Texas State Department of Public Health), and the American Type Culture Collection (ATCC).

11.2 *Culture preparation for artificially contaminated food – Vibrio* were grown to stationary phase in APW and serially diluted in APW to final concentrations likely to give fractional recovery (based on preparatory studies).

11.3 *Food samples* – Five food types were included in this study; raw ahi tuna, raw shrimp, cooked shrimp, oysters, and raw scallops.

Raw tuna was artificially inoculated with *V. cholera*, cooked shrimp were artificially inoculated with *V. parahaemolyticus*, and raw scallops were artificially inoculated with *V. vulnificus*, while naturally occurring flora was tested in raw shrimp and raw oysters. Reference method enrichment varied

according to the sample type examined. Tuna and raw shrimp were tested on a plus/minus basis according to the FDA-BAM protocols for *V. cholera*. Though much of the FDA-BAM *Vibrio* chapter is MPN-based, and thus the MPN-based methods were used to validate the effectiveness of the assay, it is anticipated that the BAX ® test kit will primarily be used to screen on a presence/absence basis so additional samples were tested to validate this type of screening. That is, samples were tested using the FDA-BAM enrichment conditions and culture confirmation with BAX ® testing from each of the MPN replicates, but with additional unpaired 25g samples enriched in 225 ml of enrichment media before BAX ® testing as a complement. Each 25g sample enrichment was also culture confirmed using the FDA-BAM methodology.

Analysis – BAX® system methodS

12.1 *Prepare equipment* - Turn on heating blocks (37ºC and 95ºC). Check that cooling blocks have been refrigerated overnight. Turn on power to cycler/detector, then to computer. Launch BAX® system application. If instrument diagnostics recommends verification, follow Verification Wizard screen prompts for procedure.

12.2 *Create rack file* – Follow prompts in the Rack Wizard to enter identifying data on the entire rack and on the individual samples.

12.3 *Perform lysis* –Add 5 µL of enrichment from the top of each enrichment to 200 µL of protease reagent in a cluster tube. Place in heating block at 37±1°C for 30 minutes. Transfer tubes to 95°C heating block for 10 minutes. Transfer to cooling block (2–8°C) for 5 minute.

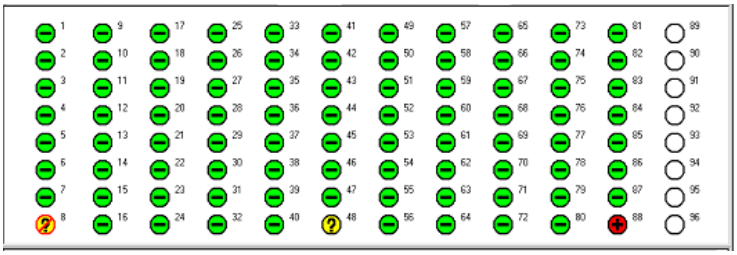
12.4 *Warm up cycler/detector* - Select RUN FULL PROCESS from the menu bar of the application window to heat the instrument to the set temperature (90ºC for the block, 100ºC for the lid).

12.5 *Hydrate PCR tablets with lysate* - Place PCR tube holder over insert of the PCR cooling block (solid side in rear). Place one PCR tube per sample into the holder. Loosen all caps, and remove caps from a row of tubes. Using a multi-channel pipette, transfer 30 µL of lysate to the row of PCR tubes for the *Vibrio* assay. Seal tubes with replacement optical caps. Using new tips, repeat transfer for each row until all samples have been transferred into PCR tubes.

12.6 *Amplify and detect* - Follow screen prompts at the PCR Wizard for loading samples into the cycler/detector and begin the program. The Full Process program takes about 75 min to complete. When finished, the PCR Wizard will prompt you to unload the samples and will automatically display the results.

Interpretation and test result report

Review results on screen as a grid of wells



Negative - Circle with (-) symbol
Positive - Circle with (+) symbol
Indeterminate - Circle with (?) symbol
Error (low signal) - Circle with (?) symbol and slash (/)

Food method comparison studies

Methodology – In accordance with an AOAC-RI approved study design, DuPont Qualicon compared the BAX® system method to the FDA-BAM [9] method for detecting *Vibrio* species in food samples.

Tuna (*V. cholera*) – Internal Qualicon and Independent Laboratory Shared Matrix

For tuna testing, a strain of *V. cholera* was taken from the DuPont Qualicon culture collection and struck for purity on a T_1N_1 agar plate. A single colony was inoculated into a tube containing 10 ml of APW broth, and incubated 18 hrs at 35ºC. The stationary phase culture was enumerated by plating dilutions on T_1N_3 and TSA agar plates. Based on preparatory studies, a dilution factor was established to give inoculation levels appropriate for achieving fractional positive results for the tuna matrix. Samples were

inoculated as a master sample of sliced tuna, and mixed well by shaking and hand massaging in a biohazard bag. Samples were divided into analytical size portions into blender jars if they were to be blended or stomacher bags if they were to be processed by scissors and held at 4°C for 48-72 hours before enrichment (Qualicon tested by scissors processing while the independent laboratory tested by blending). Following this cold stress/acclimation, if processing with scissors, portions of tuna were removed and processed with scissors which were decontaminated with ethanol and allowed to air dry before preparation of another sample. Samples prepared in this way were cut into approximately 1g pieces (~25 pieces per analytical unit). If processing with blending, portions were blended at high speed for 1 min. If processing with blending, portions were blended at high speed for 1 min. Three each samples of 100g, 10g and 1g were also prepared from this mix for MPN analysis.

Tuna portions were mixed as described above in 225 ml of APW and incubated at 35°C for 22 +/- 2 hrs total with reference method plating performed at 6-8 hrs and concurrently with BAX® testing after 16-20 hrs of incubation.

At each reference culture sample point, a 3 mm loop was used to streak for isolation onto dried plates of TCBS, mCPC, and CHROMagar *Vibrio* agar plates. Three or more typical colonies from each agar media when present were struck onto T_1N_3 agar plates and subjected to the initial biochemical screenings specified in the FDA BAM. Colonies which were phenotypically consistent with *Vibrio* (with a preference for *V. cholera* for this spiked study) were subjected to API-20E testing as described in the FDA BAM. If PCR positive samples' culture results had been inconsistent with *V. cholera*, up to 24 additional colonies would have been picked for characterization, but this was not needed for this matrix.

Raw Shrimp (*V. cholera*)
For raw frozen shrimp in an ongoing retail survey, Qualicon found shrimp with a low enough level of naturally occurring *V. cholera* to give fractionally positive results. Twenty samples of 25g each were removed from this batch and blended at high speed for 2 min at high speed in 225 ml of APW and incubated at 35°C overnight (18 +/- 2 hrs) with reference method plating performed at 6-8 hrs and concurrently with BAX® testing after overnight incubation onto TCBS, mCPC, and CHROMagar. Plates were incubated at 35-37°C overnight.

At each reference culture sample point, a 3 mm loop was used to streak for isolation onto dried plates of TCBS, mCPC, and CHROMagar *Vibrio* agar plates. Three or more typical colonies from each agar media were struck onto T_1N_3 agar plates and subjected to the initial biochemical screenings specified in the FDA BAM. Presumptive *V. cholera* was given preference for selection, despite the fact that there were many more colonies consistent with *V. parahaemolyticus,* and most enrichments (11/20) in this study were PCR positive for the presence of this species. Though not part of this study, all *V. parahaemolyticus* PCR positive enrichments did culture confirm for the presence of this species, and none of the PCR negative samples were culture positive. Colonies which were consistent with *Vibrio* in initial screening were subjected to API-20E testing as described in the FDA BAM. In two of the BAX ® positive enrichments, no culture confirmed isolates were initially obtained. Additional isolates were picked (up to 24 per plating media where available) and characterized. In both cases one or more *V. cholera* isolates were recovered. Samples from which one or more confirmed *V. cholera* isolates were obtained were considered reference method positive in this study.

Cooked Shrimp (*V. parahaemolyticus*)
Frozen, cooked shrimp were tested for artificially introduced *V. parahaemolyticus*. Cooked refrigerated shrimp were spiked as master samples at two levels with *V. parahaemolyticus* strain TD3129 in which at least one level was likely to be informative of method performance when compared to the reference MPN method. Shrimp were held at 4°C for 48-72 hrs to acclimate the introduced *Vibrio*. For the FDA BAM method, from the spiked master samples, five replicates of 50g of shrimp were weighed into blender jars and homogenized at high speed for 90 sec and used for analysis. The entire animal was used

for blending. PBS (450 ml) was added and blended for 1 min at 8,000 RPM. This constituted the 1:10 dilution. Two further serial dilutions were prepared in PBS for final 1:100 and 1:1000 dilutions (in testing of artificially contaminated product, since very low spike levels were used, no further dilutions were performed). Since this was a cooked product, 3 x 10 ml portions of the 1:10 dilution were transferred into 3 tubes containing 10 ml of 2X APW. This represented the 1 g portion. Similarly, 3 x 1 ml portions of the 1:100 and 1:1000 dilutions were inoculated into 10 ml of single-strength APW. APW enrichments were incubated overnight at 35 ±2°C (18 +/- 2 hrs). A 3-mm loopful from the top 1 cm of each APW tube was struck for isolation onto TCBS, mCPC, and *Vibrio* Chromagar plates. Concurrently with plating, a BAX ® PCR assay was performed from each MPN tube. TCBS and Chromagar plates were incubated at 35 ±2°C and mCPC at 39-40 °C overnight.

Additionally, five 25g samples from the same master sample were directly stomached (2 min at 100 rpm) with APW. For enrichment and plating, the 25g enrichments were treated as described above for MPN analysis.

V. parahaemolyticus appear as round, opaque, green or bluish colonies (usually), 2 to 3 mm in diameter on TCBS agar. Interfering, competitive *V. alginolyticus* colonies are, large, opaque, and yellow (usually). Isolates were struck for purity on T_1N_3 agar plates and subjected to initial screening by oxidase and string tests. Isolates giving expected reactions were subjected to further screening using the API 20E test kit as modified in the FDA-BAM by using 2% NaCl as the diluent.

Raw Scallops (*V. vulnificus*)

Raw scallops were spiked with *V. vulnificus* strain TD3149 at a level likely to be informative of method performance (in which at least one dilution of the MPN analysis was fractionally positive) when compared to the reference MPN method. For the FDA BAM method, from the spiked master samples, five replicates of 50g of scallops were weighed into blender jars and homogenized at high speed for 90 sec and used for analysis. Scallops were held at 4°C for 48-72 hrs to acclimate the introduced *Vibrio*. PBS (450 ml) was added and blended for 1 min at 8,000 RPM. This constituted the 1:10 dilution. One further serial dilution was prepared in PBS for a final 1:100 dilution (in testing of artificially contaminated product, since very low spike levels were used, no further dilutions were performed). 3 x 10 ml portions of the 1:10 dilution were transferred into 3 tubes containing 10 ml of 2X APW. This represented the 1 g portion. Similarly, 3 x 1 ml portions of the 1:10 and 1:100 dilutions were inoculated into 10 ml of single-strength APW. APW enrichments were incubated overnight at 35 ± 2°C (18 +/- 2 hrs). A 3-mm loopful from the top 1 cm of each APW tube was struck for isolation onto TCBS, mCPC, and *Vibrio* Chromagar plates. Concurrently with plating, a BAX ® PCR assay was performed from each MPN tube. TCBS and Chromagar plates were incubated at 35 ±2°C and mCPC at 39-40 °C overnight (18 +/- 2 hrs).

Additionally, five 25g samples from the same master sample were directly stomached (2 min at 100 rpm) with APW. For enrichment and plating, the 25g enrichments were treated as described above for MPN analysis.

V. vulnificus appear as purple colonies on mCPC agar. Isolates were struck for purity on T_1N_3 agar plates and subjected to initial screening by oxidase and string tests. Isolates giving expected reactions were subjected to further screening using the API 20E test kit as modified in the FDA-BAM by using 2% NaCl as the diluent.

Oysters (*V. parahaemolyticus* and *V. vulnificus*)

BAX ® lysates were prepared as described above for scallops (with the exception that dilutions were carried out to 10^{-6}) from samples tested using the MPN procedures of the FDA-BAM in collaboration with the FDA Dauphin Island Seafood Laboratory. The FDA-BAM protocol with *tlh* (thermo-labile hemolysin) pcr based isolate confirmation for *V. parahaemolyticus* and with *vvh*-a (cytolysin) pcr based

isolate confirmation for *V. vulnificus* was used for these studies. BAX ® results were compared to the results from the appropriate species specific FDA-BAM PCR for the presence of *V. parahaemolyticus* and *V. vulnificus* in the MPN tubes. To demonstrate the utility of the protocol across a wide level of contamination density, three sets of oysters were examined. One set was stored overnight after harvest at 3°C, another set at 25°C overnight, and a third set at 35°C. For molluscan shellfish, ~12 animals were pooled and blended 90 sec with an equal vol of PBS (1:2 diln). A 1:10 dilution was prepared by weighing (weighing is recommended because air bubbles in the 1:2 dilution prevent accurate volumetric transfer) of the 1:2 homogenate to 4 X ml of PBS. Additional 10-fold dilutions were prepared volumetrically (i.e. 1ml of 1:10 to 9.0ml of PBS for a 1:100 dilution).

Three 100 ml portions (the 10g samples) were added to 100 ml 2X APW. Three 10 ml portions of the 1:10 dilution were inoculated into 3 tubes containing 10 ml of 2X APW. This represented the 1 g portions. Similarly, 3 x 1 ml portions of the 1:10, 1:100, 1: 1000, and 1:10,000 dilutions were inoculated into 10 ml of single-strength APW. APW was incubated overnight (18 +/- 2 hrs) at 35 ±2°C. A 3-mm loopful was struck from the top 1 cm of all APW tubes onto TCBS, mCPC, and CC agars.

1.1 TCBS plates were incubated at 35 ±2°C overnight (18 +/- 2 hrs) while mCPC and CC plates were incubated at 39-40°C. *V. parahaemolyticus* appear as round, opaque, green or bluish colonies, 2 to 3 mm in diameter on TCBS agar. Interfering, competitive *V. alginolyticus* colonies are, large, opaque, and yellow. Most strains of *V. parahaemolyticus* will not grow on mCPC or CC agar. On mCPC and CC agars, *V. vulnificus* colonies are round, flat, opaque, yellow, and 1 to 2 mm in diameter. Presumptive isolates (three typical isolates per species per MPN tube where available) were purified as described previously and inoculated onto T_1N_3 plates and into 96 well plates for freezing and subsequent FDA-BAM colony confirmation pcr testing.

1.1.1 Isolates with typical morphology from each MPN tube were identified as *V. parahaemolyticus* or *V. vulnificus* by pcr as described in the FDA-BAM and the following sections.

Confirmation of *V. vulnificus* by polymerase chain reaction

1. Isolates obtained by the MPN procedure plating were confirmed by PCR as described in the FDA-BAM.
2. Primers for PCR vvhA (519 base amplicon) are from base 785 to 1303 of the cytolysin gene. The following primers should be used:

   Vvh-785F 5' ccg cgg tac agg ttg gcg ca 3'
   Vvh-1303R 5'cgc cac cca ctt tcg ggc c 3'
3. The follow reaction was used:

Reagent	Reaction vol.
dH2O	28.2 µl
10X Buffer.MgCl2	5.0 µl
dNTPs	8.0 µl
primer mix (6 primers)	7.5 µl
template	1.0 µl
Taq polymerase	0.3 µl
Total vol	50.0 µl

4. The following PCR conditions were used:

   PCR conditions:

denature	94°C 10 min	
denature	94° C 1 min	
anneal	62°C 1 min	25 cycles
extend	72°C 1 min	
final extend	72°C 10 min	
hold	8°C indefinite	

5. Agarose gel analysis of PCR products. For each isolate, 10 µl PCR product was combined with 2 µl 6X loading gel and loaded into wells of a 1.5% agarose gel containing 1 µg/ml ethidium bromide submerged in 1X TBE. A constant voltage of 5 to 10 V/cm was applied. Gels were illuminated with a UV transluminator (Gel Doc 1000 System, BioRad, Hercules, CA) and bands were visualized relative to molecular weight marker migration. Positive and negative culture controls and reagent controls were included with each PCR run. Isolates were confirmed with the presence of a 519 bp for the species specific pcr product.

Confirmation of *V. parahaemolyticus* by polymerase chain reaction

1. Isolates obtained by the MPN procedure plating were confirmed by PCR as described in the FDA-BAM.
2. The following primer sets were used (final concentration in each reaction for each primer 0.2µM):
   tlh gene species specific (450 bp)
   L-TL 5' aaa gcg gat tat gca gaa gca ctg 3'
   R-TL 5' gct act ttc tag cat ttt ctc tgc 3'
3. The following PCR reagents were used:

Reagent	Reaction vol.
dH2O	28.2 µl
10X Buffer.MgCl2	5.0 µl
dNTPs	8.0 µl
primer mix (6 primers)	7.5 µl
template	1.0 µl
Taq polymerase	0.3 µl
Total vol	50.0 µl

4. The following PCR conditions were used:
   PCR conditions:

denature	94°C 3 min	
denature	94° C 1 min	
anneal	60°C 1 min	25 cycles
extend	72°C 2 min	
final extend	72°C 3 min	
hold	8°C indefinite	

5. Agarose gel analysis of PCR products. For each isolate, 10 µl PCR product was combined with 2 µl 6X loading gel and loaded into wells of a 1.5% agarose gel containing 1 µg/ml ethidium bromide submerged in 1X TBE. A constant voltage of 5 to 10 V/cm was applied. Gels were illuminated with a UV transluminator (Gel Doc 1000 System, BioRad, Hercules, CA) and bands were visualized relative to molecular weight marker migration. Positive and negative culture controls and reagent controls were included with each PCR run. Isolates were confirmed with the presence of the 450 bp band for the species specific pcr product.

Table 1. BAX vs. Reference Results for Presence/Absence Testing

Sample type	MPN or Spike Level	Samples	BAX pos	BAX Confirmed	Reference pos	Sensitivity[1]	Specificity[2]	Chi Square[3]
Tuna	0.5 MPN/25g (*V. cholerae*)	20	3	3	3	100%	100%	-
	1.9 MPN/25g (*V. cholerae*)	20	13	13	13	100%	100%	-
	3.75 MPN/25g (*V. cholerae*)	20	19	19	19	100%	100%	-
	0 cfu/25g	5	0	0	0		100%	
Tuna (Independent Laboratory)	6 MPN/25g (*V. cholerae*)	20	9	9	9	100%	100%	-
	0 cfu/25g	5	0	0	0		100%	
Frozen raw shrimp	Naturally contaminated (*V. cholerae*)	20	5	5	5	100%	100%	-

[1] Sensitivity - Total number of confirmed positive test portions by the method divided by total number of confirmed positive test portions by both the alternative and reference methods.

[2] Specificity - Total number of analyzed negative test portions by the method divided by total number of confirmed negative test portions by both the alternative and reference methods.

[3] McNemar Chi-Square test statistic used for calculating significance

Table 2. BAX System Results for Samples with Presence/Absence and MPN Testing								
	Presence/Absence in 25g sample			MPN (3 tube, 3 dilution – 1g, 0.1g, 0.01g)				
Sample type	Inoculation level	BAX positive / confirmed	Reference positive / confirmed	Sample	BAX positive (1g, 0.1g, 0.01g)	Reference positive (1g, 0.1g, 0.01g)	BAX MPN[1]	Reference MPN[1]
Cooked shrimp (*V. parahaemolyticus*)	1.8 cfu/g	5/5	5/5	1	1, 0, 0	1, 0, 0	0.36/g	0.36/g
				2	1, 0, 0	1, 0, 0	0.36/g	0.36/g
				3	1, 0, 0	1, 0, 0	0.36/g	0.36/g
				4	1, 0, 0	1, 0, 0	0.36/g	0.36/g
				5	1, 0, 0	1, 0, 0	0.36/g	0.36/g
Cooked shrimp (*V. parahaemolyticus*)	18 cfu/g	5/5	5/5	1	2, 0, 0	2, 0, 0	0.92/g	0.92/g
				2	2, 2, 0	2, 2, 0	2.1/g	2.1/g
				3	2, 0, 0	2, 0, 0	0.92/g	0.92/g
				4	3, 0, 0	3, 0, 0	2.3/g	2.3/g
				5	2, 1, 0	2, 1, 0	1.5/g	1.5/g
Scallops (*V. vulnificus*)	1.4×10^4 cfu/g	5/5	5/5	1	1, 0, 0	1, 0, 0	0.36/g	0.36/g
				2	0, 0, 0	0, 0, 0	<0.3/g	<0.3/g
				3	2, 0, 0	2, 0, 0	0.92/g	0.92/g
				4	0, 0, 0	0, 0, 0	<0.3/g	<0.3/g
				5	0, 0, 0	0, 0, 0	<0.3/g	<0.3/g

[1] MPN values determined using the FDA-BAM MPN tables.

Table 3. BAX System Results for Oysters with MPN Testing *V. parahaemolyticus* (3 tube, 8 dilution)				
Sample Set	BAX positive (10g, 1g, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6})	Reference positive (10g, 1g, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6})	BAX MPN[1]	Reference MPN[1]
3°C	3, 3, 3, 1, 0, 0, 0, 0	3, 3, 3, 1, 0, 0, 0, 0	42 MPN/g	42 MPN/g
25°C	3, 3, 3, 3, 3, 3, 3, 2	3, 3, 3, 3, 3, 3, 3, 2	1.1×10^6 MPN/g	1.1×10^6 MPN/g
35°C	3, 3, 3, 3, 3, 3, 3, 3	3, 3, 2, 3, 3, 3, 3, 3	>1.1×10^6 MPN/g	>1.1×10^6 MPN/g *

[1] MPN values determined using the FDA-BAM MPN tables.

***An MPN of 3,3,3 for the Reference MPN was used for the 10^{-4}, 10^{-5} and 10^{-6} replicates. This MPN calculation assumes that the one 10^{-1} g MPN tube from which no confirmed *V. parahaemolyticus* strain was recovered was a failure to pick a true typical isolate present in the background of non-*V. parahaemolyticus* which exhibited typical morphology for the target. Since all three replicates for the MPN tubes up to 5 orders of magnitude more dilute than the 10-1 tube were culture confirmed, it is unlikely that the culture result from this one discordant tube was correct.**

Table 4. BAX System Results for Oysters with MPN Testing *V. vulnificus* (3 tube, 8 dilution)				
Sample Set	BAX positive (10g, 1g, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6})	Reference positive (10g, 1g, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6})	BAX MPN[1]	Reference MPN[1]
3°C	3, 3, 1, 0, 0, 0, 0, 0	3, 3, 1, 0, 0, 0, 0, 0	4.6 MPN/g	4.6 MPN/g
25°C	3, 3, 3, 3, 3, 1, 0, 0	3, 3, 3, 3, 3, 1, 0, 0	4,200 MPN/g	4,200 MPN/g
35°C	3, 3, 3, 3, 3, 2, 0, 1	3, 2, 3, 3, 3, 2, 0, 1	14,000 MPN/g	14,000 MPN/g *

[1] MPN values determined using the FDA-BAM MPN tables

*** An MPN of 2,0,1 for the Reference MPN was used for the 10^{-4}, 10^{-5} and 10^{-6} replicates. This MPN calculation assumes that the one 1 g MPN tube from which no confirmed *V. vulnificus* strain was recovered was a failure to pick a true typical isolate present in the background of non-*V. vulnificus* which exhibited typical morphology for the target. Since all three replicates for the MPN tubes up to 3 orders of magnitude more dilute than the 10-1 tube were culture confirmed, it is unlikely that the culture result from this one discordant tube was correct..**

Table 5. BAX vs. Reference Results Aggregate									
Sample type	Target Level by MPN or cfu per 25 gram	Samples or Number of MPN Tubes	BAX pos	Reference pos	Sensitivity %[1]	Specificity %[2]	False Pos %[3]	False Neg %[4]	Chi Square[5]
Tuna	0.5 MPN/25g	20	3	3	100	100	0	0	-
	1.9 MPN /25g	20	13	13	100	100	0	0	-
	3.75 MPN /25g	20	19	19	100	100	0	0	-
	0 cfu/25g	5	0	0		100	0	0	-
Tuna (Independent Laboratory Study)	MPN/25g	20	9	9	100	100	0	0	-
	0 cfu/25g	5	0	0		100	0	0	-
Frozen raw shrimp	Naturally contaminated	20	5	5	100	100	0	0	-
Cooked shrimp (MPN)	1.8 cfu/g	45	5	5	100	100	0	0	-
Cooked shrimp (25g)	1.8 cfu/g	5	5	5	100		0	0	-
Cooked shrimp (MPN)	18 cfu/g	45	14	14	100	100	0	0	-

Cooked shrimp (25g)	18 cfu/g	5	5	5	100		0	0	-
Frozen Scallops (MPN)	1.4 x 10^4 cfu/g	45	3	3	100	100	0	0	-
Frozen Scallops (25g)	1.4 x 10^4 cfu/g	5	5	5	100		0	0	-
Oysters 3°C	Naturally contaminated – *V. parahaemolyticus*	24	10	10	100	100	0	0	-
Oysters 25°C Abuse		24	23	23	100	100	0	0	-
Oysters 35°C Abuse		24	24	23	100	96	4	0	0
Oysters 3°C	Naturally contaminated – *V. vulnificus*	24	7	7	100	100	0	0	-
Oysters 25°C Abuse		24	16	16	100	100	0	0	-
Oysters 35°C Abuse		24	18	17	100	94	6	0	0

[1] Sensitivity - Total number of confirmed positive test portions by the method divided by total number of confirmed positive test portions by both the alternative and reference methods.
[2] Specificity - Total number of analyzed negative test portions by the method divided by total number of confirmed negative test portions by both the alternative and reference methods.
[3] False negative rate is calculated as BAX (-) Ref (+) BAX enrichment samples / Tot Ref (+) samples
[4] False positive rate is calculated as BAX (+) Ref (-) / Tot Ref (-) samples
[5] McNemar Chi-Square test statistic used for calculating significance of results

Results and Discussion of Food Studies

Data from these studies exhibits near complete equivalence between test and reference method results. In all studies except the oyster trials, complete equivalence was found. From two enrichments in the oyster studies, there was a discordant result, one for *V. parahaemolyticus* and one for *V. vulnificus*. In both of these cases the result occurred in an MPN tube that was well under the highest dilution that tested positive and was thus likely indicative of a failure to be able to isolate the target when it was truly present in the enrichment. Since selective and differential media for Vibrio do not give complete inhibition against many other genre there was likely a relatively high number of non-target similar appearing bacterial colonies on the plate, and none of the selected colonies were found to be the target species by phenotypic characterization from these two enrichment tubes.

Since the BAX ® test kit returns a result in about 24 hours versus the 3-5 days needed for culture based methods; the test kit can lead to a significantly faster increase in release of product.

Inclusivity / Exclusivity Study

Choice of Strains

V. cholera (n=46), *V. parahaemolyticus* (n=47), and *V. vulnificus* (n=33) strains were tested by the BAX ® assay for inclusivity. Most isolates were originally obtained from naturally contaminated food and environmental samples (many from the laboratory of Dr. Judy Johnson, collected when she was on faculty at the University of Maryland) and an effort is being made to more accurately determine source for non-ATTC isolates shown below. Additionally, 36 strains were obtained through an ongoing retail shrimp study at Qualicon. Identifications were confirmed biochemically using either the API 20E test kit as modified in the FDA-BAM or using the biochemical characterization scheme described in Table 1 of the FDA-BAM *Vibrio* chapter (9), some *V. cholera* isolates (see table 6) were also characterized by serology.

Culture Enrichment

For each inclusivity strain, one colony from an overnight T_1N_3 agar plate was inoculated into a tube containing alkaline peptone water (APW) and incubated at 37°C overnight, giving a cell density of approximately 10^8 cfu/ml. Isolates were diluted 1:1000 in APW to reach the target enrichment level of 10^5 cfu/mL before processing in the BAX® system.

Each non-*Vibrio* exclusivity strain was incubated at 37°C overnight in Brain Heart Infusion (BHI) broth. Isolates were diluted 1:10 in BHI before processing in the BAX® system. *Vibrio* strains in the exclusivity panel were grown at 35°C overnight in APW, then diluted 1:10 in APW before processing in the BAX® system.

Results

Table 6. Inclusivity Results for *Vibrio cholerae/parahaemolyticus/vulnificus*

Strain ID	Other strain designation	Source	Location of testing	Species (serotype)	Result *V. cholera*	Result *V. parahaemolyticus*	Result *V. vulnificus*
VcJVY212		Unknown	UF	*V. cholerae*	Pos	Neg	Neg
VcJVB52		Unknown	UF	*V. cholerae*	Pos	Neg	Neg
Vc5439/62		Unknown	UF	*V. cholerae*	Pos	Neg	Neg
Vc569B		Unknown	UF	*V. cholerae*	Pos	Neg	Neg
VcS171		Unknown	UF	*V. cholerae*	Pos	Neg	Neg
VcNAG12		Unknown	UF	*V. cholerae*	Pos	Neg	Neg
VcATCC25874		Unknown	UF	*V. cholerae*	Pos	Neg	Neg
Vc8		Unknown	UF	*V. cholerae*	Pos	Neg	Neg
VcB1307 Dacca		Unknown	UF	*V. cholerae*	Pos	Neg	Neg
VcA5		Unknown	UF	*V. cholerae*	Pos	Neg	Neg
VcI10		Unknown	UF	*V. cholerae*	Pos	Neg	Neg
Vc646 Ogawa01		Unknown	UF	*V. cholerae*	Pos	Neg	Neg
Vc395 Classical Ogawa01		Unknown	UF	*V. cholerae*	Pos	Neg	Neg
TD3192		Unknown	Qualicon	*V. cholerae*	Pos	Neg	Neg
TD7000	ATCC 9459	Unknown	Qualicon	*V. cholerae*	Pos	Neg	Neg
DD9892		Unknown	Qualicon	*V. cholerae*	Pos	Neg	Neg
DD13084	ATCC 14035	Unknown	Qualicon	*V. cholerae*	Pos	Neg	Neg
TD3161		Unknown	Qualicon	*V. cholerae (non-O1, O139)*	Pos	Neg	Neg
TD3162		Unknown	Qualicon	*V. cholerae (non-O1, O139)*	Pos	Neg	Neg
TD3163		Unknown	Qualicon	*V. cholerae (non-O1, O139)*	Pos	Neg	Neg
TD3164		Unknown	Qualicon	*V. cholerae (non-O1, O139)*	Pos	Neg	Neg
TD3165		Unknown	Qualicon	*V. cholerae (non-O1, O139)*	Pos	Neg	Neg
TD3167		Unknown	Qualicon	*V. cholerae (non-O1, O139)*	Pos	Neg	Neg
TD3170		Unknown	Qualicon	*V. cholerae (non-O1, O139)*	Pos	Neg	Neg
TD3171		Unknown	Qualicon	*V. cholerae (non-O1, O139)*	Pos	Neg	Neg
TD3173		Unknown	Qualicon	*V. cholerae (non-O1, O139)*	Pos	Neg	Neg
TD3180		Unknown	Qualicon	*V. cholerae O1*	Pos	Neg	Neg
TD3183		Unknown	Qualicon	*V. cholerae O1*	Pos	Neg	Neg
TD3185		Unknown	Qualicon	*V. cholerae O1*	Pos	Neg	Neg
TD3186		Unknown	Qualicon	*V. cholerae O1*	Pos	Neg	Neg

Table 6. Inclusivity Results for *Vibrio cholerae/parahaemolyticus/vulnificus*							
Strain ID	Other strain designation	Source	Location of testing	Species (serotype)	Result *V. cholera*	Result *V. parahaemolyticus*	Result *V. vulnificus*
TD3187		Unknown	Qualicon	*V. cholerae O1*	Pos	Neg	Neg
TD3858		Unknown	Qualicon	*V. cholerae O1*	Pos	Neg	Neg
TD3859		Unknown	Qualicon	*V. cholerae O1*	Pos	Neg	Neg
TD3860		Unknown	Qualicon	*V. cholerae O1*	Pos	Neg	Neg
TD3861		Unknown	Qualicon	*V. cholerae O1*	Pos	Neg	Neg
TD3862		Unknown	Qualicon	*V. cholerae O1*	Pos	Neg	Neg
TD3863		Unknown	Qualicon	*V. cholerae O1*	Pos	Neg	Neg
TD3864		Unknown	Qualicon	*V. cholerae O1*	Pos	Neg	Neg
TD3203		Unknown	Qualicon	*V. cholerae O139*	Pos	Neg	Neg
TD3211		Unknown	Qualicon	*V. cholerae O139*	Pos	Neg	Neg
TD3213		Unknown	Qualicon	*V. cholerae O139*	Pos	Neg	Neg
TD3214		Unknown	Qualicon	*V. cholerae O139*	Pos	Neg	Neg
VpTx2103		Unknown	UF	*V. parahaemolyticus*	Neg	Pos	Neg
VpTx3547		Unknown	UF	*V. parahaemolyticus*	Neg	Pos	Neg
VpDAL1094		Unknown	UF	*V. parahaemolyticus*	Neg	Pos	Neg
Vp17802		Unknown	UF	*V. parahaemolyticus*	Neg	Pos	Neg
Vp43996		Unknown	UF	*V. parahaemolyticus*	Neg	Pos	Neg
DD2633	ATCC 17802	Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3129		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3130		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3131		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3132		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3133		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3134		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3135		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3153		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3154		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3155		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3156		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3157		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3159		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3160		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg

Table 6. Inclusivity Results for *Vibrio cholerae/parahaemolyticus/vulnificus*							
Strain ID	Other strain designation	Source	Location of testing	Species (serotype)	Result *V. cholera*	Result *V. parahaemolyticus*	Result *V. vulnificus*
Vv FLA141		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
Vv FLA126		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA134		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
Vv Fla 129		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA127		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA135		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA115		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA149		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvB3-313/98		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA121		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA137		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvB3-302/99		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA119		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA116		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA102		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvB2-2		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA108		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
TD3121		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD3148		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD3149		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD3204		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD3207		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD3208		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD3210		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD3212		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD3217		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD3219		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD4527	ATCC 27562	Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
DD13082	ATCC BAA-86	Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
DD13231		Shrimp	Qualicon	*V. cholera*	Pos	Neg	Neg
DD13232		Shrimp	Qualicon	*V. cholera*	Pos	Neg	Neg
DD13208		Shrimp	Qualicon	*V. cholera*	Pos	Neg	Neg

Table 6. Inclusivity Results for *Vibrio cholerae/parahaemolyticus/vulnificus*							
Strain ID	Other strain designation	Source	Location of testing	Species (serotype)	Result *V. cholera*	Result *V. parahaemolyticus*	Result *V. vulnificus*
DD13209		Shrimp	Qualicon	*V. cholera*	Pos	Neg	Neg
DD13212		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13216		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13217		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13218		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13211		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13222		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13223		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13224		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13225		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13226		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13228		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13229		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13230		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13233		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13234		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13235		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13236		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13204		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13207		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13200		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13202		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13201		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13203		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13211		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13214		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13215		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13210		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13205		Shrimp	Qualicon	*V. vulnificus*	Neg	Neg	Pos
DD13206		Shrimp	Qualicon	*V. vulnificus*	Neg	Neg	Pos
DD13227		Shrimp	Qualicon	*V. vulnificus*	Neg	Neg	Pos
DD13213		Shrimp	Qualicon	*V. vulnificus*	Neg	Neg	Pos

Table 7. Inclusivity Results for *Vibrio cholerae/parahaemolyticus/vulnificus*

Strain ID	Other strain designation	Source	Species	Result *V. cholera*	Result *V. parahaemolyticus*	Result *V. vulnificus*
DD2558		Unknown	*Citrobacter freundii*	Neg	Neg	Neg
DD383		Unknown	*Citrobacter freundii*	Neg	Neg	Neg
DD2560		Unknown	*Citrobacter kosei*	Neg	Neg	Neg
DD2561		Unknown	*Citrobacter kosei*	Neg	Neg	Neg
DD12835		Unknown	E. coli O157:H7	Neg	Neg	Neg
DD1450		Unknown	E. coli O157:H7	Neg	Neg	Neg
DD1979		Unknown	E. coli O157:H7	Neg	Neg	Neg
TD8136		Unknown	E. coli O157:H7	Neg	Neg	Neg
DD2554		Unknown	*Enterococcus faecalis*	Neg	Neg	Neg
DD6523		Unknown	*Klebsiella oxytoca*	Neg	Neg	Neg
DD2546		Unknown	*Klebsiella pneumoniae*	Neg	Neg	Neg
DD1144		Unknown	*Listeria monocytogenes*	Neg	Neg	Neg
DD1283		Unknown	*Listeria monocytogenes*	Neg	Neg	Neg
DD1309		Unknown	*Listeria monocytogenes*	Neg	Neg	Neg
DD3572	ATCC 9459	Unknown	*Listeria innocua*	Neg	Neg	Neg
DD3376		Unknown	*Listeria ivanovii*	Neg	Neg	Neg
DD2874	ATCC 14035	Unknown	*Listeria seeligeri*	Neg	Neg	Neg
DD3354		Unknown	*Listeria welshimeri*	Neg	Neg	Neg
DD3411		Unknown	*Listeria welshimeri*	Neg	Neg	Neg
DD2357		Unknown	*Proteus mirabilis*	Neg	Neg	Neg
DD374		Unknown	*Proteus mirabilis*	Neg	Neg	Neg
DD13148		Unknown	*Pseudomonas aeruginosa*	Neg	Neg	Neg
DD3982		Unknown	*Pseudomonas aeruginosa*	Neg	Neg	Neg
DD3019		Unknown	*Salmonella ser.* Dublin	Neg	Neg	Neg
DD706		Unknown	*Salmonella ser.* Enteritidis	Neg	Neg	Neg
DD1261		Unknown	*Salmonella ser.* Newport	Neg	Neg	Neg
DD13060		Unknown	*Salmonella ser.* Senftenburg	Neg	Neg	Neg
DD586		Unknown	*Salmonella ser.* Typhimurium	Neg	Neg	Neg
DD1083		Unknown	*Shigella flexneri*	Neg	Neg	Neg
DD699		Unknown	*Shigella soneii*	Neg	Neg	Neg
DD10156		Unknown	*Staphylococcus aureus*	Neg	Neg	Neg
DD7426		Unknown	*Staphylococcus aureus*	Neg	Neg	Neg

Table 7. Inclusivity Results for *Vibrio cholerae/parahaemolyticus/vulnificus*

Strain ID	Other strain designation	Source	Species	Result *V. cholera*	Result *V. parahaemolyticus*	Result *V. vulnificus*
DD9775		Unknown	*Staphylococcus aureus*	Neg	Neg	Neg
DD11233		Unknown	*Vibrio alginolyticus*	Neg	Neg	Neg
TD3146		Unknown	*Vibrio alginolyticus*	Neg	Neg	Neg
TD3195		Unknown	*Vibrio alginolyticus*	Neg	Neg	Neg
TD3200		Unknown	*Vibrio alginolyticus*	Neg	Neg	Neg
TD3658		Unknown	*Vibrio alginolyticus*	Neg	Neg	Neg
TD4501		Unknown	*Vibrio anguillarum*	Neg	Neg	Neg
TD4498		Unknown	*Vibrio carchariae*	Neg	Neg	Neg
TD3194		Unknown	*Vibrio damsela*	Neg	Neg	Neg
TD4524		Unknown	*Vibrio damsela*	Neg	Neg	Neg
DD2631		Unknown	*Vibrio fluvialis*	Neg	Neg	Neg
TD4526		Unknown	*Vibrio fluvialis*	Neg	Neg	Neg
TD4497		Unknown	*Vibrio harveyi*	Neg	Neg	Neg
DD11232		Unknown	*Vibrio mimicus*	Neg	Neg	Neg
DD13083		Unknown	*Vibrio mimicus*	Neg	Neg	Neg
TD3137	ATCC 17802	Unknown	*Vibrio mimicus*	Neg	Neg	Neg
TD3147		Unknown	*Vibrio mimicus*	Neg	Neg	Neg
TD3216		Unknown	*Vibrio mimicus*	Neg	Neg	Neg
TD4500		Unknown	*Vibrio natriegens*	Neg	Neg	Neg
TD4528		Unknown	*Vibrio pelagia*	Neg	Neg	Neg
TD4523		Unknown	*Vibrio tubiashii*	Neg	Neg	Neg
DD2399		Unknown	*Yersinia aldovae*	Neg	Neg	Neg
DD592		Unknown	*Yersinia enterocolitica*	Neg	Neg	Neg

Results – ALL TARGET *VIBRIO* ISOLATES GAVE EXPECTED POSITIVE RESULTS AND ALL NON-*VIBRIO* AND NON-TARGET *VIBRIO* SPECIES GAVE EXPECTED NEGATIVE RESULTS.

Stability Study

Methodology – BAX ® system test kits were evaluated in experiments to determine a reasonable shelf-life using both accelerated and non-accelerated storage conditions (see table below). *V. cholera* TD 3858, *V. cholera* TD 3192, *V. parahaemolyticus* TD 3129, *V. parahaemolyticus* TD 4496, and *V. vulnificus* DD 13082 were assayed using purified DNA at a level equivalent to one order of magnitude over the product's claimed sensitivity level (i.e. 10^5 cfu/mL) by the BAX ® assay. Additionally, two non-target *Vibrio* and non-*Vibrio* strains, *Pseudomonas aeruginosa* DD 962 and *Vibrio mimicus (non-target Vibrio species)* DD 13083 were tested using purified DNA at levels corresponding to 10^8 cfu/ml in an enriched sample. Three replicates of each strain at each temperature condition at each time point were assayed. Also, for each condition, for each time point, three non-spiked lysis buffer controls were tested.

Results – All results except for one *V. vulnificus* test at the 23 day 25°C treatment gave the expected result (tests spiked with positive target tested BAX ® positive while non-target and non-spiked tests tested BAX ® negative). It is not known why this one result was atypical, though it is possible this was due to a procedural error such as a pipette tip not properly affixed during the 5 µl lysate preparation step or an accidental loading of a non-target replicate into what was supposed to be a target reaction. The results of the accelerated stability study showed no difference in the performance of this test kit after being stored for 122 days at 25°C and 37ºC as compared to the 4ºC control. Based on these results and applying the Q_{10} rule of the Arrhenius equation, a 32 month shelf life has been assigned to these test kits.

Predicted Stability = Accelerated Stability X $2^{\Delta t/10}$
For example: Stability of a product at 50°C is 32 days.
Recommended storage temperature is 25°C and n = (50 - 25)/10 = 2.5
Qn = (2)2.5 = 5.66 The predicted shelf life is 32 days X 5.66 = 181 days

Accelerated stability studies are continuing and it is anticipated that the next lot of test kits will be assigned a 36 month shelf life. Real-time testing at 4ºC has shown stability for 122 days and is continuing.

Table 8. Summary of stability study

Time Point (days)	Storage Temp (°C)	BAX ® Positive *Vibrio cholera/parahaemolyticus/ vulnificus*	BAX ® Positive non-target organisms and non-spiked controls
23	4	15/15	0/9
	25	14/15	0/9
	37	15/15	0/9
60	4	15/15	0/9
	25	15/15	0/9
	37	15/15	0/9
122	4	15/15	0/9
	25	15/15	0/9
	37	15/15	0/9

Lot-to-lot study

Methodology –BAX® system test kits from three lots with different expiration dates were tested in an experiment to determine any evidence of lot-to-lot performance differences. *V. cholera* TD 3858, *V. cholera* TD 3192, *V. parahaemolyticus* TD 3129, and *V. vulnificus* DD 13082 were assayed using dilutions of overnight cultures at levels equivalent to approximately one order of magnitude over the product's claimed sensitivity level (i.e. ~10^5 cfu/mL) by the BAX ® assay. Additionally, two non-target *Vibrio* and non-*Vibrio* strains, *Pseudomonas aeruginosa* DD 962 and *Vibrio mimicus (non-target Vibrio species)* DD 13083 were tested using cells at levels of approximately 10^8 cfu/ml. Two replicates of each strain at each temperature condition at each time point were assayed. Also, for each condition, for each time point, two non-spiked lysis buffer controls were tested.

Table 9. Lot to Lot Test Kit Comparison

Lot #	Expiration Date	*Vibrio* spiked positives	Non-*Vibrio* spiked positives
030508	12/05/2010	8/8	0/6
061008	02/09/2011	8/8	0/6
8263	08/23/2011	8/8	0/6

Results – This lot to lot comparison study found no evidence of performance differences.

Ruggedness Study

Methodology –The BAX® system was evaluated to determine whether it performs as expected despite variations in operational parameters. Since the entire amplification and detection phases are fully automated, independent variables were selected from the enrichment and sample preparation phases. Eight variables believed to have the largest potential for impact on performance were selected, as shown in Table 10 with associated low and high levels:

Table 10. Variables in ruggedness study

Variable	Normal level	Low level	High level
1) Sample volume	5 µL	4	6
2) Incubation temperature (lysis)	37ºC	34	40
3) Incubation time (lysis)	20 minutes	15	30
4) Inactivation temperature (lysis)	95ºC	91	99
5) Inactivation time (lysis)	10 minutes	8	12
6) Total hydration volume	30 µL	27	33
7) Enrichment temperature	35ºC	32	38

For assay factors (1-6) each factor was varied, both high and low level as well as a normal level, for three replicates of 6 strains (4 different *Vibrio target strains* and 2 different non-*target strains*). Additionally, two non-inoculated samples were assayed for each variable/level studied.

For inoculated samples, *V. cholera* TD 3858, *V. cholera* TD 3192, *V. parahaemolyticus* TD 3129, and *V. vulnificus* TD 3121 were serially diluted to just above the product's claimed sensitivity level (i.e. 10^5 cfu/ml of enriched culture) and prepared for the BAX® assay. Additionally, two non-target *Vibrio* and non-*Vibrio* strains, *Vibrio mimicus (non-target Vibrio species)* TD 3147 and *Salmonella* Newport DD 1261 were grown and diluted to attain inoculation levels of ~10^7-10^8 cfu/ml as described in the lot to lot study. Uninoculated samples were freshly prepared with APW and treated in an analogous manner to the inoculated samples.

For the enrichment factor (7) low levels of ~10 cfu of *V. cholera* TD 3858, *V. cholera* TD 3192, *V. parahaemolyticus* TD 3129, and *V. vulnificus* TD 3121 and high levels (~10^5 cfu) of non-target strains *Salmonella* Newport DD 1261 and *Vibrio mimicus (non-target Vibrio species)* TD 3147 were added to 225 ml aliquots of APW with replicates for each variable for each strain and assayed for high (n=2), low (n=2), and normal (n=2) conditions.

Table 11. Results of ruggedness study

Variable	Normal level	Positive *Vibrio*	Positive Non- *Vibrio*	Positive Uninoc.	Low level	Positive *Vibrio*	Positive Non- *Vibrio*	Positive Uninoc.	High level	Positive *Vibrio*	Positive Non- *Vibrio*	Positive Uninoc.
1) Sample volume	5 µL	12/12	0/6	0/2	4	12/12	0/6	0/2	6	12/12	0/6	0/2
2) Incubation temperature	37ºC	12/12	0/6	0/2	34	12/12	0/6	0/2	40	12/12	0/6	0/2
3) Incubation time	20 min	12/12	0/6	0/2	15	12/12	0/6	0/2	30	12/12	0/6	0/2
4) Inactivation temperature	95ºC	12/12	0/6	0/2	91	12/12	0/6	0/2	99	12/12	0/6	0/2
5) Inactivation time	10 min	12/12	0/6	0/2	8	12/12	0/6	0/2	12	12/12	0/6	0/2
6) Total hydration volume	30 µL	12/12	0/6	0/2	27	12/12	0/6	0/2	33	12/12	0/6	0/2
7) Enrichment temperature	35ºC	8/8	0/4	0/2	32	8/8	0/4	0/2	38	8/8	0/4	0/2

Results – The results of the ruggedness study are shown in Table 11. All *Vibrio*-inoculated samples returned positive results. All non-*Vibrio* inoculated and un-inoculated samples were negative. These results indicate that the variables studied did not affect the performance of the BAX® system assay within the ranges tested.

Discussion

In initial development studies, some enriched samples were found to test positive by the BAX® pcr assay but negative by the reference culture method. Often, this is the case when non-target competitive flora, either non-*Vibrio*, or non-target *Vibrio* species are present in an enrichment with cell densities at a much higher level than the target organism. In such cases, an additional plating media, CHROMagar *Vibrio*, has been found to be useful. For each sample tested for most studies (with the exception of the oyster studies performed at Dauphin Island), a CHROMagar *Vibrio* plate was also struck from each enriched sample to reflect this fact. In one study (the naturally contaminated frozen raw shrimp work) two samples were found to be pcr positive/culture negative. For these samples that tested pcr positive, but from which no confirmed colonies of a positive species were found from the FDA-BAM media, more colonies than required by the FDA BAM procedure were picked from the TCBS, mCPC and CHROMagar *Vibrio* plates into cluster tubes containing 500 µl APW (up to 24 per sample per media where available). Individual isolates were allowed to grow in the cluster tubes overnight at room temperature and tested by BAX® assay. Presumptive positive cluster tubes were struck onto TCBS or T_1N_3 agar and confirmed using the FDA-BAM methods. Both of these samples were then found to be positive using this enhanced protocol, yielding at least one confirmed *V. cholera* isolate. Qualicon has also demonstrated the presence of atypical *V. parahaemolyticus* strains (confirmed by DNA sequence-based characterization) that do not present with typical characteristics on *Vibrio* selective and differential agars. All enrichments which tested positive by PCR, with the exception of two MPN tubes from the oyster study, were also positive for typical confirmed colonies on one or more of the three agars above. In the oyster studies, only three typical colonies per MPN tube were selected as per the FDA-BAM protocols, and a greater number of colonies selected per tube would have made the experiment unmanageable. This highlights a potential issue with the reference method in that typical colony morphology on plates is a critical step in the reference method and the complex microbial ecology of an oyster can potentially lead to less than optimal results when non-target isolates with a typical phenotype on *Vibrio* selective agars are present in significant numbers relative to the levels of target *Vibrio*. In other non-AOAC studies conducted at Qualicon some instances of PCR positive enrichments have yielded phenotypically atypical isolates that test positive by PCR. These isolates have been characterized by sequence-based identification (microSeq ®, Applied Biosystems, Foster City, CA) as target *Vibrio* species and are being shared with the community of *Vibrio* experts for further characterization (data not shown). The above described work supports continued work on the natural phenotypic and genetic variation of pathogenic species of *Vibrio* occurring in foods.

Conclusion

The data in these studies, within their statistical uncertainty, support the product claims of the BAX® System PCR Assay for Detecting *Vibrio cholera, parahaemolyticus, and vulnificus* with the tested foods, including raw frozen shrimp, cooked shrimp, raw oysters, raw ahi tuna, and raw scallops.

References

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Proposal Subject: Domoic Acid Test Kit

Specific NSSP Guide Reference: Section IV. Guidance Documents, Chapter II. Growing Areas .10 Approved National Shellfish Sanitation Program Laboratory Tests: Microbiological and Biotoxin Analytical Methods.

Text of Proposal/ Requested Action

Mercury Science Inc., in collaboration with the NOAA Center for Coastal Fisheries and Habitat Research has developed a new quantitative immunoassay for the detection of domoic acid. The assay has been commercialized and is currently sold for research use as the Domoic Acid Test Kit (product # DAK-36) (Information online at http://mercuryscience.com/DA).

This product underwent thorough testing by Mercury Science to define the performance characteristics of the assay prior to commercialization. In addition, the product has been independently validated in several labs in a variety of matrices. The results of these internal and external validation studies strongly suggest that the Domoic Acid Test Kit is a rapid, low-cost, and accurate method for analysis of food, water and phytoplankton samples.

At this time, Mercury Science would like to submit a partially complete Method Application to the ISSC Laboratory Methods Review Committee. Please note that the Method Application at this time does not include the completed Single Lab Validation report. The DA analyses to complete Section C. Validation Criteria are currently in progress and will continue throughout the summer. My laboratory has just received funding from the North Pacific Research Board and will be running ISSC Single Laboratory Validation Testing on butter clams (*Saxidomus giganteus*), blue mussels (*Mytilus edulis*), geoducks (*Panopea abrupta*), manila clams (*Venerupis japonica*), oysters (*Crassostrea virginica*) and razor clams (*Siliqua patula*) from Alaska later this summer. The NOAA CCFHR laboratory has similarly received their MERHAB funds last week and will be conducting a parallel Single Laboratory Validation study on butter clams, blue mussels, geoducks, manila clams, oysters, and razor clams from California, Oregon and Washington, oysters from North Carolina and quahogs (*Mercenaria mercenaria*) from Georges Bank, Massachusetts. The goal is to test a broad array of commercial species to ensure that matrix affects do not affect the assay. The results will be made available to the ISSC as they become available.

The work to date includes 1) publishing the complete ELISA methodology and initial validation studies in the December 2008 issue of the Journal of Shellfish Research and 2) completing the first validation series using oysters from North Carolina. The technique was also independently validated by the Quinault tribe in Washington State. They ran the ELISA on razor clam samples gathered by the tribe for a year and sent duplicate samples to the Washington Department of Health HPLC for analyses and have made their results available for inclusion in this preliminary application.

The purpose of this submission is to bring the new method to the attention of the committee in a manner that enables the method to be evaluated in a timely way. I am also seeking the committee's advice and guidance on the validation studies that will be conducted this coming summer by my laboratory and that of Wayne Litaker at NOAA. In the initial study using the oyster tissues I have closely followed the ISSC guidelines, but wanted to ensure that my interpretation was correct. I would therefore request the committee to review the methodology used in the initial oyster validation study to ensure the procedures used meet current requirements and that no additional data need to be gathered. If necessary, the protocol can be altered to meet the committee requirements.

Please find in association with this cover letter a series of materials relevant to the evaluation of the Domoic Acid Test Kit by the ISSC Laboratory Methods Review Committee.

These items included:

- ISSC Method Application with Section A, Section B**,** and Section D completed (see below).
- A pdf file containing the User Guide for the Domoic Acid Test Kit (DAK-36) that is included in the commercial product. (Also available online at: http://www.mercuryscience.com/DA User Guide 2007A.pdf)
- A pdf file containing a reprint of the research paper entitled " RAPID ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETECTION OF THE ALGAL TOXIN DOMOIC ACID," published in the December, 2008 issue of Journal for Shellfish Research. This paper describes correlation data comparing the Domoic Acid Test Kit versus HPLC analysis using several sample matrices. (Also available online at: http://mercuryscience.com/LitakerStewartDec2008.pdf)
- An Excel file showing the results of a study done by the Quinault Indian Nation and the Washington Department of Health comparing razor clam analysis performed by the Domoic Acid Test Kit versus HPLC analysis. This independent study used samples collected over a nineteen month period and was planned and performed without any input from Mercury Science or NOAA. (also available online at: http://mercuryscience.com/QINWDOHdata.xls)
- Preliminary tests using oyster spiked materials (see below)

The ELISA method has been used independently in six laboratories and provided results equivalent to those obtained using HPLC, FMOC-HPLC and LC-MS. This is detailed in the Litaker et al. 2008 publication listed above. Based on the correlation studies conducted so far, I request that this method be considered for interim approval by the LMR committee until the remaining validation data can be provided over the next six months. Upon completion of the SLV, consideration for approval of the assay as a Level 4 method will be requested.

Public Health Significance: The regulatory method for DA detection sanctioned by the Interstate Shellfish Sanitation Conference is a high performance liquid chromatography (HPLC) assay. Though accurate, these analyses are generally run by centralized state facilities with results typically not available for 3 to 14 days after the samples are collected. In more remote communities, many of which depend heavily on subsistence clam harvests, these long delays and the costs of sample analysis are causes for public health concern. The average cost of approximately $100 per sample limits the number of samples that can be analyzed (Harold Rourk, Washington State Department of Health, personal communication). Resource managers in coastal communities have expressed their desire for a cost-effective method for rapid and accurate determination of DA concentrations in shellfish and phytoplankton samples.

Cost Information (if available): Anticipated cost is $7.00 per duplicate reaction

Proposed Specific Research Need/Problem to be Addressed:	This research focuses on the development is an accurate, rapid, cost-effective ELISA for use by environmental managers and public health officials to monitor Domoic Acid concentrations in environment samples. The regulatory method for DA detection sanctioned by the Interstate Shellfish Sanitation Conference is a high performance liquid chromatography (HPLC) assay. Though accurate, these analyses are generally run by centralized state facilities with results typically not available for 3 to 14 days after the samples are collected. In more remote communities, many of which depend heavily on subsistence clam harvests, these long delays and the costs of sample analysis are causes for public health concern. The average cost of approximately $100 per sample limits the number of samples that can be analyzed (Harold Rourk, Washington State Department of Health, personal communication). Resource managers in coastal communities have expressed their desire for a cost-effective method for rapid and accurate determination of DA concentrations in shellfish and phytoplankton samples. The high throughput capacity of the assay also allows for much faster response times when domoic acid events occur. The relatively low cost of the assay means that significantly more sampling is also possible on the same or smaller budget.
How will addressing this research support/ improve the mission/role of the ISSC/NSSP/ Industry? Support need with literature citations as appropriate.	This Assay will allow better protect public health and provide a rapid response capability when DA outbreaks occurs. It can also be adapted to monitoring phytoplankton samples so that toxic blooms can be identify and tracked. Toxic phytoplankton cells generally appear several weeks before the shellfish become toxic and can be used as an early warning system for when shellfish are likely to become toxic/ More detailed information on the assay and its potential uses is provided in a recently published article: RAPID ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETECTION OF THE ALGAL TOXIN DOMOIC ACID, Journal of Shellfish Research, Vol. 27, No. 5, 1301–1310, 2008. Available online at: http://mercuryscience.com/LitakerStewartDec2008.pdf
Estimated Cost:	$7.00 per duplicate sample (~$200.00 for ELISA kit capable of analyzing 36 duplicate samples in 1.5 h)
Relative Priority Rank in Terms of Resolving Research Need:	Immediate ☐ Important ☐ Required ☐ Other ☐ Valuable ☐
Proposed Sources of Funding/ Support:	Grants have been awarded by NPRB and NOAA MERHAB program for the completion of the validation studies.
Time Frame Anticipated:	Validation should be completed by January or February 2010.
Action by 2009 Laboratory Methods Review Committee	Recommended referral of Proposal 09-105 to the appropriate committee as determined by the Conference Chairman.
Action by 2009 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 09-105.

Action by 2009 General Assembly	Adopted recommendation of 2009 Task Force I on Proposal 09-105.
Action by USFDA 02/16/2010	Concurred with Conference action on Proposal 09-105.
Action by 2011 Laboratory Methods Review Committee	Recommended referral of Proposal 09-105 to the appropriate committee as determined by the Conference Chairman to await further data to be provided by Mercury Science the developer of the method to determine if the method is fit for purpose within the NSSP as a screening tool.
Action by 2011 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 09-105.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 09-105.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 09-105.

Journal of Shellfish Research, Vol. 27, No. 5, 1301–1310, 2008.

RAPID ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETECTION OF THE ALGAL TOXIN DOMOIC ACID

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ABSTRACT Domoic acid (DA) is a potent toxin produced by bloom-forming phytoplankton in the genus *Pseudo-nitzschia*, which is responsible for causing amnesic shellfish poisoning (ASP) in humans. ASP symptoms include vomiting, diarrhea, and in more severe cases confusion, loss of memory, disorientation, and even coma or death. This paper describes the development and validation of a rapid, sensitive, enzyme linked immunosorbent assay test kit for detecting DA using a monoclonal antibody. The assay gives equivalent results to those obtained using standard high performance liquid chromatography, fluorenylmethoxycarbonyl high performance liquid chromatography, or liquid chromatography—mass spectrometry methods. It has a linear range from 0.1–3 ppb and was used successfully to measure DA in razor clams, mussels, scallops, and phytoplankton. The assay requires approximately 1.5 h to complete and has a standard 96-well format where each strip of eight wells is removable and can be stored at 4°C until needed. The first two wells of each strip serve as an internal control eliminating the need to run a standard curve. This allows as few as 3 or as many as 36 duplicate samples to be run at a time enabling real-time sample processing and limiting degradation of DA, which can occur during storage. There was minimal cross-reactivity in this assay with glutamine, glutamic acid, kainic acid, epi- or iso-DA. This accurate, rapid, cost-effective, assay offers environmental managers and public health officials an effective tool for monitoring DA concentrations in environment samples.

KEY WORDS: ASP, domoic acid poisoning, ELISA, mussels, scallops, razor clams, test kit

INTRODUCTION

Domoic acid (DA) is a potent toxin produced by bloom-forming phytoplankton in the genus *Pseudo-nitzschia* (Fig. 1). It is a glutamate analog, which acts as a potent excitatory neurotransmitter and causes amnesic shellfish poisoning (ASP) in humans (Quilliam & Wright 1989, Quilliam et al. 1989b, Wright et al. 1989). Symptoms include vomiting, diarrhea, and in more severe cases confusion, loss of memory, disorientation, and even death. As a tricarboxylic acid, fully ionized at seawater pH, DA can behave as a potent trace metal ligand (Rue & Bruland 2001, Wells et al. 2005). DA can bioaccumulate and rapidly transvectors throughout the food chain *via* clams, mussels, crabs, filter feeding fish, and other organisms (Horner & Postel 1993, Scallet et al. 2005, Vigilant & Silver 2007). DA poisoning was first recognized after a lethal event on Prince Edward Island, Canada in 1987 (Wright et al. 1989). Since that time, a number of toxic events have occurred on the United States west coast where DA has been shown to commonly accumulate in the edible parts of razor clams (*Siliqua patula*), mussels (*Mytilus californianus* or *edulis*), and Dungeness crabs (*Cancer magister*) (Wekell et al. 1994, Horner et al. 1997). High levels of DA in razor clams in Oregon and Washington are responsible for beach closures that can last for more than a year. Losses of more than $20 million annually result from these closures caused by lost tourism and reduced recreational and commercial and tribal clam harvests (Adams et al. 2000). DA has also been implicated in the death and illness of brown pelicans (*Pelecanus occidentalis*) and Brandt's cormorants (*Phalacrocorax penicillatus*) (Fritz et al. 1992, Work et al. 1993), California sea lions (*Zalophus californianus*) (Scholin et al. 2000, Trainer et al. 2000, Brodie et al. 2006), sea otters (*Enhydra lutris*) (Kreuder et al. 2003), and possibly whales (Lefebvre et al. 2002).

The regulatory method for DA detection sanctioned by the Interstate Shellfish Sanitation Conference (Quilliam et al. 1989a, Quilliam et al. 1995) is a high performance liquid chromatography (HPLC) assay (Quilliam et al. 1991, Hatfield et al. 1994). Though accurate, these analyses are generally run

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by centralized state facilities with results typically not available for 3–14 days after the samples are collected. In more remote communities, many of which depend heavily on subsistence clam harvests, these long delays and the costs of sample analysis are causes for public health concern. The average cost of approximately $100 per sample limits the number of samples that can be analyzed (Harold Rourk, WA State Department of Health, pers. comm.). Resource managers in coastal communities have expressed their desire for a cost-effective method for rapid and accurate determination of DA concentrations in shellfish and phytoplankton samples. This paper describes the development and optimization of a robust monoclonal antibody based enzyme-linked immunosorbent assay (ELISA) test kit for DA that will meet management needs for rapid detection of DA in environmental samples.

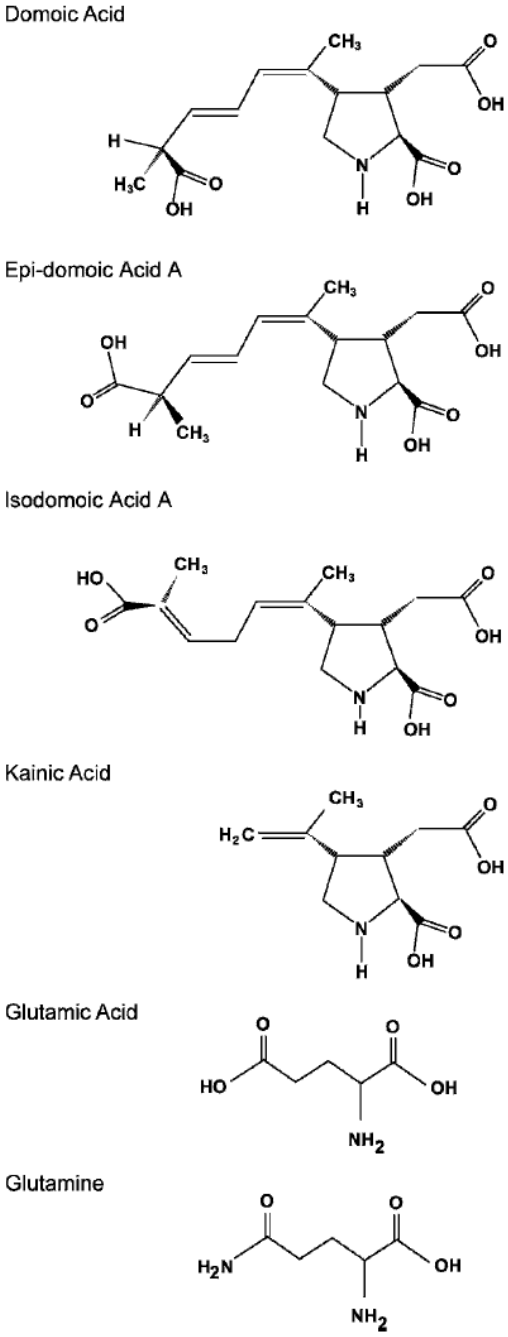

Figure 1. Structure of domoic acid, the isomers epi-domoic acid, isodomoic acid, and two analogues kainic acid and glutamic acid.

MATERIALS AND METHODS

Assay Kit Overview

The DA assay kit was developed jointly by NOAA's National Centers for Coastal Ocean Science, National Ocean Service, and the Northwest Fisheries Science Center, together with an industry partner Mercury Science, Inc., Durham, NC (NOAA/MSI). It was designed as a sequential competitive enzyme linked immunosorbent assay (ELISA) utilizing a high avidity monoclonal antibody (mAb) to DA to ensure assay specificity and consistency across production lots. In the current format, a fixed number of anti-DA mAb binding sites are incubated with dissolved DA in the sample followed by the addition of a DA—horseradish peroxidase (HRP) conjugate. As these binding events occur, the anti-DA mAb molecules are simultaneously captured by antimouse antibodies affixed to the surface of the microtiter plate wells. Subsequent HRP derived color development, readable on standard microplate readers, was inversely proportional to the concentration of DA in the sample matrix. The assay reagents were titrated so that the amount of mAb and the DA–HRP conjugate added produced a maximal absorbance signal of 3 absorbance units when no DA was present. The implementation of this ELISA system required the development and validation of two essential reagents, a high avidity monoclonal antibody to DA and a stable DA-HRP conjugate recognized by the same mAb.

Production of the Anti-Domoic Acid Antibody

Domoic acid (Sigma-Aldrich, St. Louis, MO), was conjugated with bovine serum albumin (BSA) using dicyclohexyl carbodiimide and N-hydroxysuccinimide by a two-step synthetic pathway (Adamczyk et al. 1994). Ten mice were immunized with the DA-BSA immunogen. Serum titers were determined five days after each boost. A fusion was performed on the three mice that showed the greatest response. Hybridoma cell lines and monoclonal antibody production was performed according to the method of Fenderson et al. (1984). The 10 clones with highest affinity mAbs were selected for further growth and their affinity to DA was compared. The most sensitive clone was ultimately selected as the primary mAb for use in the assay development.

DA-HRP Conjugate

Domoic acid (Sigma) was cross-linked to horseradish peroxidase (HRP) using the procedure of Yoon et al. (1993). The reagent was tested for stability and was used to screen for high affinity mAbs after the fusion and for assay development.

Domoic Acid Standards

The DA standards used to calibrate the assay were purchased from the Certified Reference Materials Program at the National Research Council of Canada Institute for Marine Biosciences (Halifax, Nova Scotia, Canada).

Assay Calibration

A series of dose response curves using varying amounts of antibody and DA-HRP were performed to optimize the assay sensitivity. The optimal assay conditions were found to have an effective linear range from approximately 0.1–3.0 ppb. These conditions were used in all the subsequent phases of assay development. The antibody was also tested for cross-reactivity with varying concentrations of kainic acid, glutamine and glutamic acid. These compounds are structurally similar to various portions of DA molecule and have the potential to cross-react with anti-DA mAbs. Glutamine and glutamic acid, in particular, are common in animal tissues, including shellfish.

Calculation of the Parameters Needed to Construct an Internal Domoic Acid Standard for Each Well Strip

Using the optimized DA assay, multiple dose response curves were made using the NRC standards diluted to between 0 and 10 ppb (1–10 ng mL^{-1}) in the assay reaction buffer. The average response derived from each of the individual response curves was calculated and a dose response curve was generated using a four parameter logit-log curve fitting analysis (Ritchie et al. 1981; Fig. 2). Four parameters were derived from this analysis. This first was B_o, the maximal signal, which occurred when no sample DA was present (Fig. 3A). The second was B, the signal produced by a known amount of sample DA. The third was the slope of the logistic transformed data [proportional to the linear portion of the sigmoidal curve describing the relationship between the ln sample DA concentration versus signal (B)]. And the fourth was ED_{50}, the DA concentration at the mid point of the slope curve where half the available anti-DA mAbs in the well are bound to DA-HRP (Fig. 3A). Because the concentration ratio of anti-DA antibody and DA–HRP conjugates are standardized within reagent lots, the kinetics of the reaction were fixed between assay runs (assuming constant temperature), such that the slope and ED_{50} values remain constant. This made it possible to calculate DA concentrations using the four parameter model.

$$\text{DA concentration} = ED_{50}[(B_o/B) - 1]^{-\text{slope}}$$

Because the slope and ED_{50} are constants, all that was needed to calculate the DA concentrations was an accurate B_o and the B estimates from individual samples. In the assay, the mean value for B_o for each strip of wells was determined by adding sample dilution buffer lacking DA to the first two wells in that strip. Duplicate aliquots from each of three extracted samples diluted with sample buffer were then added to the six remaining wells to obtain the B values. Duplicates were run to ensure assay replicability. It should be noted that B_o (the maximal value with no DA added) can have noticeable variation between assays depending on differences in temperature and development time as shown in Figure 2A. However, when the B values for each strip are divided by B_o, the kinetics of the curve become normalized (i.e., replicable between strips and between runs) (see Fig. 2B). In this way the average B_o values serves as an internal standard that can be used in place of a standard curve provided the variation in the B_o is not above or below certain limits, which are specified in the calculation software described later.

Domoic Acid ELISA Test Kit Procedure

The 96-well assay tray used in the assay contained 12 strips. Each strip of 8 wells could be removed and stored until it was needed. The first two wells of each strip were used as a control (no DA added). The remaining six wells were used to analyze three samples in duplicate. This format provided the flexibility of running anywhere from 3–36 duplicate samples at a time. For unknown sample analysis, extracts were diluted to a final concentration ranging from 0.3–3 to ppb using the sample buffer (phosphate salt solution, pH 7.8, containing casein). For

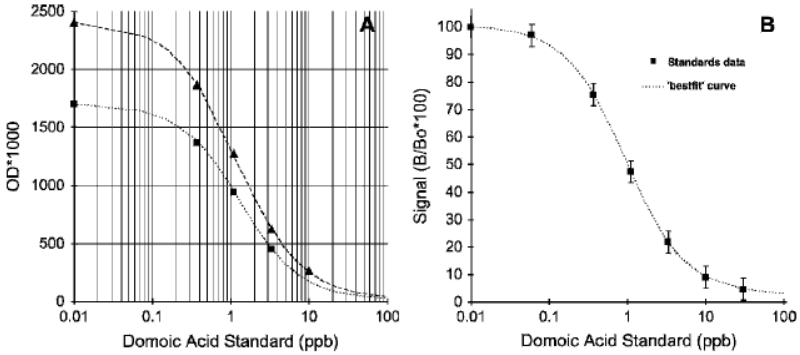

Figure 2. (A) Representative dose response curves for domoic acid analyzed on different days. It should be noted that B_o (the average of the maximal 450 nm absorbance values from the first two wells of a strip to which no DA is added) can vary noticeably between assays depending on differences in ambient temperature and development time. (B) The mean and SD in signal from eight normalized domoic acid dose response curves carried out over the course of several weeks. These data were specifically normalized by dividing each of the resultant absorbance values by B_o. The result of this normalization process, given that the concentrations of antidomoic acid antibody and HRP-domoic acid conjugate are fixed, is that the resultant curves are replicable between rows and between assays done on different days. The black squares and error bars indicate the mean value at each given domoic acid concentration ± 1 SD.

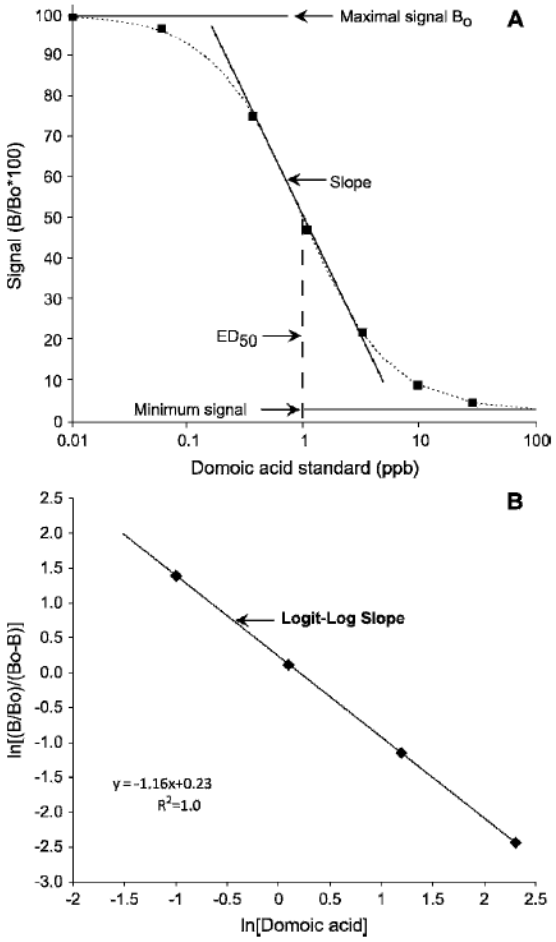

Figure 3. (A) DA concentrations versus the corresponding ELISA absorbance values, which were normalized by dividing by maximal (B_o) absorbance value. (B) Log-logit transform of the data shown in Fig. 3A. From this analysis it was possible to calculate the parameters needed to accurately calculate domoic acid concentrations using the ELISA assay. These parameters include B_o, the maximal absorbance value at 450 nm obtained from the first two wells of a strip to which no free domoic acid is added and B, the 450 nm absorbance value for a given sample, slope of the logit-log transformed data, which were proportional to the linear portion of the sigmoidal curve describing the relationship between the ln DA concentration versus signal (B), and ED_{50}, the mid point of the slope curve where half the available anti-DA mAbs are bound to DA.

clam tissues containing DA, sample dilutions of 1:50 and 1:1000 were typically used. Preliminary tests with razor clam extracts showed that a 25-fold dilution in sample dilution buffer eliminated matrix effects in ELISA analysis.

The assay was initiated by adding 50 µL of the anti-DA antibody to each well using a multi channel pipettor. Next, 50 µL of the control solution (sample buffer without DA) was added to the first two wells in each row. Duplicate 50 µL aliquots from the diluted DA extracts were then added to the remaining wells in each strip and the plate incubated at room temperature for 30 min on an orbital shaker set to vigorously mix the solution in each well (PerkinElmer Waltham, MA 1296–004 DELFIA Plateshake set on high). Vigorous mixing is key to obtaining replicable results from one run to the next. In this step, the bulk of the native DA will bind to available mAbs in proportion to the DA concentration. At the end of the incubation, 50 µL of DA HRP conjugate was added to each well and the plate incubated a second time for 30 min at room temperature on an orbital shaker. The DA-HRP will then bind to remaining available mAb sites. After the incubation, the plate was washed three times with wash solution [Tris-HCl buffered salt solution (pH 7.8) containing Tween 20 and sodium azide as a preservative] using a commercial plate washer, making certain the fluid was completely aspirated from all the wells. Alternatively, these washes can be done manually by adding wash solution to wells using a multichannel pipettor and then flicking all fluid from the wells. The manual method may result in slightly higher variability. Next, 100 µL of K-Blue TMB substrate (5.5′-tetramethylbenzidine, Neogen Corporation, Lexington, KY) was added to each well. The plate was placed on an orbital shaker for no more than 5 min, or until adequate color development was observed. Color development was terminated by adding 100 µL stop solution (1N hydrochloric acid) to each well. The absorbance in each well was measured at 450 nm using a Thermo Ascent MultiSkan plate reader (Thermo Scientific, Waltham, MA). The DA concentrations were determined using the sample (B) and control (B_o) absorbances, the original tissue weights, and the volume of 20% or 50% methanol used to extract each sample. The actual calculations were made using a Microsoft Excel work sheet (Microsoft Corporation, Redmond, WA), which incorporates the constants for the four parameter model described above. This worksheet can be downloaded from Stewart (2008). Processing time for this assay was ~1.5 h.

Routine Tissue Extraction

In the case of razor clams and scallops, pooled samples of 10–12 individual shellfish were cleaned, and ground to a smooth and uniform homogenate in a commercial blender (Waring model HGBSS56, Torrington, CT). Clams were pooled because previous studies of DA in razor clams from the Washington coast indicated that the coefficient of variation for DA between clams in a population exceeded 100% (Wekell et al. 2002). If the homogenate appeared to be forming a gel caused by unusually high lipid content, an equal weight of water was added and the dilution noted. Approximately 2 g of homogenized tissue were added to a tared 50 mL conical tube and the weight recorded to the nearest 0.01 g. Next, 18 mL of 50% methanol were added and the samples mixed at high speed on a vortex mixer for 2 min. Once the extraction was completed the tubes were spun in a table top centrifuge for 20 min at 10,000× g or until a tight pellet and clear supernatant were obtained. If the samples did not clear despite the spinning at high speed, the supernatant was poured into a syringe, then passed through a 0.45 µm Millex HA syringe filter (Millipore, Billerica, MA) to remove proteins and other compounds that can form micelles, whereas soluble DA remained in the filtrate. At this point the homogenate was ready for analysis by ELISA and HPLC. If necessary, the sample was stored at 4°C for up to 24 h in an explosion proof refrigerator prior to analysis.

Phytoplankton Extraction

Approximately 0.1–1.0 L of cultured cells or sea water samples were filtered onto a GF/F filter, which was immediately frozen at –80°C until the filter could be processed. For processing, the filter was placed in a 5 mL conical BD Falcon Tube (Becton Dickinson, Franklin Lakes, NJ) and 3 mL of 20% methanol were added. The samples were then sonicated using a Thermo Fisher Scientific Model 100 Sonic Dismembrator with a 1/8 inch probe (model 15-338-80, Fisher Scientific, Waltham, MA) until the filter was completely homogenized. Care was taken to prevent the probe from rupturing the tube. The sonicator probe was cleaned very carefully with 20% methanol between samples to prevent cross-contamination. Next the homogenate was centrifuged at 3000 *g* for 10 min. The supernatant was then passed through a disposable Whatman GD/X 0.2 µm syringe filter (Florham Park, NJ) into a 5 mL tube. At this point the sample was split for analysis using both the ELISA and HPLC assays.

HPLC Validation of DA concentration from Razor Clam Tissues

HPLC is the accepted standard method for measuring DA and is the basis of the current official method for regulatory action in the U.S. (AOAC Official Method 991.26). The lower detection level for the standard assay is ~0.5 ppm. This technique was used to validate the DA concentration in the razor clams in this study. Briefly, 10–15 mL of the clarified supernatant prepared as described above was transferred into a 25 mL disposable plastic syringe and filtered through 0.45 micron HA Millipore filter (Bedford, MA) into a labeled scintillation vial. Salt clean-up was done with solid phase extraction columns (Hatfield et al. 1994). Strong anion exchange (SAX) solid phase extraction (SPE) cartridges (Whatman, Florham Park, NJ) were conditioned by washing successively with 6 mL of methanol, 6 mL of deionized water, and 6 mL of 50% methanol. The SPE clean up also removes tryptophan, which is a major source of false positives in HPLC-UV detection of DA because it coelutes with DA. Each sample was then drawn through a conditioned SAX SPE cartridge at a rate of 1 drop per second using a vacuum manifold. Flow was stopped when the meniscus was just above the top of the column. The columns were washed with 5 mL of 0.1 M NaCl in 10% aqueous acetonitrile (10% acetonitrile: 90% deionized water). The columns were immediately moved to a new row in the vacuum manifold and the DA eluted from the SPE cartridge using 5 mL of 0.5 M NaCl in aqueous 10% acetonitrile (10:90, acetonitrile:deionized water) and collected in 5 mL graduated centrifuge tubes. Flow was stopped when eluant reached 4.9 mL in the graduated centrifuge tube. The graduated centrifuge tube was removed from the manifold and the actual volume recorded. The graduated centrifuge tubes were capped and the eluant immediately mixed by shaking the tube vigorously 5–10 times. Tissues from the other invertebrate species examined (Table 1) were processed similarly, except that the extracts were filtered through Nanospec MF GHP 0.45 µm centrifugal filters (Pall, Ann Arbor, MI) instead of SPE columns before HPLC analysis. Eluted samples were transferred to HPLC analysis vials. The HPLC conditions were as follows: Vydac TP210 column (Grace, Deerfield, IL), 2.1 by 250 mm, 40°C, elution of DA in 10% acetonitrile containing 0.1% trifluoroacetic acid (TFA). Twenty µl of each sample were injected into the column and eluted isocratically at 0.3 mL per min. The retention time for the DA peak was about 6–8 min depending on the column. Canadian NRC DACS standards at concentration of 1 ppm in 10% acetonitrile solution were run simultaneously (Hardstaff et al. 1990).

HPLC Detection of Domoic Acid in Phytoplankton Using Fluorenylmethoxycarbonyl (FMOC) Derivatization

A more sensitive fluorescent fluorenylmethoxycarbonyl chloride (FMOC) derivatization method (Pocklington et al. 1990) was used to determine particulate DA concentrations in phytoplankton samples, which typically contained less DA than shellfish tissues. The samples were processed on a Hewlett-Packard 1090 HPLC using a Vydac 201TP, 5 µm, 25 cm column, HP 1046A fluorescence detector, and column heater set to 40°C with the following modification. In our analysis, solvents A (HPLC Water with 0.1% v/v TFA) and B (acetonitrile with 0.1% v/v TFA) were pumped at 0.2 mL/min and the linear gradient elution was changed allowing for increased separation and resolution of the domoic acid peak. The initial gradient went from 70% A and 30% B at time of injection to

TABLE 1.

Intertidal invertebrates sampled from several locations around Monterey Bay in November 2006. HPLC-UV analysis detected significant levels of compounds comigrating with iso- and epi-domoic acid standards. These crude methanolic extracts were used to challenge the NOAA and Biosense ELISAs. The goal was to establish the extent to which the ELISA assays are confounded by the presence of coeluting compounds called as the domoic acid isomers epi- and iso-domoic acid by HPLC-UV assay. Nondetect samples are represented as 0 values.

Organism	Combined epi and iso-DA by HPLC (ppb)	DA Concentration by NOAA ELISA (ppb)	% Total DA Detected by NOAA ELISA	DA Concentration by Biosennse ELISA (ppb)	% Total DA Detected by Biosense ELISA
Chthamalus fissus/dalli	281.7	0.00	0.00	0.02	0.01
Chthamalus fissus/dalli	1,137.1	15.41	1.36	1.53	0.13
Littorina scutulata	198.7	10.57	5.32	3.02	1.52
Littorina scutulata	682.0	15.98	2.34	1.02	0.15
Littorina scutulata	119.5	0.00	0.00	0.17	0.14
Lottia digitalis	236.7	0.00	0.00	0.10	0.04
Lottia digitalis	477.9	13.91	2.91	0.09	0.02
Lottia digitalis	390.6	10.31	2.64	0.78	0.20

60% A and 40% B over 0–10 min, then held constant for 10 min; adjusted to 0% A and 100% B from 20–30 min, held isocratic for 2 min; adjusted from 0% A and 100% B to 70% A and 30% B over 2 min, and then held constant at these (initial) conditions until the end of the run at 45 min. Dihydrokainic acid was used as an internal standard, as described by Pocklington et al. (1990).

A subset of phytoplankton samples was validated to confirm the presence of DA (by mass) using liquid chromatography-mass spectrometry (LC-MS) on a ThermoFinnigan Quantum Discovery Max TSQ ESI Mass Spectrometer coupled to a HP 1100 series binary pump HPLC, following the general protocol of Quilliam et al. (1989a). Samples for LC-MS were prepared as for HPLC, but were then dried down under vacuum and redissolved in 100% methanol prior to injection. The HPLC conditions for the reverse phase were programmed for a linear gradient elution of 10:90% acetonitrile:deionized water (both containing 0.1% formic acid) up to 0:100% water:acetonitrile over 30 min.

Testing Cross-Reactivity of the ELISA Against Glutamine, Kainic Acid and Putative Isomers Epi-DA and Iso-DA

Domoic acid is structurally similar to glutamine, glutamic acid and kainic acid, all of which can potentially co-occur with DA in sample extracts (Fig. 1). To test for potential cross-reactivity with these compounds, the NOAA/MSI ELISA kit was run using concentrations of glutamine, glutamic acid and kainic acid ranging from 10 ppb to 5 ppm. The ED_{50} for each compound was calculated and then divided by ED_{50} for DA and multiplied by 100 to determine percent cross-reactivity (Table 2). A majority of DA in razor clams and phytoplankton is in the form shown at the top of Figure 1. However, samples sometimes contain a larger quantity of compounds closely eluting with DA on standard HPLC runs that have been identified as the DA conformers epi- and iso-DA (Wright et al. 1990, Kotaki et al. 2005). To determine if the mAb used in this assay could detect these DA isomers, and the extent of interference by such coeluting compounds present in crude extracts of intertidal barnacle, limpet, and snail samples, crude methanolic extracts of these tissues were assays using HPLC-UV and both the NOAA/MSI and Biosense (Biosense Laboratories, Bergen, Norway) ELISA methods. These intertidal invertebrate extracts exhibited high levels of the putative epi-DA and iso-DA isomers as called by comigration on HPLC chromatograms. These compounds are generally near detection limits in razor clams, crabs, and to a lesser extent in mussels, and therefore these extracts provided novel matrices for evaluating the accuracy of NOAA/MSI ELISA.

TABLE 2.

Cross-reactivity of the NOAA/MSI ELISA with kainic acid. glutamine, and glutamic acid.

Analyte	% Reactivity in the Domoic Acid Assay
Domoic acid	100
Kainic acid	0.3
Glutamine	<0.1
Glutamic acid	<0.1

Data Analyses

Analytical results for DA concentrations determined from razor clams, mussels, scallops and phytoplankton cells determined by HPLC, FMOC-HPLC, LC-MS and the NOAA/MSI ELISA were compared using linear regression analysis (Sokal & Rohlf 1995). The performance of the NOAA/MSI and Biosense ELISA kits was also compared using a subset of the phytoplankton samples. This comparison involved simultaneously analyzing phytoplankton extracts using the two kits and comparing the results with those obtained using FMOC-HPLC. All samples were run within a 24 h period to prevent differential degradation of DA, which may occur in some samples. Data were compared using linear regression analysis.

RESULTS AND DISCUSSION

The NOAA/MSI ELISA accurately measured NRC standard DA concentrations (Fig. 4) and gave equivalent results for razor clam (Fig. 5), mussel (Fig. 6), scallop (Fig. 7), and phytoplankton extracts (Fig. 8) as obtained when using HPLC, FMOC-HPLC, or LC-MS methods. When the variability in the NOAA/MSI ELISA and FMOC-HPLC method were compared using replicate phytoplankton extracts they were found to be comparable (Fig. 9). The primary advantage of the NOAA/MSI ELISA over HPLC methods, besides a significantly lower cost per sample was much higher throughput. As many as 36 samples can be completed in <1.5 h after tissue extraction.

The NOAA/MSI format was also flexible. An internal control was incorporated into each strip, which eliminated the necessity of running a standard curve each time the assay was performed. Any unused strips could be removed and stored in a desiccator pouch at 4°C for at least six months without compromising assay performance. This allowed as few as 3 samples to be run in real time thereby avoiding the degradation of DA that can occur during storage, particularly once the samples have been extracted (Smith et al. 2006). For example, when phytoplankton samples were run within 24 h using the Biosense ELISA kit, which has been validated by an international collaborative study, and is officially approved by the AOAC International for regulatory detection of DA in shellfish,

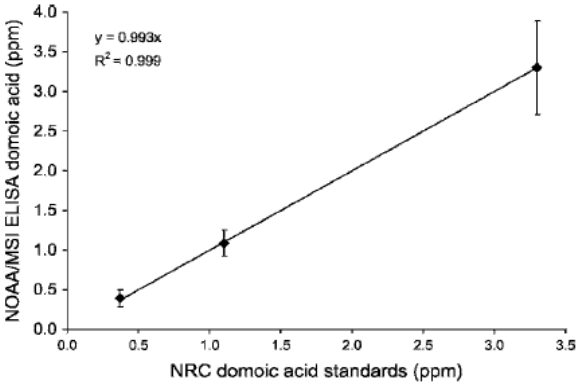

Figure 4. Relationship between various concentrations of National Research Council of Canada (NRC) domoic acid standards and the resultant NOAA/Mercury Science (NOAA/MSI) ELISA values determined using 10 different plates.

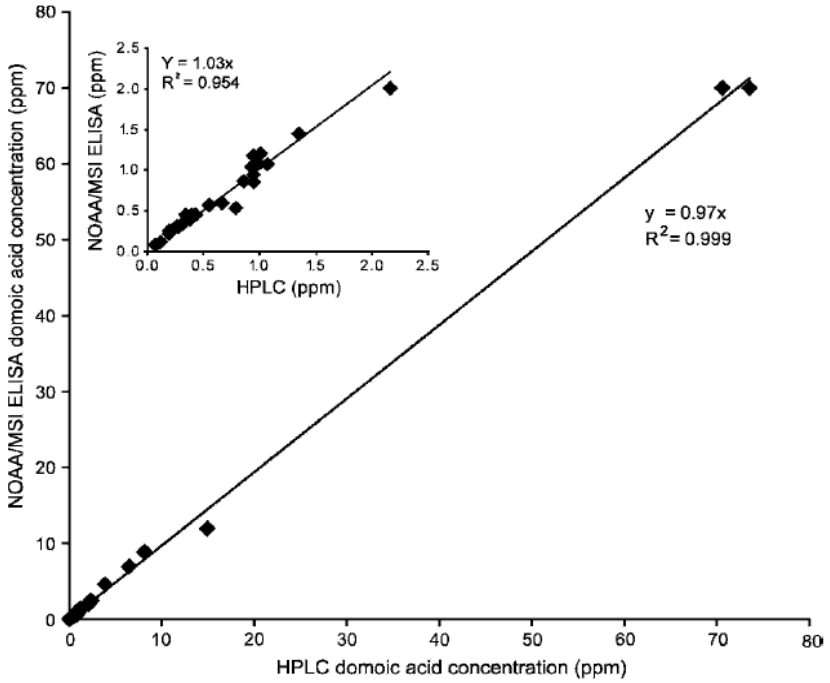

Figure 5. Domoic acid concentrations in razor clam tissues determined from replicate tissue extracts analyzed using HPLC and NOAA/Mercury Science (NOAA/MSI) ELISA. The inset shows an expanded version of the regression analysis for sample containing less than 2.5 ppm domoic acid.

and the NOAA/MSI ELISA kit, equivalent results were obtained (Fig. 10, $r^2 = 0.97$). In contrast, when samples were run two weeks apart the correlation dropped to $r^2 = 0.79$, indicating DA degradation.

The ability to efficiently run a small number of samples in real time was not incorporated into other DA ELISA formats. For example, the Biosense DA ELISA kit includes reagents for only two standard curves (product insert), therefore, only two batches of samples can be run per kit. This means that when small numbers of samples are being collected, they may have to be stored until a sufficient number of samples have been accumulated to maximize the number of samples per kit. This could lead to sample degradation and a critical delay in reporting when samples surpass the regulatory limit of 20 ppm.

Another advantage of the NOAA/MSI assay is that it could be run in either a quantitative or screening mode when assaying shellfish tissues. For quantitative analysis, several dilutions were assayed simultaneously to obtain an accurate DA concentration.

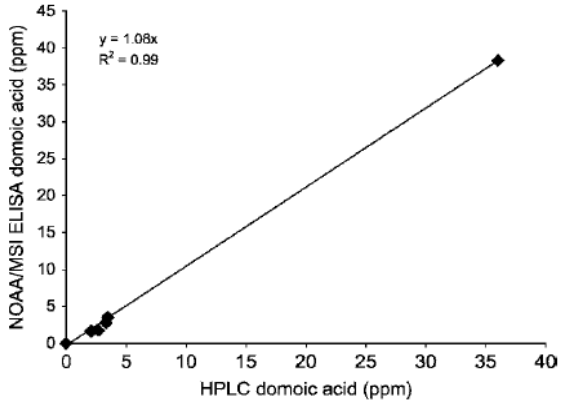

Figure 6. Domoic acid concentrations in mussel tissues determined using HPLC and the NOAA/Mercury Science (NOAA/MSI) ELISA. Aliquots from each sample were run simultaneously.

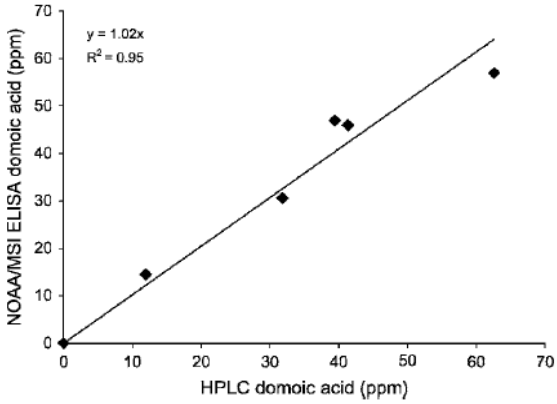

Figure 7. Concentration of domoic acid in scallop tissues extracted from the scallop (*Pecten maximus*) using the standard NOAA/Mercury Science (NOAA/MSI) protocol.

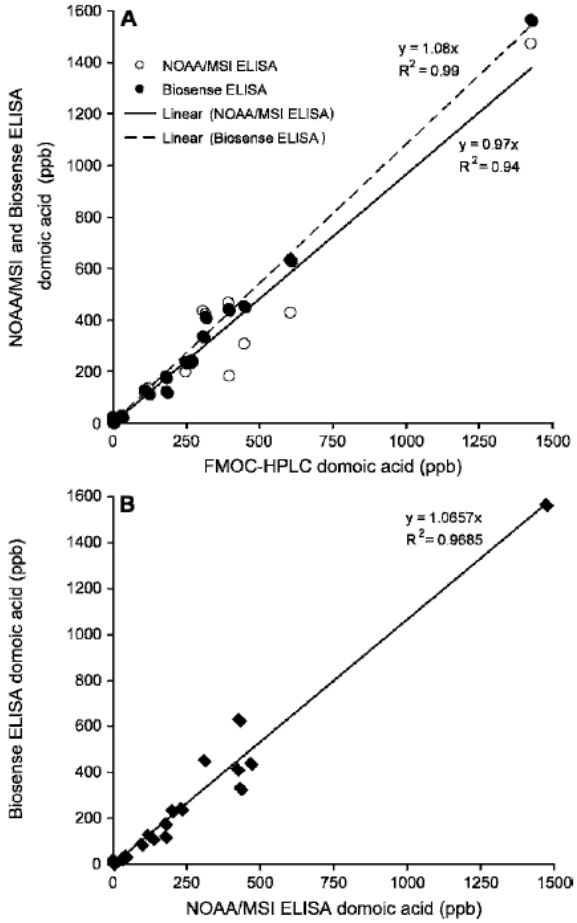

Figure 8. (A) Domoic acid concentrations measured from split phytoplankton sample extracts, which were measured within 24 h by FMOC-HPLC and either the NOAA/Mercury Science (NOAA/MSI) or Biosense ELISAs. (B) Comparison of domoic acid concentrations measured in split samples by either HPLC or ELISA.

Alternatively, to rapidly screen for DA concentrations of concern, the sample extracts were diluted 1:1,000 before running the assay. Taking into account the 1:10 dilution that occurred during the extraction process, the 1:1,000 dilution reduced samples in the 20 ppm DA range to ~2 ppb in the diluted sample. This concentration was within the linear range of the assay (0.1–3 ppb). Tissue samples with 5–10 fold less DA, and far below levels of concern, would show no detectible DA at this dilution. Tissues containing initial DA concentrations >30 ppm would be off scale and indicate a significant DA concentration requiring action. Any samples from this rapid screening that were of concern could then be diluted and run again to obtain an accurate concentration. The NOAA/MSI ELISA test kit also comes with a simple Excel spreadsheet, which allowed the toxin concentrations to be quickly and easily calculated in either a quantitative or rapid screening mode. All that had to be entered was the B_o (no DA added) and sample absorbance data from each strip, the weight of the extracted tissue samples, and the extraction volumes.

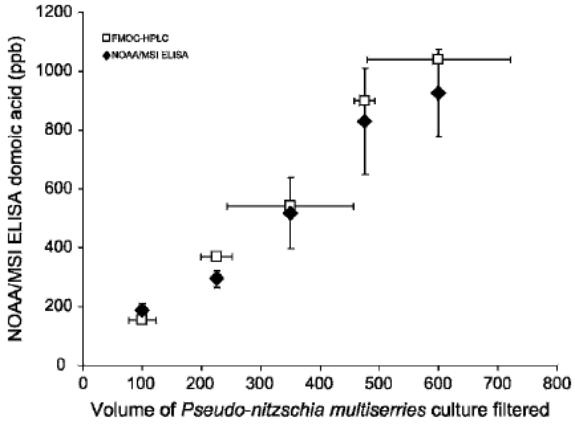

Figure 9. Comparison of the variability between phytoplankton extracts measured using FMOC-HPLC (□) and the NOAA/Mercury Science ELISA (♦). Error bars indicate ±1 SD. Because the error bars largely overlap, for clarity, the standard deviation for the FMOC-HPLC is plotted in the horizontal direction and the NOAA ELISA in the vertical direction.

The NOAA/MSI and Biosense ELISA kits were tested against crude methanolic extracts of several intertidal invertebrates, which HPLC identified as containing >100 ppb levels of epi-DA and iso-DA. These compounds are reported to be less toxic DA congeners based on receptor binding assays (Sawant et al. 2007). Results from both ELISA kits revealed the presence of only trace amounts of DA equivalents in the extracts. The NOAA/MSI ELISA cross-reactivity with these compounds ranged from 0% to 5.3% and the Biosense ELISA cross-reactivity from 0.01% to 1.5% (Table 1) indicating that the ELISA assays are relatively insensitive to cogener interference. It should also be noted that the regulatory methods for assessing human safety are currently based on measuring DA alone, not the combination of DA, iso-DA and epi-DA. These results indicated that both the NOAA/MSI and Biosense

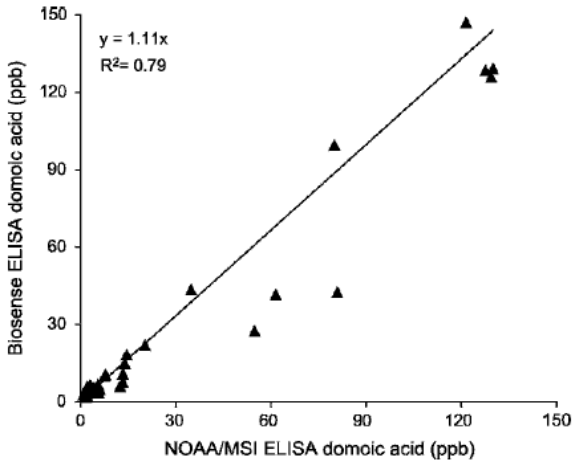

Figure 10. NOAA/MSI ELISA versus Biosense ELISA for phytoplankton samples when the two assays were run several weeks apart showing the increased variability, caused by differential degradation or absorption rates, when samples are not measured within the same 24 h period.

ELISA methods provide DA values comparable to the HPLC values currently used as a basis for regulatory decisions.

Measuring low concentrations of DA in real time is particularly important because the presence or absence of DA contamination is frequently patchy and associated with variable onshore transport of toxic phytoplankton blooms (e.g., Trainer et al. 2002). Depending on prevailing winds and currents, one harvest area can become highly contaminated over a short period whereas adjacent regions remain uncontaminated (Trainer et al. 2000). These differentially affected regions frequently include areas where significant commercial and recreational clam harvests occur. This variability complicates monitoring programs designed to protect human health. The current standard practice involves shipping shellfish samples to a centralized facility for HPLC analyses, introducing delays between 3–14 days from the date of sample collection to reporting results. This turnaround time is too slow to adequately protect subsistence shellfish harvesters who rely on clams consumed within a day or two of harvest. The cost of HPLC analysis is also relatively high per sample and requires a substantially higher capital investment compared with the NOAA/MSI ELISA method. Having an economical technique for better assessing the degree of contamination locally, and in real time, is of great value for local resource managers and public health officials.

The ability to detect DA in phytoplankton using the NOAA/MSI kits would further benefit environmental monitoring programs designed to detect the early onset of toxic *Pseudo-nitzschia* blooms. It is known that increases in the *Pseudo-nitzschia* capable of producing DA often precedes the contamination of shellfish and other filter feeders by a week or two (Trainer & Suddleson 2005). A combination of cell counts and direct toxicity measurements should provide timely predictions for marine resource managers and public health officials. The kit is now commercially available with MSI authorized to market, manufacture and distribute the 96-well plate format test kits. We anticipate completing the necessary validation procedures to qualify the 96 well plate format for regulatory use by public health officials. We are also developing a field test kit that can be used to detect DA levels in shellfish tissues above or below 20 ppm within 10 min after extraction. The test will require no laboratory equipment other than a homogenizer and can be used directly in the field by non-technical personnel, including shellfish harvesters and members of citizen monitoring groups and local volunteers.

In summary, the NOAA/MSI ELISA test kit provides an accurate, flexible and cost effective method for measuring DA in clam, mussel and scallop tissues, as well as in phytoplankton samples. The assay yields concentrations for DA that are indistinguishable from those obtained by HPLC. With further validation, the NOAA/MSI ELISA kit is expected to be approved as a regulatory method for making decisions concerning public health. The short assay (1.5-h) processing time, and relatively low cost, compared with HPLC analysis, mean that the ELISA can be used in more remote locations by environmental managers and public health officials to provide near real-time monitoring capacities.

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Doc. 2007A

Domoic Acid Screening Test Kit

Colorimetric Immunoassay
for the detection of
Domoic Acid
in environmental samples

Instructions and User Guide

FOR SCIENTIFIC RESEARCH USE

Manufactured by
Mercury Science Inc.
Tel: (866) 861-5836

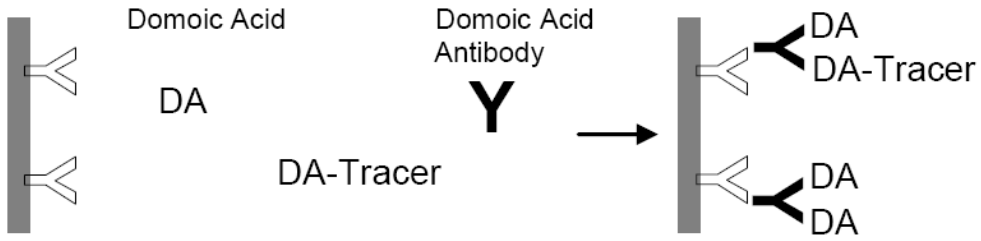
Domoic Acid Screening Test Kit

For Scientific Research Use Only.
This product is not to be used for In Vitro or In Vivo Diagnosis.

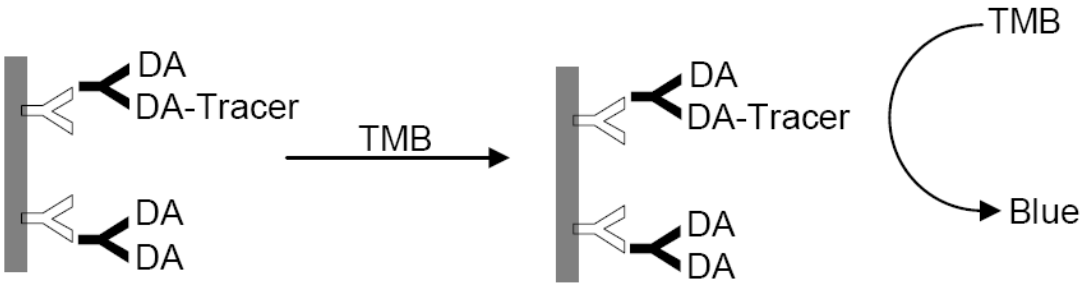
PRINCIPLES OF THE ASSAY
This product contains an antibody (Ab) that binds Domoic Acid and has been developed for the semi-quantitative detection of Domoic Acid in sample extracts. The signal of samples and a control are compared to determine the amount of Domoic Acid present.

The Domoic Acid assay is a solid phase colorimetric immunoassay, based on competition between Domoic Acid and enzyme-labelled Domoic Acid (DA-Tracer) for anti-Domoic Acid antibody. Samples containing Domoic Acid inhibit the binding of the DA-Tracer to the antibody molecules. Both the Ab-Domoic Acid and Ab-DA-Tracer complexes are captured on the surface of the microtiter plate wells.

Following a wash step, the addition of an enzyme substrate (TMB) forms a color proportional to the amount of DA-Tracer in the well. The amount of color measured is inversely proportional to the concentration of Domoic Acid in the sample.



Solid phase
anti-mouse IgG



TEST KIT CONTENTS Each Domoic Acid test kit contains reagents for testing a maximum of 36 samples in duplicate.
The expiry date of the test kit is stated on the outer label.
Store the kit between 2°C and 8°C.

Reagents Store the reagents between 2°C and 8°C when not in use.

Component	Quantity	
Control Solution	1 vial	2 mL
The control is a phosphate-buffered salt solution with casein. Contains sodium azide as a preservative.		
Sample Dilution Buffer	1 bottle	50 mL
Ready-to-use phosphate buffered (pH 7.8) salt solution with casein. Contains sodium azide as a preservative.		
Domoic Acid- Tracer	1 vial	7.5 mL
The tracer is in a MOPS-buffered solution containing bovine protein as a stabilizer and methylisothiazolone, bromonitrodioxane, and Proclin 300 as preservatives.		
Domoic Acid Antibody	1 vial	7.5 mL
The antibody is in phosphate-buffered salt solution with casein. Contains sodium azide as a preservative.		
Wash Concentrate	1 bottle	40 mL
A 25-fold concentration of Tris-HCl buffered (pH 7.8) salt solution with Tween 20. Contains sodium azide as a preservative. Prepare for use by mixing entire contents with 960 mL of distilled water and placing in platewasher WASH Bottle.		
Substrate Solution	1 bottle	15 mL
Tetramethylbenzidine and H_2O_2	Keep away from direct sunlight.	
Stop Solution	1 bottle	15 mL
1 N Hydrochloric Acid		
Anti-Mouse IgG Microtitration Strips	1 plate (12 x 8 wells)	

WARNINGS AND PRECAUTIONS
For research use only. Handle all samples as potentially hazardous.
Disposal of all waste should be in accordance with local regulations.

SCREENING ASSAY PROCEDURE

Perform each determination in duplicate for the Control and unknowns. All sample extracts should be filtered prior to analysis. All reagents and samples should be brought to room temperature prior to use. Use only the number of strips needed. Keep unused strips stored in their aluminum foil pouch with the included desiccant until needed.

1. Pipet 50 uL of the diluted Domoic Acid Antibody solution into each well.

2. Pipet 50 uL of each Control or sample into a well using the sequence shown in the table below. **Always use wells A and B on each strip as Controls.** Always perform duplicate analyses of samples. Three samples can be tested per strip. The example below shows the testing of eight samples.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Con-trol	Con-trol	Con-trol									
B	Con-trol	Con-trol	Con-trol									
C	1st Unk	4th Unk	7th Unk									
D	1st Unk	4th Unk	7th Unk									
E	2nd Unk	5th Unk	8th Unk									
F	2nd Unk	5th Unk	8th Unk									
G	3rd Unk	6th Unk										
H	3rd Unk	6th Unk										

3. Shake the wells for 30 minutes.

4. Pipet 50 uL of the Domoic Acid Tracer solution into each well.

5. Shake the wells for 30 minutes.

6. Wash the strips 3 times on the platewasher. Tap the strips upside-down firmly on a paper towel to blot away any excess wash solution that may remain in the wells.

7. Add 100 uL of Substrate Solution to each well. Shake the plate for five minutes.

8. Add 100 uL of Stop Solution to each well. Shake the plate briefly.

9. Measure the absorbance in each well. Note: If Control absorbance is greater than 3.0 AU, remove 50 uL from ALL WELLS and measure absorbance.

10. The data can be analyzed using the Excel worksheet available at the following link:

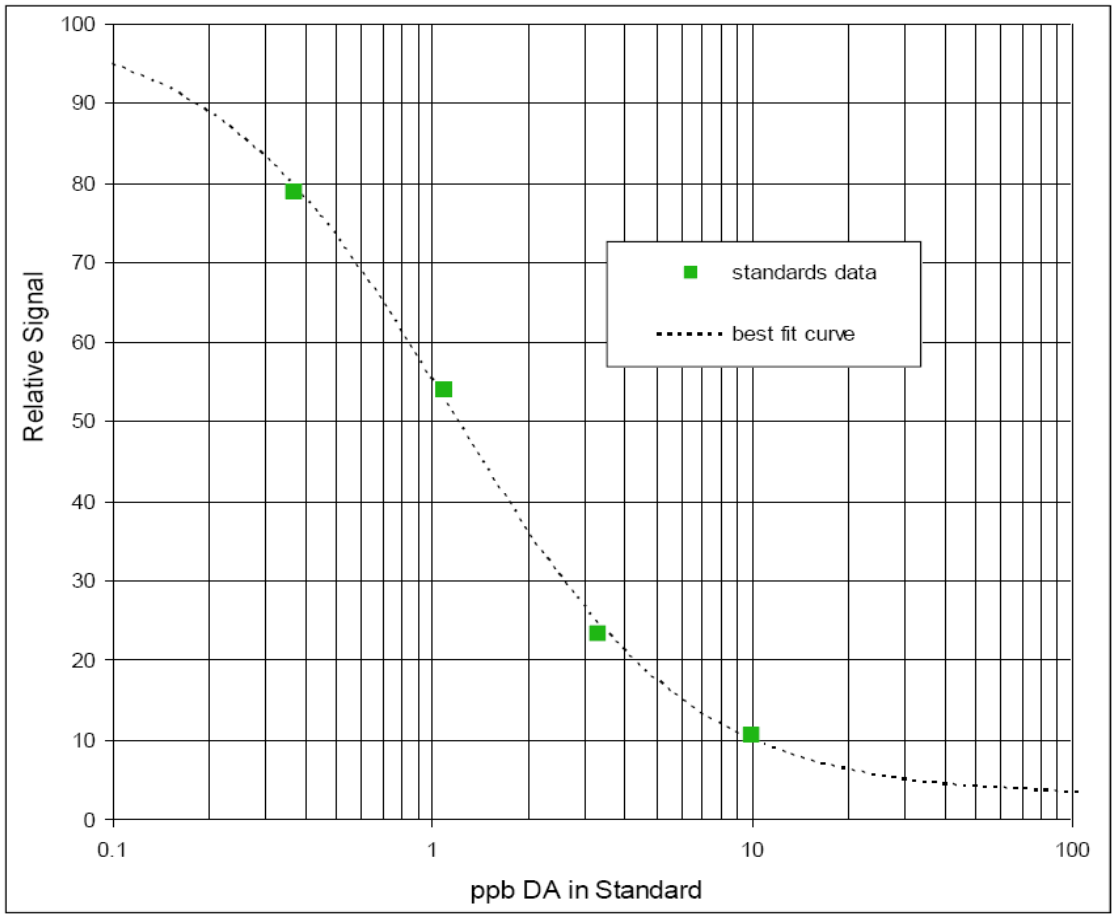
http://mercuryscience.com/Domoic Acid Quantitation 8Well Strip.xls

PERFORMANCE CHARACTERISTICS

Reproducibility

Inter-Assay Standard Curve

The average values and standard deviation of 5 separate standard curves is shown below.



Intra-assay Signal Precision

Analysis of 12 replicates for five different samples

	A	B	C	D	E
Signal (% of Control)	99.5	76.5	47.5	23.5	10.4
Standard Deviation	1.4	1.2	2.0	2.3	1.1
% Coeff. Var.	1.4	1.6	4.2	9.8	10.9

Intra-assay Concentration Precision

Analysis of 3 different samples measured in 6 separate quantitative assays.

	A	B	C
Average Conc. (ppb)	0.56	1.54	3.66
Standard Deviation (ppb)	0.01	0.13	0.19
% Coeff. Var.	2.1	8.6	5.3

PERFORMANCE CHARACTERISTICS (Cont.)

Detection Limit

The detection limit is defined as the minimum concentration of Domoic Acid that can be distinguished from a blank standard with 95% confidence. A detection limit of 0.1 ppb Domoic Acid in extraction buffer has been demonstrated with this assay.

Cross Reactivity

This assay is specific for the detection of domoic acid. The ability of the assay to detect structurally related compounds is shown in the following table.

Analyte	% Reactivity
Domoic Acid	100
Kainic Acid	0.3
Glutamic Acid	less than 0.1
Glutamine	less than 0.1

PROCEDURAL NOTES

Please read all instructions thoroughly before using this kit. Do not mix reagents from kits having different lot numbers. Do not use kits after the expiration date printed on the kit label.

Reagents should be at room temperature when used.

During washing steps, check that each well is completely filled during wash solution additions. After washing is complete, invert the wells and tap them gently against a paper towel to remove excess liquid.

The platewasher should be rinsed with distilled water at the end of each day of use to prevent clogging of the dispensing and aspirating ports. Prime the platewasher with wash solution before the first wash each day.

Care must be taken during each step to prevent contamination of reagents and equipment Do not use the same pipet tip in two different reagents.

For Technical Assistance, contact Mercury Science Inc: (866) 861-5836.

Additional Information

MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT

The Domoic Acid test kit is part of a complete system of immunodiagnostic reagents and instrumentation. The system requires the following equipment.

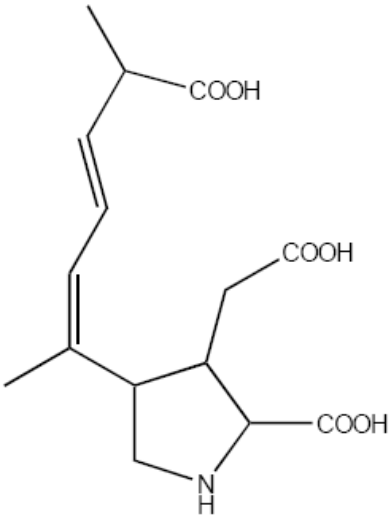
1. Microtiterplate Reader able to measure Absorbance at 450 nm
2. Platewasher
3. Plate Shaker
4. 8 Channel pipet
5. Pipetmen (P10, P200 and P1000)

Other Notes:

- Perform each Control and Sample in duplicate wells.
- All sample extracts should be filtered prior to analysis.
- All reagents and samples should be brought to room temperature prior to use.
- Use only the number of strips needed.
- Keep unused strips stored in their aluminum foil pouch with the included desiccant until needed.
- If Control absorbance is greater than 3.0 AU, remove 100 uL from ALL WELLS and repeat absorbance measurement.

An Excel worksheet has been developed to analyze results and quantitate the amount of domoic acid in extracts. Send your request for the "Domoic Acid Quantitation Worksheet - DAK-36" to: info@mercuryscience.com

Structure of Domoic Acid

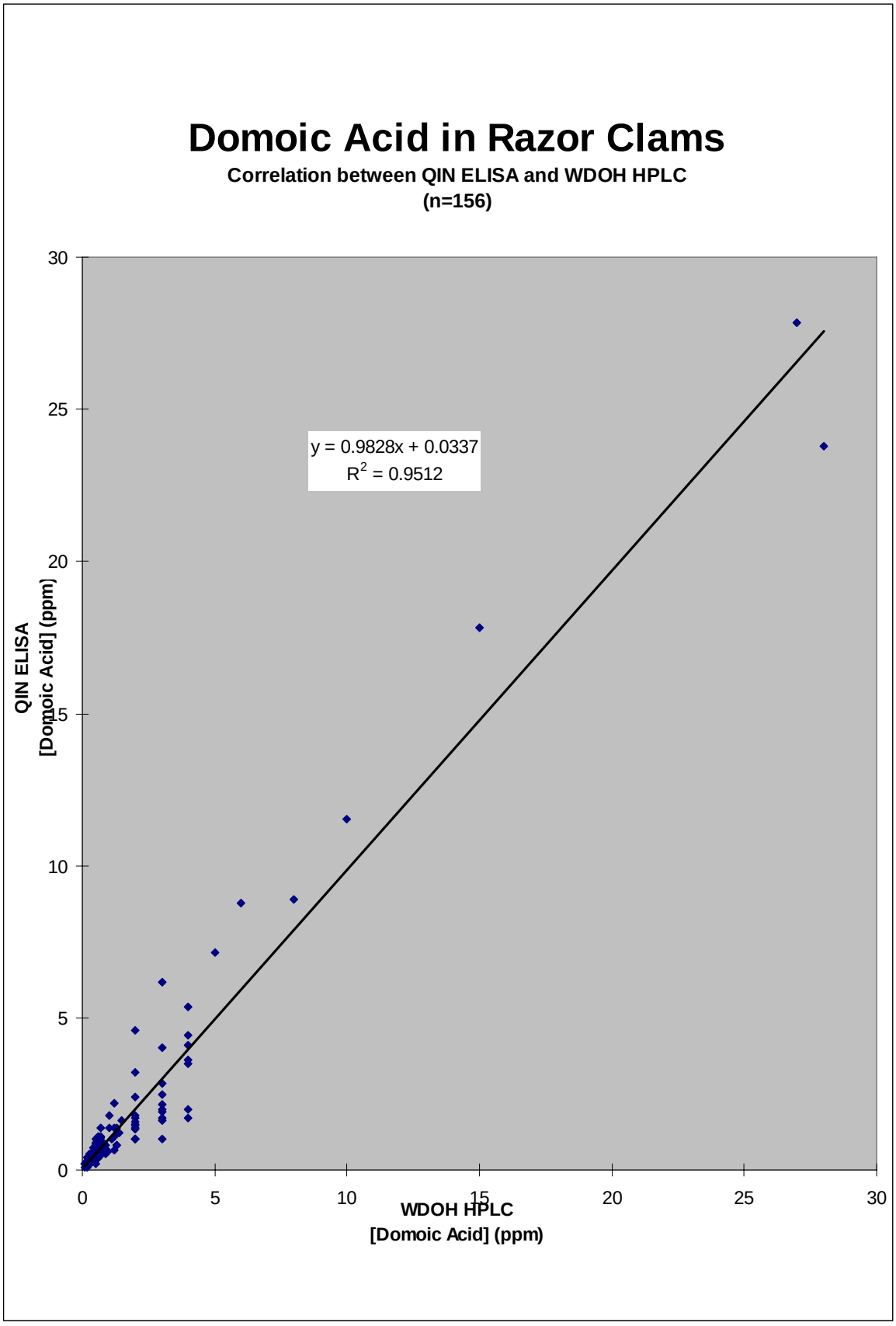


Domoic Acid Test Kit

Summary Protocol Sheet

Add Antibody	50 uL
Add Control and Samples	50 uL
Incubate	Shake for 30 minutes
Add Tracer	50 uL
Incubate	Shake for 30 minutes
Wash	"3 WASHES" program
TMB	Add 100 uL, shake for 5 minutes
Stop	Add 100uL
Measure	Absorbance at 450 nm

Note: If Control absorbance is greater than 3.0 AU, remove 100 uL from ALL WELLS and repeat absorbance measurement.


Domoic Acid in Razor Clams
Correlation between QIN ELISA and WDOH HPLC
(n=156)
y = 0.9828x + 0.0337
R2 = 0.9512
QIN ELISA
[Domoic Acid] (ppm)
WDOH HPLC
[Domoic Acid] (ppm)
0
5
10
15
20
25
30

I. Method Application and Single Lab Validation Checklist For Acceptance of a Method for Use in the NSSP

(http://www.issc.org/client_resources/lmr%20documents/i.%20issc%20lab%20method%20application%20checklist.pdf)

ISSC Method Application and Single Lab Validation Checklist For Acceptance of a Method for Use in the NSSP

The purpose of single laboratory validation in the National Shellfish Sanitation Program (NSSP) is to ensure that the analytical method under consideration for adoption by the NSSP is fit for its intended use in the Program. A Checklist has been developed which explores and articulates the need for the method in the NSSP; provides an itemized list of method documentation requirements; and, sets forth the performance characteristics to be tested as part of the overall process of single laboratory validation. For ease in application, the performance characteristics listed under validation criteria on the Checklist have been defined and accompany the Checklist as part of the process of single laboratory validation. Further a generic protocol has been developed that provides the basic framework for integrating the requirements for the single laboratory validation of all analytical methods intended for adoption by the NSSP. Methods submitted to the Interstate Shellfish Sanitation Conference (ISSC) Laboratory Methods Review (LMR) Committee for acceptance will require, at a minimum, six (6) months for review from the date of submission.

Name of the New Method	DOMOIC ACID RAPID ENZYME-LINKED IMMUNOSORBENT ASSAY – 96 Well Format
Name of the Method Developer	Mercury Science Inc. and the National Oceanic and Atmospheric Administration
Developer Contact Information	Attn: Tom Stewart 4802 Glendarion Dr. Durham, NC 27713 Phone: (866) 861-5836

Checklist	Y/N	Submitter Comments
A. Need for the New Method		
1. Clearly define the need for which the method has been developed.	Y	Faster, more affordable DA analysis
2. What is the intended purpose of the method?	Y	Monitoring shellfish and water samples for DA
3. Is there an acknowledged need for this method in the NSSP?	Y	Faster analysis decreases public health risks
4. What type of method? i.e. chemical, molecular, culture, etc.	Y	Enzyme-Linked Immunosorbent Assay (ELISA)
B. Method Documentation		
1. Method documentation includes the following information:		
Method Title	Y	DOMOIC ACID RAPID ENZYME-LINKED IMMUNOSORBENT ASSAY – 96 Well Format
Method Scope	Y	For the analysis of food, phytoplankton, and water
References	Y	Peer Reviewed Publication, Independent Correlation Study
Principle	Y	Competitive ELISA
Any Proprietary Aspects	Y	Unique Antibody and Enzyme Conjugate
Equipment Required	Y	Equipment is listed for this method
Reagents Required	Y	Reagents are listed for this method
Sample Collection, Preservation and	Y	Requirements are described for this method

Storage Requirements		
Safety Requirements	Y	Normal Good Lab Practices
Clear and Easy to Follow Step-by-Step Procedure	Y	See User Guide supplied with DA Test kit.
Quality Control Steps Specific for this Method	Y	Described below
C. Validation Criteria		
1. Accuracy / Trueness		SLV Testing in Progress – see preliminary results using oysters
2. Measurement Uncertainty		SLV Testing in Progress– see preliminary results using oysters
3. Precision Characteristics (repeatability and reproducibility)		SLV Testing in Progress– see preliminary results using oysters
4. Recovery		SLV Testing in Progress– see preliminary results using oysters
5. Specificity		SLV Testing in Progress
6. Working and Linear Ranges		See publication Dec 2008 issue Journal Shellfish Research - 0.3 to 3 ppb
7. Limit of Detection		Linear range
8. Limit of Quantitation / Sensitivity		SLV Testing in Progress
9. Ruggedness		SLV Testing in Progress
10. Matrix Effects		SLV Testing in Progress
11. Comparability (if intended as a substitute for an established method accepted by the NSSP)		Results from one independent study are included
D. Other Information		
1. Cost of the Method	Y	$200 per 36 duplicate samples
2. Special Technical Skills Required to Perform the Method	Y	Some ELISA experience or training required
3. Special Equipment Required and Associated Cost	Y	See list
4. Abbreviations and Acronyms Defined	Y	See list
5. Details of Turn Around Times (time involved to complete the method)	Y	90 minutes
6. Provide Brief Overview of the Quality Systems Used in the Lab	Y	See attached
Submitters Signature 	Date: June 18, 2009	
Submission of Validation Data and Draft Method to Committee	Date:	
Reviewing Members	Date:	
Accepted	Date:	
Recommendations for Further Work	Date:	

II. DEFINITIONS

1. **Accuracy/Trueness** - Closeness of agreement between a test result and the accepted reference value.
2. **Analyte/measurand** - The specific organism or chemical substance sought or determined in a sample.
3. **Blank** - Sample material containing no detectable level of the analyte or measurand of interest that is subjected to the analytical process and monitors contamination during analysis.
4. **Comparability** – The acceptability of a new or modified method as a substitute for an established method in the NSSP. Comparability must be demonstrated for each substrate or tissue type by season and geographic area if applicable.
5. **Fit for purpose** – The analytical method is appropriate to the purpose for which the results are likely to be used.
6. **HORRAT value** – HORRAT values give a measure of the acceptability of the precision characteristics of a method.[4]
7. **Limit of Detection** – the minimum concentration at which the analyte or measurand can be identified. Limit of detection is matrix and analyte/measurand dependent.[4]
8. **Limit of Quantitation/Sensitivity** – the minimum concentration of the analyte or measurand that can be quantified with an acceptable level of precision and accuracy under the conditions of the test.
9. **Linear Range** – the range within the working range where the results are proportional to the concentration of the analyte or measurand present in the sample.
10. **Measurement Uncertainty** – A single parameter (usually a standard deviation or confidence interval) expressing the possible range of values around the measured result within which the true value is expected to be with a stated degree of probability. It takes into account all recognized effects operating on the result including: overall precision of the complete method, the method and laboratory bias and matrix effects.
11. **Matrix** – The component or substrate of a test sample.
12. **Method Validation** – The process of verifying that a method is fit for purpose.[1]
13. **Precision** – The closeness of agreement between independent test results obtained under stipulated conditions.[1, 2] There are two components of precision:
    a. **Repeatability** – The measure of agreement of replicate tests carried out on the same sample in the same laboratory by the same analyst within short intervals of time.
    b. **Reproducibility** – The measure of agreement between tests carried out in different laboratories. In single laboratory validation studies reproducibility is the closeness of agreement between results obtained with the same method on replicate analytical portions with different analysts or with the same analyst on different days.
14. **Quality System** - The laboratory's quality system is the process by which the laboratory conducts its activities so as to provide data of known and documented quality with which to demonstrate regulatory compliance and for other decision–making purposes. This system includes a process by which appropriate analytical methods are selected, their capability is evaluated, and their performance is documented. The quality system shall be documented in the laboratory's quality manual.
15. **Recovery** – The fraction or percentage of an analyte or measurand recovered following sample analysis.
16. **Ruggedness** – The ability of a particular method to withstand relatively minor changes in analytical technique, reagents, or environmental factors likely to arise in different test environments.[4]
17. **Specificity** – The ability of a method to measure only what it is intended to measure.[1]
18. **Working Range** – The range of analyte or measurand concentration over which the method is applied.

REFERENCES:

1. Eurachem Guide, 1998. The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics. LGC Ltd. Teddington, Middlesex, United Kingdom.
2. IUPAC Technical Report, 2002. Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis, Pure Appl. Chem., Vol. 74, (5): 835-855.
3. Joint FAO/IAEA Expert Consultation, 1999. Guidelines for Single-Laboratory Validation of Anilytical Methods for Trace-Level Concentrations of Organic Chemicals.
4. MAF Food Assurance Authority, 2002. A Guide for the Validation and Approval of New Marine Biotoxin Test Methods. Wellington, New Zealand.
5. National Environmental Laboratory Accreditation. , 2003. Standards. June 5.
6. EPA. 2004. EPA Microbiological Alternate Procedure Test Procedure (ATP) Protocol for Drinking Water, Ambient Water, and Wastewater Monitoring Methods: Guidance. U.S. Environmental Protection Agency (EPA), Office of Water Engineering and Analysis Division, 1200 Pennsylvania Avenue, NW, (4303T), Washington, DC 20460. April.

III. Single Laboratory Validation (SLV) Protocol For Submission to the Interstate Shellfish Sanitation Conference (ISSC) For Method Approval

Single Laboratory Validation (SLV) Protocol
For Submission to the Interstate Shellfish Sanitation Conference (ISSC)
For Method Approval

Information: Applicants shall attach all procedures, with materials, methods, calibrations and interpretations of data with the request for review and potential approval by the ISSC. The ISSC also recommends that submitters include peer-reviewed articles of the procedure (or similar procedures from which the submitting procedure has been derived) published in technical journals with their submittals. Methods submitted to the ISSC LMR committee for acceptance will require, at a minimum, 6 months for review from the date of submission.

Note: The applicant should provide all information and data identified above as well as the following material, if applicable:

Justification for New Method

- Name of the New Method.

  DOMOIC ACID RAPID ENZYME-LINKED IMMUNOSORBENT ASSAY – 96 Well Format
  (Marketed by Mercury Science Inc. as Product # DAK-36 Domoic Acid Test Kit.)

- Specify the Type of Method (e.g., Chemical, Molecular, or Culture).

  Enzyme linked immunosorbent assay (ELISA) using an anti-domoic acid monoclonal antibody

- Name of Method Developer.

  The DA assay kit was developed jointly by NOAA's National Centers for Coastal Ocean Science, National Ocean Service, and the Northwest Fisheries Science Center, together with an industry partner Mercury Science, Inc., Durham, North Carolina

- Developer Contact Information [e.g., Address and Phone Number(s)].

  Mercury Science Inc.
  Attn: Tom Stewart
  4802 Glendarion Dr.
  Durham, NC 27713

Phone: (866) 861-5836

• Date of Submission.

June 18, 2009

• Purpose and Intended Use of the Method.

The method is an accurate, rapid, cost-effective tool for use by environmental managers and public health officials to monitor Domoic Acid concentrations in environment samples.

• Need for the New Method in the NSSP, Noting Any Relationships to Existing Methods.

The regulatory method for DA detection sanctioned by the Interstate Shellfish Sanitation Conference is a high performance liquid chromatography (HPLC) assay. Though accurate, these analyses are generally run by centralized state facilities with results typically not available for 3 to 14 days after the samples are collected. In more remote communities, many of which depend heavily on subsistence clam harvests, these long delays and the costs of sample analysis are causes for public health concern. The average cost of approximately $100 per sample limits the number of samples that can be analyzed (Harold Rourk, Washington State Department of Health, personal communication). Resource managers in coastal communities have expressed their desire for a cost-effective method for rapid and accurate determination of DA concentrations in shellfish and phytoplankton samples. The high throughput capacity of the assay also allows for much faster response times when domoic acid events occur. The relatively low cost of the assay means that significantly more sampling is also possible on the same or smaller budget.

• Method Limitations and Potential Indications of Cases Where the Method May Not Be Applicable to Specific Matrix Types.

This ELISA is sensitive to organic solvents such as methanol. Sample extracts that contain methanol can be diluted with Sample Dilution Buffer (provided in the kit) to reduce methanol concentrations to less than 1%.

• Other Comments.

The implementation of this ELISA system required the development and validation of two essential reagents, a high avidity monoclonal antibody to DA and a stable DA-HRP conjugate recognized by the same monoclonal antibody.

Method Documentation

• Method Title.

Domoic Acid Rapid Enzyme-Linked ImmunoSorbent Assay (ELISA) – 96 Well Format

• Method Scope.

The method is a sequential competitive enzyme linked immunosorbent assay (ELISA) utilizing a high avidity monoclonal antibody (mAb) to DA to ensure assay specificity and consistency across production lots. The assay is specific for Domoic Acid and can be used for the analysis of tissue extracts, phytoplankton samples, and water samples.

• References.

RAPID ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETECTION OF THE ALGAL TOXIN DOMOIC ACID, Journal of Shellfish Research, Vol. 27, No. 5, 1301–1310, 2008.
Available online at: http://mercuryscience.com/LitakerStewartDec2008.pdf

User Guide Available Online at: http://www.mercuryscience.com/DA User Guide 2007A.pdf

• Principle.

A fixed number of anti-DA mAb binding sites are incubated with dissolved DA in the sample followed by the addition of a DA – horseradish peroxidase (HRP) conjugate. As these binding events occur, the anti-DA mAb molecules are simultaneously captured by anti-mouse antibodies affixed to the surface of the microtiter plate wells. Following a wash step, subsequent HRP derived color development, readable on standard microplate readers, was inversely proportional to the concentration of DA in the sample matrix. The assay reagents were titrated so that the amount of mAb and the DA–HRP conjugate added produced a maximal absorbance signal of approximately 2.5 absorbance units when no DA was present.

• Analytes/Measurands.

Domoic Acid

• Proprietary Aspects.

The assay uses a unique monoclonal antibody and enzyme conjugate developed by Mercury Science Inc.

• Equipment.

Microtiterplate orbital shaker
Automated microtiterplate washer
Multichannel pipette
Pipetman (P20, P200, P1000) or equivalent
Microtiterplate reader (capable of reading at 450nm)

• Reagents.

1. anti-DA antibody
2. DA-HRP conjugate
3. Assay Buffer
4. Control Solution
5. Wash solution
6. TMB substrate
7. Stop solution

• Media.

Tissue samples are extracted using a solvent of Methanol:Water (50:50, v:v)
Extracts are diluted into an aqueous sample buffer prior to analysis by the ELISA.

Water samples are filtered and buffered prior to analysis by the ELISA.

Phytoplankton samples are ruptured by appropriate methods in aqueous sample buffer prior to analysis by the ELISA.

• Matrix or Matrices of Interest.

Butter clam (*Saxidomus giganteus*), blue mussel (*Mytilus edulis*), geoduck (*Panopea abrupta*), manila clam (*Venerupis japonica*), oyster (*Crassostrea virginica*), quahog (*Mercenaria mercenaria*) and razor clam (*Siliqua patula*) tissues, as well as phytoplankton and water samples

• Sample Collection, Preservation, Preparation, Storage, Cleanup, etc.

Shellfish preparation: In the case of shellfish, pooled samples of 10-12 individuals are cleaned, and ground to a smooth and uniform homogenate in a commercial blender. Approximately 2 g of homogenized tissue are added to a tared 50 mL conical tube and the weight recorded to the nearest 0.01g. Next, 18 mL of 50% methanol are added and the samples mixed at high speed on a vortex mixer for 2 min. Once the extraction is complete, the tubes are spun in a table top centrifuge for 20 min at 10,000xg or until a tight pellet and clear supernatant are obtained. If the samples do not clear despite the spinning at high speed, the supernatant is passed through a 0.45 µm syringe filter. The extract is then diluted 1:100 or 1:1000 into Sample Dilution Buffer and is ready for analysis by ELISA. If necessary, the sample may be stored at 4ºC for up to 24 h in a refrigerator prior to analysis.

Phytoplankton preparation: Approximately 0.1 to 1.0 L of cultured cells or sea water samples are filtered onto a GF/F filter which can be immediately frozen at -80^{o}C until the filter can be processed or processed immediately. For processing, filters are placed in a 5mL conical tube and 3 mL of 20% methanol are added. The samples are sonicated until the filter is completely homogenized. Care is needed to prevent the probe from rupturing the tube. The sonicator probe is cleaned carefully with 20% methanol between samples to prevent cross-contamination. Next the homogenate is centrifuged at 3000xg for 10 minutes. The supernatant is passed through a 0.2 µm syringe filter. The extract is then diluted into Sample Dilution Buffer and is ready for analysis by ELISA.

Storage of test kit: Any unused strips can be removed and stored in a desiccator pouch at 4^{o}C for at least six months without compromising assay performance

• Safety Requirements.

General Good Laboratory Practices should be followed at all times.
Safety Glasses should be worn at all times.
The Stop solution in the assay contains 1 M hydrochloric acid. Care must be taken to avoid skin or eye contact with the Stop solution.

• Other Information (Cost of the Method, Special Technical Skills Required to Perform the Method, Special Equipment Required and Associated Cost, Abbreviations and Acronyms Defined and Details of Turn Around Times [Time Involved to Complete the Method]).

Cost of the Method: The DAK 36 Domoic Acid Test Kit costs $200 and contains sufficient assay reagents to perform 36 sample analyses (less than $6 per sample)

Special Technical Skills Required to Perform the Method: It is recommended that users have prior experience performing ELISA assays or receive training from Mercury Science Inc.

Special Equipment Required and Associated Cost (estimated):

- Microtiterplate orbital shaker $500
- Automated microtiterplate washer $5,000
- Multichannel pipette $700
- Pipetmen (P20, P200, P1000) (or equivalent) $1,500
- Microtiterplate reader (capable of reading at 450nm) $6,500

This equipment is commonly available in most state laboratories.

Abbreviations and Acronyms Defined:

ELISA – Enzyme-Linked Immunosorbent Assay
HRP – Horseradish Peroxidase
TMB – Tetramethylbenzidine
DA – Domoic Acid
mAb – monoclonal Antibody

Details of Turn Around Times: As many as 36 sample extracts can be analyzed in <1.5 hours.

• Test Procedures, (Be Specific and Provide Easy-to-Follow Step-by-Step Procedures and indicate critical steps.).

The 96 well assay tray used in the assay contains 12 strips. Each strip of 8 wells can be removed and stored until it is needed. The first two wells of each strip are used as a control (no DA added). The remaining six wells are used to analyze 3 samples in duplicate. This format provided the flexibility of running anywhere from 3 to 36 duplicate samples at a time.

1. For unknown sample analysis, extracts are diluted to a final concentration ranging from 0.3 to 3 to ppb using the Sample Dilution Buffer [phosphate salt solution, pH 7.8, containing casein]. For clam tissues containing DA, sample dilutions of 1:100 and 1:1000 are typically used. (Preliminary tests with razor clam extracts showed that a 25-fold dilution in sample dilution buffer eliminates matrix effects in ELISA analysis.)
2. The immunoassay is started by adding 50 µl of the anti-DA antibody reagent to each well using a multi-channel pipette.
3. Next, 50 µl of the Control solution (sample buffer without DA) is added to the first two wells in each strip.
4. Duplicate 50 ul aliquots from the diluted DA extracts are then added to the remaining wells in each strip and the plate is shaken at room temperature for 30 minutes on an orbital shaker set to vigorously mix the solution in each well. **Vigorous mixing is key to reaching equilibrium in the allotted time and obtaining replicable results from one run to the next.** In this step, DA in the sample binds to available mAb in proportion to [DA].
5. At the end of the incubation, 50 µl of DA HRP conjugate is added to each well and the plate is shaken a second time for 30 min at room temperature on an orbital shaker. The DA-HRP will bind to available mAb sites.
6. Following Step 5, the plate is washed three times with wash solution [Tris-HCl buffered salt solution (pH 7.8) containing Tween 20 and sodium azide as a preservative] using a commercial plate washer, making certain the fluid is completely aspirated from all the wells. *Alternatively, these washes can be done manually by adding wash solution to wells using a multichannel pipettor and then flicking all fluid from the wells*. The manual method may result in slightly higher variability.
7. Next, 100 µL of SureBlue TMB substrate (5,5'-tetramethylbenzidine, kpl.com) is added to each well.
8. The plate is placed on an orbital shaker for no more than 5 minutes, or until adequate color development is observed.

9. Color development is terminated by adding 100 µL stop solution (1N hydrochloric acid) to each well.
10. The absorbance in each well is measured at 450 nm using a plate reader.
11. The DA concentrations are determined using the sample (B) and control (B_o) absorbances, the original tissue weights, and the volume of 20% or 50% methanol used to extract each sample. The actual calculations are made using a Microsoft Excel work sheet that incorporates the constants for a four parameter model (DA concentration $=ED_{50}(B_o/B\ -1)^{-slope}$). This worksheet can be downloaded from:

    http://www.mercuryscience.com/Domoic%20Acid%20Quantitation%208Well%20Strip.xls

Processing time for this assay is approximately 1.5 hours.

- Quality Control (Provide Specific Steps.).

  Bo signals should be greater than 1.5 AU and less than 3.0 AU. When Bo values are greater than 3.0, the user can remove 50 ul of the yellow solution from ALL wells on that strip and re-read the signal.

  Duplicate signals should be within 10% of their average value. For example: Two duplicate wells having AU values of 1.500 and 1.600 are acceptable because the difference between the values and their average (1.550) is less than 10%. If two duplicate wells have AU values of 1.000 and 1.400, this result is invalid and should be retested because the variation between the values is too great because: (1.200 - 1.000)/1.000 = 20%

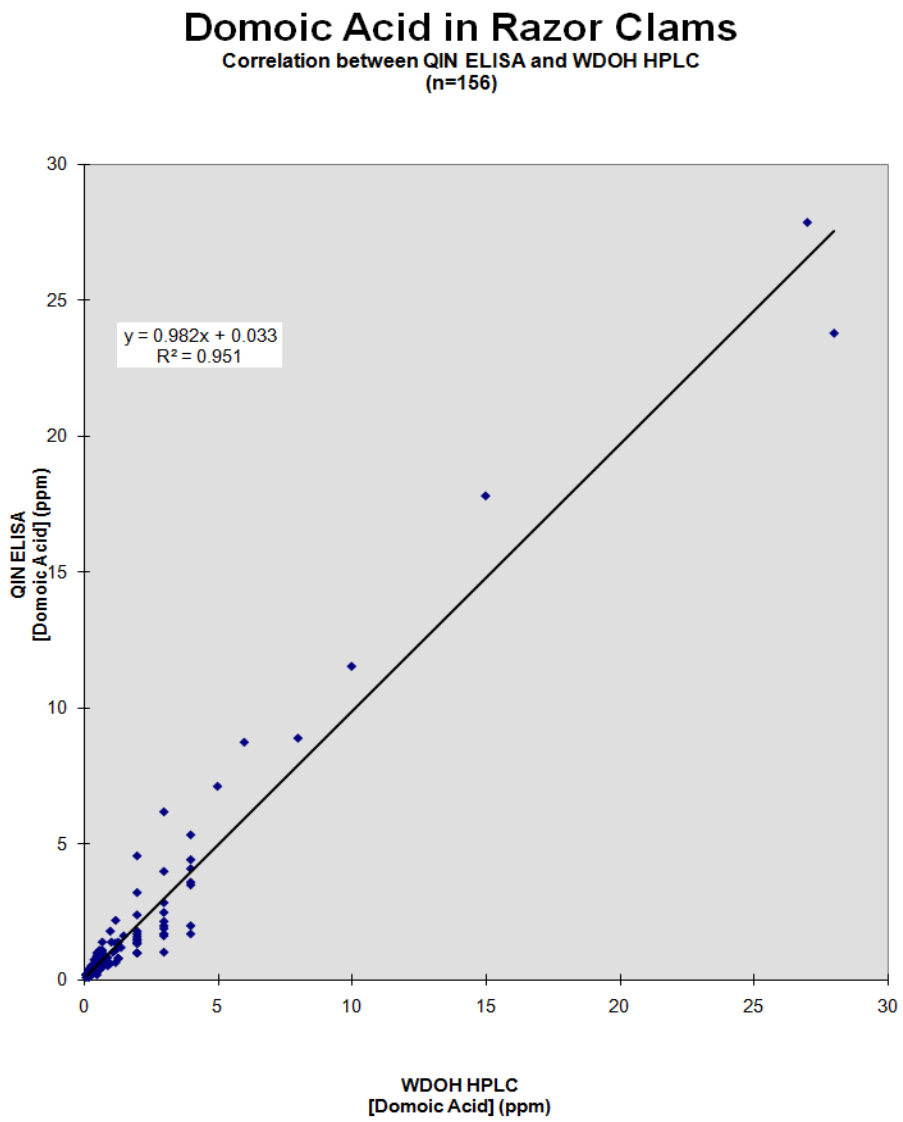
  Domoic Acid standard solutions can be run as needed to QC the accuracy of the assay. QC protocols can be developed on a case-by-case basis with assistance provided by Mercury Science Inc.

- Validation Criteria (Include Accuracy / Trueness, Measurement Uncertainty, Precision [Repeatability and Reproducibility], Recovery, Specificity, Working and Linear Ranges, Limit of Detection, Limit of Quantitation / Sensitivity, Ruggedness, Matrix Effects and Comparability (if intended as a substitute for an established method accepted by the NSSP).

  A preliminary validation study using oyster tissue has been completed and provided to the committee for feedback. Oysters were selected because they were locally available and could be run prior to the submission date. These data should be considered preliminary. In addition, an informal validation study was conducted by the Quinault Tribe and the Washington Department of Health and included below. The remaining validation studies are will be done in the latter part of the summer and fall 2009. Results will be provided to the LRM committee as they become available.

  During internal validation studies at Mercury Science, the assay was found to have an effective quantitative range from approximately 0.3 to 3.0 ppb using domoic acid standard solutions.

- Comparability: The graph below shows the results of a year-long study done by the Quinault Indian Nation (QIN) and the Washington Department of Health (WDOH) comparing razor clam analysis performed by the Domoic Acid Test Kit versus HPLC analysis. One hundred fifty six samples were compared. This independent study was planned and performed without any input from Mercury Science or NOAA.



Additional correlation studies are reported in the following research paper:

RAPID ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETECTION OF THE ALGAL TOXIN DOMOIC ACID, Journal of Shellfish Research, Vol. 27, No. 5, 1301–1310, 2008.
Available online at: http://mercuryscience.com/LitakerStewartDec2008.pdf

- Data and Statistical Analyses Performed for Each Validation Criterion Tested (Be Specific and Provide Clear Easy-to-Follow Step-by-Step Procedures.). Preliminary study presented for feedback from the committee

- Calculations and Formulas Used for Each Validation Criterion Tested. Testing in Progress

- Results for Each Validation Criterion Tested. Testing in Progress

- Discussion of Each Validation Criterion Tested. Testing in Progress

- Summary of Results. Testing in Progress

Additional Requirement
If a laboratory method is found acceptable for use in the National Shellfish Sanitation Program and adopted by the Interstate Shellfish Sanitation Conference, the method submitter will draft a laboratory checklist that can be used to evaluate laboratories performing their procedure. The checklist will be submitted to the ISSC and reviewed by the Laboratory Quality Assurance Committee for Conference approval.

(For guidance: refer to the checklists in the National Shellfish Sanitation Program Guide for the Control of Molluscan Shellfish 2003, Guidance Documents, Chapter II – Growing Areas, .11 Evaluation of Laboratories by State Laboratory Evaluation Officers Including Laboratory Evaluation Checklists.)

VII. SLV Documents for Marine Biotoxin and Non-MPN Based Microbiological Methods (http://www.issc.org/lmrforms.aspx)

VII. #1 Marine Biotoxin and Non-MPN Based Microbiological Methods SOP – Accuracy/Trueness & Measurement Uncertainty

VALIDATION CRITERIA

Accuracy/Trueness is the closeness of agreement between test results and the accepted reference value. To determine method accuracy/trueness, the concentration of the targeted analyte/measurand/organism of interest as measured by the analytical method under study is compared to a reference concentration.

Measurement uncertainty is a single parameter (usually a standard deviation or confidence interval) expressing the possible range of values around the measured result within which the true value is expected to be with a stated degree of probability. It takes into account all recognized effects operating on the result including: overall precision of the complete method, the method and laboratory bias and matrix effects.

Procedure: This procedure is applicable for use with either growing waters or shellfish tissues. Make every effort to use samples free of the target analyte/measurand/organism of interest. For each shellfish type of interest use a minimum of 10-12 animals per sample. For each sample take two (2) aliquots of either the homogenate or growing water sample appropriately sized for your work and spike one(1) of the two (2) aliquots with a suitable known concentration of the target analyte/measurand/organism of interest. Do not spike the second aliquot. This is the sample blank. For microbiological methods determine the concentration of the target organism of interest used to spike each sample by plating on/in appropriate agar. Process both aliquots of sample as usual to determine the method concentration for the target analyte/measurand/organism of interest. For growing waters do twenty (20) samples collected from a variety of growing areas. For shellfish do twenty (20) samples for each shellfish tissue type of interest collected from a variety of growing areas, the same growing area harvested on different days or from different process lots. **Use a variety of concentrations spanning the range of concentrations of importance in the application of the method to spike sample homogenates or growing water samples**. Both the low and high level spike concentrations must yield determinate values when analyzed by the method under study.

Data:

Working Range _The working range is 0.3 to 3.0 ppb and samples are diluted into the effective range so the working range is 0 to over 100 ppm
Sample Type _Shellfish Tissue__
Agar used to determine spike concentration ___Not applicable__
Organism used for spiking Oyster (*Crassostrea virginica*)
Sample Spike conc/plate count Sample blank conc Spiked sample conc from analysis

The regulatory limit for DA is 20 ppm in shellfish tissue and the dynamic range of the assay was tested using oyster tissues spiked with 2.3 to 35.5 ppm domoic acid. The standard spikes of domoic acid were calibrated using the Canadian NRC standards. The following procedure was used.

Extraction:

1. Live oysters were shucked on 3/30 and 3/31/2009 and homogenized 12 at a time in a blender and stored in 50mL tubes in -80C freezer
2. Samples thawed just prior to use
3. 2 g oyster weighed out in 50mL tube and exact weigh recorded to nearest mg
4. 18mL 50% MeOH added to tube
5. DA added to the homogenate so that the final concentrations in 20 mL were 0.25, .5, 1, 2, 4 ppm. This is equivalent to 2.5,5,10,20 or 40ppm in 2g oyster that is subsequently extracted into the total 20 ml volume.
6. Each tube vortexed for 1 min

ELISA

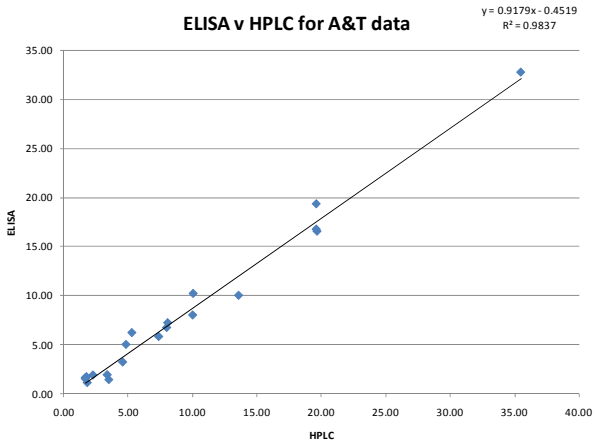
1. ~1.4mL from each tube were transferred into a 2mL microfuge tube
2. Samples in microfuge tubes centrifuged at 14,000 rpm for 5 min
3. Aliquots of the resulting supernatant were diluted with ELISA kit sample dilution buffer with a 2 step dilution series so each extract contained ~2ppb
4. Diluted extracts processed on ELISA following kit instructions

HPLC was used to determine initial spike concentration using the following procedure:

1. Spiked 50mL tubes centrifuged at 3000rpm for 20 min
2. Supernatant filtered with 25mm GF/F filter first, and then filtered with .45um syringe tip filter with 30mL syringe
3. SPE tubes pre-conditioned with 6mL MilliQ water, then 3mL 100% MeOH, then 50% MeOH
4. 5mL of extract though SPE tube, 1 drop per second
5. Washed with 5mL .1M NaCl
6. Eluted/ collected with 5 mL .5M NaCL in 15mL tube
7. ~1mL pipetted with 9 inch glass Pasteur pipette into clear HPLC vial
8. Run through HPLC- 20uL injection, .3mL/min, 15 min/sample….
9. Area and time of peak recorded
10. The DA concentration in each oyster extract was estimated using the previously determined standard curve where peak area =15.704 x DA concentration, R^2=0.9977.

Results

Sample #	Sample Spike conc (HPLC)	Sample blank conc	Spiked sample conc. from analysis (ELISA)
1	5.32	0.00	6.20
2	10.07	0.00	10.18
3	19.69	0.00	16.53
4	35.50	0.00	32.74
5	8.02	0.00	6.72
6	2.30	0.00	1.88
7	4.60	0.00	3.20
8	1.70	0.00	1.60
9	8.10	0.00	7.20
10	1.80	0.00	1.70
11	3.40	0.00	1.90
12	7.40	0.00	5.80
13	13.60	0.00	10.00
14	19.63	0.00	16.74
15	1.85	0.00	1.10
16	3.53	0.00	1.40
17	4.86	0.00	4.99
18	1.70	0.00	1.50
19	10.03	0.00	7.99
20	19.63	0.00	19.32
Average	9.14	0.00	7.93



The results of this preliminary study showed an excellent correlation between the HPLC and the ELISA assay, but with a slope of 0.92 instead of 1.0. This means the ELISA assay consistently underestimated the HPLC validated DA concentrations by ~10%. Preliminary tests using other shellfish tissues have shown a slope of approximately 1.0 (Litaker et al. 2008). I will do additional tests to determine whether or not the lower slope is due to matrix effects unique to oysters.

A consequence of this underestimation is that some of the statistical analyses below will show a significant difference between the spike concentration and the ELISA results. Given that this is the first time I have run through the calibration assay procedures I would request that the committee to wait for additional data before making any judgments concerning the robustness of the assay. Instead, I would like to use the preliminary oyster data to get the committee's feedback on whether I have adequately completed the necessary statistical analyses correctly and to obtain further clarifications concerning several of the analyses. The feedback will then be used for finalizing the subsequent analyses done in my laboratory and by the NOAA CCFHR laboratory.

For shellfish samples, repeat for each tissue type of interest.

DATA HANDLING

Accuracy/Trueness

The accuracy/trueness of a method consists of two distinct components, the portion due to the method itself regardless of the laboratory performing it and the portion contributed by the laboratory's performance. In a single laboratory method validation, it is impossible to distinguish the contribution of each to the overall accuracy/trueness of the method. Consequently, what is being estimated is the accuracy/trueness of the method as implemented by the laboratory performing the analysis. Good accuracy/trueness suggests the appropriateness of the method and the laboratory's performance of it for the intended work. Poor accuracy/trueness on the other hand indicates the potential unsuitability of the method and/or the laboratory's performance of it for the intended work.

Accuracy /trueness will be determined by calculating the closeness of agreement between the test results and either a known reference value or a reference value obtained by plate count for microbiological methods.

Measurement uncertainty

Measurement uncertainty can be determined by subtracting the results for each spiked sample from the reference value for the sample and calculating the 95% confidence interval of these differences. The confidence interval of these differences represents the range in values within which the true measurement uncertainty lies. A narrow range in values indicates that the method as implemented by the laboratory produces reliable results.

Use the log transformed data for both the plate count and the microbial results obtained from the spiked samples. If necessary use the sample blank (converted to logs for microbiological methods) to correct the spiked sample

for matrix effects and calculate the two-sided, 95% confidence interval for the difference in concentrations between the reference and the spiked samples. This range in counts represents the measurement uncertainty of the method as implemented by the laboratory.

Data Summary:
Calculated % accuracy/trueness ___86.84____

Again, the reason for the lower than expected accuracy is the fact that the slope of the relationship was 0.92 between the ELISA and HPLC for this first set of oyster samples.

Sample #	Sample Spike conc (HPLC)	Spiked sample conc. from analysis (ELISA)	Difference (ppm)
1	5.32	6.2	-0.88
2	10.07	10.18	-0.11
3	19.69	16.53	3.16
4	35.5	32.74	2.76
5	8.02	6.72	1.3
6	2.3	1.88	0.42
7	4.6	3.2	1.4
8	1.7	1.6	0.1
9	8.1	7.2	0.9
10	1.8	1.7	0.1
11	3.4	1.9	1.5
12	7.4	5.8	1.6
13	13.6	10	3.6
14	19.63	16.74	2.89
15	1.85	1.1	0.75
16	3.53	1.4	2.13
17	4.86	4.99	-0.13
18	1.7	1.5	0.2
19	10.03	7.99	2.04
20	19.63	19.32	0.31
Average	9.14	7.93	1.21
stdev			1.21832223
95% confidence interval			0.53393371

Calculated measurement uncertainty __0.5 ppm___

VII. #2 Marine Biotoxin and Non-MPN Based Microbiological Methods SOP – Ruggedness

VALIDATION CRITERIA

Ruggedness is the ability of a particular method to withstand relatively minor changes in analytical technique, reagents or environmental factors likely to arise in different test environments.

Procedure: This procedure is applicable for use with either growing waters or shellfish tissue. Make every effort to use samples free of the target analyte/measurand/organism of interest. For each shellfish type of interest use a minimum of 10 – 12 animals. For each sample take two (2) aliquots of either the growing water sample or shellfish homogenate appropriately sized for your work. Spike both aliquots with a suitable concentration of the target analyte/measurand/organism of interest. Process both aliquots of the sample as usual to determine method concentration for the target analyte/measurand/organism of interest. For the second aliquot of each sample, however, use a different batch or lot of culture media and/or test reagents as appropriate to process this aliquot. For growing waters, do ten (10) samples collected from a variety of growing waters. For shellfish do ten (10) samples for each shellfish tissue type of interest collected from a variety of growing areas, the same growing area harvested on different days or from different process lots. Use the same two batches or lots of culture media and/or test reagents to process each sample such that "batch or lot 1" is used to process the first aliquot of each sample and "batch or lot 2" is used to process the second aliquot of each sample. Use a range of concentrations which spans the range of the method's intended application to spike the sample aliquots. However both aliquots of the same sample must be spiked with the same concentration of the target analyte/measurand/organism of interest. Process samples over a period of several days.

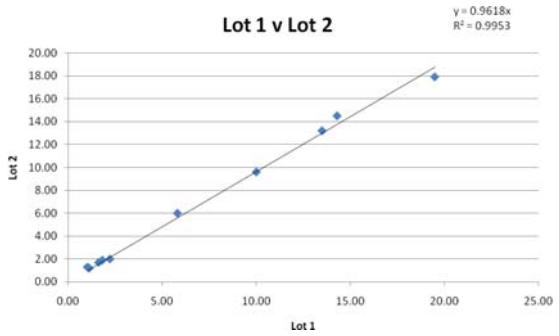
Data:

Sample type ___Oyster tissue_____
Sample Conc "Batch or Lot 1" Conc "Batch or Lot 2"
Media and/or Reagents Media and/or Reagents

Procedure:
Samples were spiked and extracted as listed in section VII. #1 Marine Biotoxin and Non-MPN Based Microbiological Methods SOP – Accuracy/Trueness & Measurement Uncertainty. After the sample was diluted in the 2-step dilution series, the sample was processed on two different ELISA kits with different lot numbers. Samples were processed between 5/19/09 and 5/27/09.

Sample #	Lot 1	Lot 2
1	1.60	1.70
2	13.50	13.20
3	2.20	2.00
4	14.30	14.50
5	1.80	1.90
6	5.80	6.00
7	10.00	9.60
8	19.50	17.90
9	1.10	1.20
10	1.00	1.30

The R^2 between the results for the two batches was 0.995 and the slope was y=0.96



For shellfish samples, repeat for each tissue type of interest.

DATA HANDLING

Ruggedness

In the day to day operations of the laboratory there will be changes in the batches/lots of culture media and/or test reagents used to process samples. Environmental factors are also likely to change over time. None of these factors, however, should adversely impact test results if the method as implemented is sufficiently rugged to be used routinely for regulatory monitoring.

Procedure: To determine whether the method as implemented is sufficiently rugged to withstand the types of changes anticipated to occur in routine use, a two-sided t-test at a significance level (α) of .05 will be used on the data to ascertain if results obtained using different culture media and/or test reagent batches/lots under slightly varying environmental conditions are significantly affected by such minor changes. Either a paired t-test or Welch's t-test will be used depending upon the shape of the distribution produced by the data for each batch/lot and their respective variances. Use log transformed data for the results obtained from microbiological methods. The appropriate t-test to be used for the analysis is determined in the following manner.

1. Test the symmetry of the distribution of results from both batch/lot 1 and batch/lot 2.
2. Calculate the variance of both batch/lot 1 and batch/lot 2 data.
3. Values for the test of symmetry for either batch/lot 1 or batch/lot 2 outside the range of -2 to +2 indicate a significant degree of skewness in the distribution.
4. A ratio of the larger of the variances of either batch/lot 1 or batch/lot 2 to the smaller of the variances of either batch/lot 1 or batch/lot 2 >2 indicates a lack of homogeneity of variance.
5. Use either the paired t-test or Welch's t-test for the analysis based on the following considerations.
    - If the distributions of the data from batch/lot 1 and batch/lot 2 are symmetric (within the range of -2 to +2) and there is homogeneity of variance, use a paired t-test for the analysis.
    - If the distributions of the data from batch/lot 1 and batch/lot 2 are symmetric (within the range of -2 to +2) but there is a lack of homogeneity of variance in the data, use Welch's t-test for the analysis.
    - If the distribution of the data from batch/lot 1 and batch/lot 2 are skewed (outside the range of -2 to +2) and the skewness for both groups is either positive for both or negative for both and there is homogeneity of variance in the data, use the paired t-test for the analysis.
    - If the distributions of the data from batch/lot 1 and batch/lot 2 are skewed and the skewness for both groups is either positive for both or negative for both but the data lacks homogeneity of variance, use Welch's t-test to analyze the data.

Paired T-test results – assumption that the variances are equal

Sample #	Lot 1	Lot 2
1	1.6	1.7
2	13.5	13.2
3	2.2	2
4	14.3	14.5
5	1.8	1.9
6	5.8	6
7	10	9.6
8	19.5	17.9
9	1.1	1.2
10	1	1.3
mean	7.08	6.93
stdev	6.7677	6.3808
t		0.0504
df		18
Significantly different		no

Welch's t-test

The t-value assuming unequal variance was 0.9599.
DF = 18
Two-tailed probability 0.3498, NS

Data Summary:
Value for the test of symmetry of the distribution of batch/lot 1 data _Not determined__
Value for the test of symmetry of the distribution of batch/lot 2 data _Not determined__
Variance of batch/lot 1 data _6.767701_____
Variance of batch/lot 2 data __6.380883_____
Ratio of the larger to the smaller of the variances of batch/lot 1 and batch/lot 2 _1.0606__
Is there a significant difference between batch/lot 1 samples and batch/lot 2 samples ____N__

Neither the paired or Welch's t-test estimates showed a significant difference between batches

VII. #3 Marine Biotoxin and Non-MPN Based Microbiological Methods SOP – Precision & Recovery

VALIDATION CRITERIA
Precision is the closeness of agreement between independent test results obtained under stipulated conditions.
Recovery is the fraction or percentage of an analyte/measurand/organism of interest recovered following sample analysis.
Procedure: This procedure is applicable for use with either growing waters or shellfish tissue. Make every effort to use samples free of the target analyte/measurand/organism of interest. For each shellfish type of interest use a minimum of 10-12 animals per sample. For each sample take four (4) aliquots of either the shellfish homogenate or growing water sample appropriately sized for the work. Spike one of the four aliquots with a low (but determinable by the method under study) concentration of the target analyte/measurand/organism of interest. Spike the second aliquot of the growing water sample or shellfish homogenate with a medium

concentration of the target analyte/measurand/organism of interest. Spike the third aliquot of the growing water sample or shellfish homogenate with a high (but determinable by the method under study) concentration of the target analyte/measurand/organism of interest. Do not spike the fourth aliquot of the growing water sample or shellfish homogenate. This is the sample blank. Spiking levels must cover the range in concentrations important to the application of the method (working range). For microbiological methods determine the concentration of the target organism of interest used to spike each aliquot by plating in/on appropriate agar. Process each aliquot including the sample blank as usual to determine the method concentration for the target analyte/measurand/organism of interest. Do two (2) replicates for each of the three (3) spiked aliquots. Replicate analysis is unnecessary for the sample blank. Do only one sample blank per sample. For growing waters, do ten (10) samples collected from a variety of growing areas. For shellfish, do ten (10) samples for each shellfish tissue type of interest collected from a variety of growing areas, the same growing area harvested on different days or from different process lots. Use the same spiking levels for each of the ten (10) samples analyzed in this exercise (i.e. 10^1, 10^3 and 10^5).

Data:
Working Range _The working range is 0.3 to 3.0 ppb and samples are diluted into the effective range so the working range is 0 to over 100 ppm
Sample Type _Shellfish Tissue__
Agar used to determine spike concentration ___Not applicable__
Organism used for spiking Oyster (*Crassostrea virginica*)

Procedure: Samples were spiked and extracted as listed in section VII. #1 Marine Biotoxin and Non-MPN Based Microbiological Methods SOP – Accuracy/Trueness & Measurand Uncertainty. Each sample was spiked with a low, medium and high concentration of approximately 2.5, 20, and 40ppm in the tissue sample. HPLC was used to determine actual spike concentration.

Sample Spike conc/Plate count/Conc of blank Conc in spiked sample from analysis

	Aliquot 1	Aliquot 2			Aliquot 3			Aliquot 4		
Sample #	Blank	L spike	L_a	L_b	M spike	M_a	M_b	H spike	H_a	H_b
1	0.00	2.60	3.00	2.50	20.14	20.50	19.40	39.93	33.70	38.50
2	0.00	2.71	2.85	2.96	19.10	19.17	19.90	39.28	31.66	33.55
3	0.00	2.26	2.11	2.19	19.64	23.42	22.29	39.84	29.32	30.24
4	0.00	2.50	1.48	1.86	19.21	16.09	16.57	35.50	32.74	30.30
5	0.00	2.62	2.08	1.87	19.11	14.01	15.92	36.56	30.95	30.84
6	0.00	2.45	2.00	2.70	15.89	17.11	13.72	34.97	26.14	27.82
7	0.00	1.99	2.06	2.31	16.42	13.00	12.36	35.32	25.44	27.08
8	0.00	1.70	1.60	1.70	14.77	13.50	13.16	27.30	19.50	19.40
9	0.00	2.14	1.80	1.70	14.60	12.50	12.40	29.48	27.40	27.70
10	0.00	1.80	1.70	1.80	14.84	12.90	12.20	30.49	26.80	30.60

~~1L 1L~~ ~~a~~
~~1L~~ ~~b~~
~~1M 1M~~ ~~a~~
~~1M~~ ~~b~~
~~1H 1H~~ ~~a~~
~~1H~~ ~~b~~
~~1B~~
~~2L 2L~~ ~~a~~
~~2 L~~ ~~b~~
~~2M 2M~~ ~~a~~
~~2M~~ ~~b~~
~~2H 2H~~ ~~a~~
~~2H~~ ~~b~~
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~~“ “~~
~~“ “~~
~~“ “~~
~~10L 10L~~ ~~a~~
~~10L~~ ~~b~~
~~10M 10M~~ ~~a~~
~~10M~~ ~~b~~
~~10H 10H~~ ~~a~~
~~10H~~ ~~b~~
~~10B~~

L, M and H refer to low, medium and high concentrations respectively. L_a, L_b, M_a, M_b, H_a and H_b refer to the replicate determinations of the sample aliquots spiked with low (L), medium (M) and high (H) concentrations of the target analyte/measurand/organism of interest. B refers to the sample blank.

For shellfish samples, repeat for each tissue type of interest.

DATA HANDLING

Precision

To determine the precision of the method as implemented by the laboratory over the range in concentrations important to the intended application of the method, the data is manipulated in the following manner:

1. Convert the plate counts and spiked sample results for the microbiological methods to logs.
2. If necessary, use the sample blank (converted to logs for the microbiological methods) to correct the results from the spiked samples for matrix effects.
3. Perform a nested or hierarchical analysis of variance (ANOVA) on the corrected spiked sample data using the following variance components.

		Low					**Medium**					**High**					
		L spike	La	(La)^2	Lb	(Lb)^2	M spike	Ma	(Ma)^2	Mb	(Mb)^2	H spike	Ha	(Ha)^2	Hb	(Hb)^2	
		2.6	3	9	2.5	6.25	20.14	20.5	420.25	19.4	376.36	39.93	33.7	1135.69	38.5	1482.25	
		2.71	2.85	8.1225	2.96	8.7616	19.1	19.17	367.489	19.9	396.01	39.28	31.66	1002.36	33.55	1125.6	
		2.26	2.11	4.4521	2.19	4.7961	19.64	23.42	548.496	22.29	496.844	39.84	29.32	859.662	30.24	914.458	
		2.5	1.48	2.1904	1.86	3.4596	19.21	16.09	258.888	16.57	274.565	35.5	32.74	1071.91	30.3	918.09	
		2.62	2.08	4.3264	1.87	3.4969	19.11	14.01	196.28	15.92	253.446	36.56	30.95	957.903	30.84	951.106	
		2.45	2	4	2.7	7.29	15.89	17.11	292.752	13.72	188.238	34.97	26.14	683.3	27.82	773.952	
		1.99	2.06	4.2436	2.31	5.3361	16.42	13	169	12.36	152.77	35.32	25.44	647.194	27.08	733.326	
		1.7	1.6	2.56	1.7	2.89	14.77	13.5	182.25	13.16	173.186	27.3	19.5	380.25	19.4	376.36	
		2.14	1.8	3.24	1.7	2.89	14.6	12.5	156.25	12.4	153.76	29.48	27.4	750.76	27.7	767.29	
		1.8	1.7	2.89	1.8	3.24	14.84	12.9	166.41	12.2	148.84	30.49	26.8	718.24	30.6	936.36	
Subgroup sample number	n(I, j, l)		10		10			10		10			10		10		
Subgroup sum	Sum (i, j, l)		20.68		21.59			162.2		157.92			283.65		296.03		Sum
Subgroup variance	[(Sum (i, j, l))^2]/n(I, j, l)		42.77		46.61			2630.88		2493.87			8045.73		8763.38		22023.24
Group sample number	n(i)		20					20					20				60
Group sum	Group sum		42.27					320.12					579.68				942.07

Group mean	Xhat (i)	2.17	16.46	30.95	
Group variance	[(Xhat (i))^2]/n(i)	89.3376	5123.84	16801.4	22014.62

C	14791.59808
Total SS	7859.977618
Among all subgroups SS	7231.65
error SS	628.33
Groups SS	7223.025403
Subgroups SS	8.62
Total DF	59
Groups DF	2
Among all subgroups DF	5
Subgroups DF	3
Error DF	54

Source of Variation	SS	DF	MS
Total	7859.98	59	
Among all subgroups	7231.65	5	
Groups	7223.03	2	3611.52
Subgroups	8.62	3	2.87
Error	628.33	54	11.64

Ho: There is no significant difference among the replicates (a,b) in affecting domoic acid concentration.
HA: There is a significant difference among replicates (a,b) in affecting domoic acid concentration.

$F = 2.87/11.64 = 0.25$ $F_{0.05(1),3,54} = 2.79$ $F < F_{0.05(1),3,54}$ Do not reject Ho.

The replicates are NOT significantly different

Ho: There is no difference in Domoic Acid concentration among the three concentrations (L, M, H).
HA: The three concentrations (L, M, H) are significantly different.

$F = 3611.52/2.87 = 1258.37$ $F_{0.05(1),2,3} = 9.55$ $F > F_{0.05(1),2,3}$ Reject H0

The concentrations are significantly different.

Source of variation Degrees of freedom Sum of Squares Mean Square
Samples 9
Concentrations in samples 20
Determinations within concentrations 30
Total 59

4. Calculate the variance ratio (F) at the 95% confidence interval for the variance components, concentrations in samples/determinations within concentrations. If the variance ratio is significant this indicates that the precision of the method as implemented by the laboratory is not consistent over the range in concentrations important to the intended application.

Per the ISSC instructions, I used F = Concentrations in samples/determinations within concentrations = 2.87/3611.52 = 0.00079

$F_{0.05(1),2,3} = 9.55$ F <<< $F_{0.05(1),2,3}$ Accept H0.

So, there is no significant difference in precision among each of the three concentrations (L,M,H)

If the variance ratio is not significant, calculate the coefficient of variation of the spiked sample data by:

1. Calculating the average concentration of the analyte/measurand/organism of interest in the spiked samples. For microbiological methods log transformed data is used for this calculation.

   Avg. concentration of Domoic acid in the spiked samples

   Low 2.17
   Med 16.46
   High 34.867

2. Calculate the standard deviation of the spiked sample data by taking the square root of the nested ANOVA variance component, **Total**.

   Standard deviation of spiked sample data

	SD
Low	0.43
Med	3.25
High	5.23

3. Divide the standard deviation of the spiked sample data by the average concentration of the analyte/measurand/organism of interest calculated for the spiked samples. For microbiological methods log transformed data is used for this calculation; and

   Low 0.20
   Med 0.20
   High 0.17

4. Multiply the quotient above by 100. This is the coefficient of variation of the method over the range of concentrations of importance in the application of the method as implemented by the laboratory.

   Low 20
   Med 20
   High 17

Recovery

The recovery of the target analyte/measurand/organisms of interest must be consistently good over the range of concentrations of importance to the application of the method under study to be of benefit in the intended work. To determine whether recovery by the method as implemented by the laboratory is consistent over the range in concentrations important to the application of the method, the data is manipulated in the following manner:

1. Convert plate count and spiked sample results for the microbiological methods to logs.
2. If necessary, use the sample blank (converted to logs for microbiological methods) to correct the results from the spiked samples for matrix effects.
3. For each sample determine the average of the replicates at each concentration such that there is only one value, the average of the two replicates at each concentration tested.
4. For each sample subtract the average for the replicates from its associated spike concentration/plate count value.

Sample	Spike	Average ELISA	Spike-ELISA
8L	1.7	1.65	0.05
10L	1.8	1.75	0.05
7L	1.99	2.18	-0.19
9L	2.14	1.75	0.39
3L	2.26	2.15	0.11
6L	2.45	2.35	0.1
4L	2.5	1.67	0.83
1L	2.6	2.75	-0.15
5L	2.62	1.97	0.65
2L	2.71	2.91	-0.2
9M	14.6	12.45	2.15
8M	14.77	13.33	1.44
10M	14.84	12.55	2.29
6M	15.89	15.41	0.47
7M	16.42	12.68	3.74
2M	19.1	19.53	-0.43
5M	19.11	14.96	4.15
4M	19.21	16.33	2.88
3M	19.64	22.86	-3.22
1M	20.14	19.95	0.19
8H	27.3	19.45	7.85
9H	29.48	27.55	1.93
10H	30.49	28.7	1.79
6H	34.97	26.98	7.99
7H	35.32	26.26	9.05
4H	35.5	31.52	3.98
5H	36.56	30.9	5.67
2H	39.28	32.61	6.68

5. Perform a one way analysis of variance (ANOVA) on the data formatted by sample concentration with the following variance components:

Source of variation Degrees of freedom Sum of Squares Mean Square
Concentration 2
Error 27
Total 29

Source of Variation	Sum of Squares	d.f.	Mean Squares	F
Between	181.9	2	90.93	20.22
Error	121.4	27	4.496	
Total	303.2	29		

Group A (low): Number of items= 10
Mean = 0.16400
95% confidence interval for Mean: -1.212 thru 1.540
Standard Deviation = 0.353
High = 0.8300 Low = -0.2000
Median = 7.5000E-02
Average Absolute Deviation from Median = 0.252

Group B (medium): Number of items= 10
Mean = 1.3660
95% confidence interval for Mean: -9.8640E-03 thru 2.742
Standard Deviation = 2.20
High = 4.150 Low = -3.220
Median = 1.795
Average Absolute Deviation from Median = 1.68

Group C (high): Number of items= 10
Mean = 5.8830
95% confidence interval for Mean: 4.507 thru 7.259
Standard Deviation = 2.92
High = 10.06 Low = 1.790
Median = 6.175
Average Absolute Deviation from Median = 2.44

The probability of this result, assuming the null hypothesis, is less than 0.0001. The highest spikes had greater variability. Those in regulatory range (Low and Medium) were less variable.

6. Calculate the variance ratio (F) at the 95% confidence interval for the mean square for concentration divided by the mean square for error. If the variance ratio or F test is significant at the 95% confidence interval, perform Tukey's Honestly Significant Difference (HSD) to compare recovery by concentration. A significant F test suggests that recovery of the method as implemented by the laboratory is not consistent over the range in concentrations important to the application of the method and may not be suitable for the work intended.

   F= 90.93/4.496 = 20.22
   Numerator degrees of freedom = 2
   Denominator degrees of freedom = 27
   Probability Value: 0.000004

   This confirms greater variability in recovery at the higher spike concentrations

   If the variance ratio or F test is not significant at the 95% confidence interval, conclude that the recovery is consistent over the range in concentrations important to the application of the method and calculate the overall percent recovery of the method as implemented by the laboratory.

   To determine the percent recovery of the method as implemented by the laboratory, the data is manipulated in the following manner:

   1. Use log transformed data for microbiological methods.
   2. If necessary use the sample blank (converted to logs for microbiological methods) to correct the results from the spiked samples for matrix effects.
   3. Calculate the average spike concentration/plate count by summing over concentrations and dividing by 30.
      18.17
   4. Calculate the average concentration of analyte/measurand/organism of interest in the spiked samples from the analysis by summing over concentrations and replicates and dividing by 60.
      15.7
   5. Divide the average concentration of analyte/measurand/organism of interest from the analysis of the spiked samples by the average concentration from the spike/plate counts then multiply by 100. This is the percent recovery of the method as implemented by the laboratory.
      86.4%

Data Summary:

- Is the variance ratio at the 95% confidence interval for the variance components, concentrations in samples/determinations within concentrations significant? **Y**
- If the variability of the method as implemented by the laboratory is consistent over the range in concentrations important to its intended applications, what is the coefficient of variation? **NA/_____%**
- Is the one way analysis of variance to determine the consistency of recovery of the method under study significant? **Y**
- At what concentrations is the one way analysis of variance significant? **NA/___?_____**
- What is the overall percent recovery of the MPN based method under study? **NA/__86.4___%**

VII. #4 Marine Biotoxin and Non-MPN Based Microbiological Methods SOP – Specificity

VALIDATION CRITERIA

Specificity is the ability of the method to measure only what it is intended to measure. To determine method specificity samples containing suspected interferences (interfering organisms/compounds/toxins) are analyzed in the presence of the analyte/measurand/targeted organism of interest.

Procedure: This procedure is applicable for use with either growing waters or shellfish tissue. Make every effort to use samples free of the targeted analyte/measurand/organism of interest. For each shellfish tissue type of interest use a minimum of 10-12 animals per sample. For each sample take three (3) aliquots of either the shellfish homogenate or growing water sample appropriately sized for the work and spike two (2) of the three (3) with a low but determinate level (by the method under study) of the targeted analyte/measurand/ organism of interest. Take one of these two (2) aliquots and also spike it with a moderate to high level of a suspected interfering organism/compound/toxin if not naturally incurred. Do not spike the third aliquot. This is the sample blank. Process each aliquot, the sample blank, the aliquot spiked with the targeted analyte/measurand/organism of interest and the aliquot spiked with the targeted analyte/measurand/organism of interest in the presence of the suspected interfering organism/compound/toxin as usual to determine the method concentration for the targeted analyte/measurand/organism of interest. Do five (5) replicates for each aliquot excluding the sample blank. Do one sample blank per analysis. Repeat this process for all suspected interfering organisms/compounds/toxins.

Data:

Glutamine and Glutamic are structurally related to domoic acid and present in shellfish tissues. Hence they represent potentially important competitors. These compounds were therefore tested to determine if high concentrations would interfere with the DA ELISA.

Name of suspected interfering organism/compound/toxin #1 _____ Glutamine _____
Sample type ____Shellfish Tissue _____
Sample blank concentration for the targeted analyte/measurand/organism of interest __0.0__
Concentration of aliquot spiked with targeted analyte/measurand/ with targeted analyte/measured: see below
Organism of interest organism: oyster

Procedure:

1. 2000 ppm solutions of Glutamine and Glutamic acid were made by mixing 26.7mg Glutamine in 13.35mL dH_2O and 26.8 mg Glutamic Acid in 13.4 mL dH2O
2. 2 g thawed oyster sample weighed into 50 mL tube
3. 17mL 50% MeOH added to tube
4. 3.34 µL 90% 1670ppm DA added to make 2.5ppm DA spike
5. Sample vortexed
6. Sample split into two 15mL tubes
7. 500 µL 50% MeOH added to DA-only tube
8. For tube spiked with interfering compound, 250mL 50% MeOH added + 250 µL 2000ppm Gulatime/Glutamic Acid for an ~55ppm spike in shellfish tissue
9. Samples then processed by ELISA and HPLC as described previously.

Replicate	Conc. of spike	Conc. of Spike Glutamine
1	1.70	1.70
2	1.60	1.70
3	1.70	1.60
4	1.90	2.10
5	1.70	2.20
Avg	1.72	1.86
mean	1.7	1.9
Standard deviation	0.1	0.2
SIavg	0.925	

Name of suspected interfering organism/compound/toxin #2 _____ Glutamic Acid _____
Sample type ____Shellfish Tissue _____
Sample blank concentration for the targeted analyte/measurand/organisn of interest __0.0__
Concentration of aliquot spiked with targeted analyte/measurand/ with targeted analyte/measured: see below
Organism of interest organism: oyster

Replicate	Conc of spike	Conc of Spike Glutamic Acid
1	1.90	1.80
2	1.60	1.80
3	1.50	1.40
4	1.30	1.50
5	1.90	1.50
Avg	1.64	1.60
Standard deviation	0.2	0.2
SIavg	1.025	

Repeat for each suspected interfering organism tested.

DATA HANDLING

The **Specificity index** will be used to test the specificity of the method in the presence of suspected interfering organisms/compounds/toxins. The **Specificity index (SI)** is calculated as indicated below:

Specificity index (SI) = Sample spiked with target of interest only
Sample spiked with both target and suspected interferences

All microbiological count data must be converted to logs before analysis. Samples spiked with both the targeted analyte/measurand/organism of interest and the targeted anaalyte/measurand/organism of interest in the presence of a suspected interfering organism/compound/toxin may have to be corrected for matrix effects before determining the Specificity index (SI). The sample blank accompanying the analysis is used for this purpose. Any corrections that may be necessary to microbiological data for matrix effects are done using log transformed data.

The Specificity index should equal one (1) in the absence of interferences. To test the significance of a Specificity index other than one (1) for any suspected interfering organism/compound/toxin, a two-sided t-test is used. For each suspected interfering organism/compound/toxin calculate the average **Specificity Index (SI)** for the 5 replicatesanalyzed for each sample by obtaining the average concentration for both the aliquot containing the targeted analyte/measurand/organism of interest only and the aliquot containing the targeted analyte/measurand/organism of interest in the presence of suspected interfering organisms/compounds/toxins and using the formula below.

SI_{avg} = Avg concentration of sample spiked with target of interest only
Avg concentration of sample spiked with both target and suspected interferences

Perform a two-sided t-test at the .05 significance level to determine if the average Specificity index (SI) obtained from the 5 replicates of each analysis differs from one (1).

Repeat for all interfering organisms/compounds/toxins tested.

Data Summary:

Interfering organism/compound/toxin #1 _____Glutamine_____ SI_{avg} 0.925_____

Significant difference from 1 _____

Interfering organism/compound/toxin #2 ____Glutamic Acid_____ SI_{avg} 1.025____

Significant difference from 1 _____

Glutamine Two tailed T-test 95% confidence level

T=2.0
DF=8
Confidence Level 91.95%
Not Significant

Glutamic Acid

T=0.3162
DF=8
Confidence Level 24.01%
Not Significant

VII. #5 Marine Biotoxin and Non-MPN Based Microbiological Methods SOP – **Linear Range, Limit of Detection, Limit of Quantitation/Sensitivity**

VALIDATION CRITERIA

Linear Range is the range within the working range where the results are proportional to the concentration of the analyte/measurand/organism of interest present in the sample.

Limit of Detection is the minimum concentration at which the analyte/measurand/organism of interest can be identified.

Limit of Quantitation/Sensitivity is the minimum concentration of the analyte/measurand/organism of interest that can be quantified with an acceptable level of precision and accuracy under the conditions of the test.

Procedure: This procedure is applicable for use with either growing waters or shellfish tissue. Make every effort to use samples free of the target analyte/measurand/organism of interest. For each shellfish type of interest use a minimum of 10-12 animals per sample. For each sample take at least six (6) aliquots of either the growing water sample or shellfish homogenate appropriately sized for your work and spike five (5) of the six (6) aliquots with five (5) different concentrations (i.e. 10^a, 10^b …10^n) of the target analyte/measurand/organism of interest spanning 50 – 150% of the working range/range of interest for the method under study. Do not spike the sixth or last aliquot of each sample. This is the sample blank. For microbiological methods determine the concentration of the target analyte/measurand/organism of interest used to spike each aliquot of each sample by plating in/on appropriate agar. Do not use aliquots of the same master solution/culture to spike all the samples in this exercise. A separate master solution /culture should be used for each sample. Process each aliquot including the sample blank as usual to determine method concentration for the target analyte/measurand/organism of interest. Do three (3) replicates for each aliquot excluding the sample blank. Do only one blank per sample. For growing waters do ten (10) samples collected from a variety of growing areas. For shellfish do ten (10) samples for each shellfish tissue type of interest collected from a variety of growing areas, the same growing area harvested on different days or from different process lots. Use the same spiking levels for each of the ten (10) samples analyzed (10^a, 10^b …10^n).

This is a section where I could use guidance by the committee. The assay has a wide dynamic range because samples are diluted into the 0.3 to 3 ppb linear range of the assay. It is this aspect of the assay which makes it difficult to implement the instructions provided above. The actual linear range was determined as by diluting the standards to various levels and testing the assay multiple times. This was a necessary step in developing the critical parameters needed by the data analysis software provided with the kit to back calculate DA values from the B and Bo values (see article published in the December 2008 issue of the Journal of Shellfish Research for details). I need to know if the data presented in the published article are sufficient to meet the committee's requirements for determining the linear range and limits of detection. If not, please recommend what procedure should be followed considering that the samples must be diluted. This is similarly true for determining the dynamic range of the assay.

Data: Testing in progress

Sample type _____
Working range/Range of interest _____
Range in spiking levels used _____
Agar used to determine spike concentration _____
Organism used for spiking _____
Aliquot 0* 1 2 3 4 5
Sample 1
Spike conc./plate count
Response, replicate 1
Response, replicate 2
Response, replicate 3
Aliquot 0* 1 2 3 4 5
Sample 2
Spike conc./plate count
Response, replicate 1
Response, replicate 2

Response, replicate 3
Sample 3
Spike conc./plate count
Response, replicate 1
Response, replicate 2
Response, replicate 3
Sample 4
Spike conc./plate count
Response, replicate 1
Response, replicate 2
Response, replicate 3
Sample 5
Spike conc./plate count
Response, replicate 1
Response, replicate 2
Response, replicate 3
Sample 6
Spike conc./plate count
Response, replicate 1
Response, replicate 2
Response, replicate 3
Sample 7
Spike conc./plate count
Response, replicate 1
Response, replicate 2
Response, replicate 3
Sample 8
Spike conc./plate count
Response, replicate 1
Response, replicate 2
Response, replicate 3
Aliquot 0* 1 2 3 4 5
Sample 9
Spike conc./plate count
Response, replicate 1
Response, replicate 2
Response, replicate 3
Sample 10
Spike conc./plate count
Response, replicate 1
Response, replicate 2
Response, replicate 3
*** Unspiked sample blank**

Response is the signal data (absorbance, florescence, Ct value), colonies, plaques, etc resulting from the analysis.
For shellfish samples repeat for each tissue type of interest**.**

DATA HANDLING

Linear Range

To determine the range within the working range where the results are proportional to the concentration of the target analyte/measurand/organism of interest present, the data is manipulated in the following manner.

1. Convert the plate counts and spiked sample results for the microbiological methods to logs.
2. If necessary, use the sample blank (converted to logs for microbiological methods) to correct the results from the spiked samples for matrix effects.
3. Divide the response obtained for each replicate tested by the concentration of the spiked analyte/measurand/organism of interest which gave rise to it. Use log values for the microbiological data.
4. Plot the data obtained above on the y-axis against the log of the concentration of the spiked analyte/measurand/organism of interest which gave rise to the respective data point on the x-axis. Connect the points. This is the relative response line.
5. Calculate the mean of the values obtained (in step 3) when the response for each replicate tested is divided by the concentration of the spiked analyte/measurand/organism of interest which gave rise to it.
6. Plot this value on the y-axis of the graph obtained in step 4 at each log concentrations of the analyte/measurand/organism of interest spiked into the samples. Connect the points to form a horizontal line. This constitutes the line of constant response
7. Multiply the value obtained in step 5 by 0.95 and 1.05.
8. Plot these values on the y-axis of the graph obtained in steps 4 and 6 at each log concentration of the analyte/measurand /organism of interest spiked into the samples. Connect the points to form two horizontal lines which bracket the line of constant response.
9. The method is linear up to the point where the relative response line (obtained in step 4) intersects either of the lines obtained above.
10. The linear range of the method as implemented by the laboratory is comprised of the range in concentrations obtained by taking the antilogs of the concentrations of the spiked analyte/measurand/organism of interest bracketed within the horizontal lines of the plot obtained in step 8 above.

Limit of Detection and Limit of Quantitation/Sensitivity

To determine the minimum concentration at which the analyte/measurand/organism of interest can be identified and subsequently quantified with an acceptable level of precision and accuracy under the conditions of the test, the data is manipulated in the following manner.

1. Calculate the coefficient of variation or relative standard deviation for each concentration of analyte/measurand/organisn of interest spiked into the samples. Use the log transformed data for manipulating microbiological results.
2. Plot the coefficient of variation/relative standard deviation on the y-axis for each concentration of analyte/measurand/organism of interest spiked into the samples and plotted on the x-axis. Use log transformed concentration values for the microbiological data.
3. Fit the curve and determine from the graph the concentration of analyte/measurand/organism of interest which gave rise to a coefficient of variation/relative standard deviation of 10%. This is the limit of quantitation/sensitivity of the method as implemented by the laboratory.
4. Divide the value for the limit of quantitation/sensitivity obtained from step 3 above by 3.3 or determine the concentration of analyte/measurand/organism of interest that gave rise to a coefficient of variation/relative standard deviation of 33%. This value is the limit of detection of the method as implemented by the laboratory.

For single laboratory validation, the concepts of "blank + 3σ" and "blank + 10σ" generally suffice for determining the limit of detection and the limit of quantitation/sensitivity. Since the blank is in theory zero (0), then the limit of detection and the limit of quantitation /sensitivity become 3σ and 10σ respectively. An absolute standard deviation of 3 and 10 equates to a coefficient of variation/relative standard deviation of 33% and 10% respectively. Accordingly the limit of detection and the limit of quantitation/sensitivity become the concentration of analyte/measurand/organism of interest which give rise to these values.

Data Summary:

Linear range of the method as implemented _____

The limit of detection of the method as implemented _____

The limit of quantitation/sensitivity of the method as implemented _____

IX. SLV Documents for New or Modified Methods as Alternatives to NSSP Methods http://www.issc.org/client_resources/lmr%20documents/ix%20%20_1%20new%20or%20modified%20methods%20as%20alternatives.pdf

IX. #1 SOP for the Single Laboratory Validation of New or Modified Analytical Methods Intended as Alternatives to Officially Recognized NSSP Methods – Comparing Methods

VALIDATION CRITERIA

Comparability is the acceptability of a new or modified analytical method as a substitute for an established method in the NSSP. To be acceptable the new or modified method must not produce a significant difference in results when compared to the officially recognized method. Comparability must be demonstrated for each substrate or tissue type of interest by season and geographic area if applicable.

Comparison of Methods:

New or modified methods demonstrating comparability to officially recognized methods must not produce significantly different results when compared

Procedure to compare the new or modified method to the officially recognized method: This procedure is applicable for use with either growing waters or shellfish tissue. For each shellfish type of interest use a minimum of 10-12 animals per sample. For each sample take two (2) aliquots and analyze one by the officially recognized method and the other by the alternative method. Actual samples are preferable; but, in cases where the occurrence of the analyte/measurand/organism of interest is intermittent (such as marine biotoxins), spiked samples can be used. Samples having a variety of concentrations which span the range of the method's intended application should be used in the comparison. Analyze a minimum of thirty (30) paired samples for each season from a variety of growing areas for a total of at least 120 samples over the period of a year for naturally incurred samples. For spiked samples analyze a minimum of ten (10) samples for each season from a variety of growing areas for a total of at least 40 samples over the period of a year.

Data:

Sample type ____Shellfish tissue- oyster_____

Date Sample/Station # Conc. Recognized method Conc. Alternative Method

Data still being gathered to answer this question.

~~1~~
~~2~~
~~3~~
~~4~~
~~5~~
~~6~~
~~7~~
~~8~~
~~9~~
~~10~~
~~n~~
~~n is the last sample in the comparison~~

For shellfish samples, repeat for each tissue type of interest

Data handling to compare the new or modified method to the officially recognized

Two methods of analysis are considered to be comparable when no significant difference can be demonstrated in their results. To determine whether comparability in methods exists, a two-sided t-test at a significance level (α) of .05 will be used to test the data. Either a paired t-test or Welch's t-test will be used depending upon the shape of the distributions produced by the data for each method and their respective variances. Use log transformed data for the results obtained from microbiological methods. The appropriate t-test to be used for the analysis is determined in the following manner.

1. Test the symmetry for the distribution of results from both the officially recognized analytical method and the proposed alternative analytical method.
2. Calculate the variance of the data for both the officially recognized analytical method and the proposed alternative analytical method.
3. Values for the test of symmetry for either method outside the range of -2 to +2 indicate a significant degree of skewness in the distribution.
4. A ratio of the larger of the variances of either method to the smaller of the variances of either method >2 indicates a lack of homogeneity of variance.
5. Use either the paired t-test or Welch's t-test for the analysis of the data based on the following considerations.
    - If the distribution of the data from the officially recognized analytical method and the proposed alternative analytical method are symmetric (within the range of -2 to +2) and there is homogeneity of variance use a paired t-test for the data analysis.
    - If the distributions of the data for both analytical methods are symmetric (within the range -2 to +2) but there is a lack of homogeneity of variance in the data, use Welch's t-test for the analysis of the data.
    - If the distributions of the data from the officially recognized and proposed alternative analytical methods are skewed (outside the range -2 to +2) and the skewness for both methods is either positive for both or negative for both and there is homogeneity of variance in the data, use the paired t-test for the analysis of the data.
    - If the distributions of the data from the officially recognized and the proposed alternative analytical methods are skewed and the skewness for both analytical methods is either positive or negative for both but the data lacks homogeneity of variance, use Welch's t-test to analyze the data.

Data summary for the comparison of the new or modified method to the officially recognized method:
Value for the test of symmetry for the distribution of the data generated by the officially recognized method _____
Value for the test of symmetry for the distribution of the data generated by the proposed alternative method _____
Variance of the data generated from the officially recognized analytical method _____
Variance of the data generated from the proposed alternative analytical method _____
Ratio of the larger to the smaller of the variances generated by the officially recognized and proposed analytical methods _____
Is there a significant difference between the analytical methods Y/N

Proposal Subject: Saxitoxin (PSP) ELISA Kit

Specific NSSP Guide Reference: Section IV. Guidance Documents, Chapter II Growing Areas, .10 Approved National Shellfish Sanitation Program Laboratory Tests: Microbiological and Biotoxin Analytical Methods

Section II. Model Ordinance Chapter III. Laboratory @.02 Methods C. Biotoxin

Text of Proposal/ Requested Action See attached ISSC Method Application

Faster, easier, and/or more reliable methods are needed to satisfy the needs of the regulatory community and shellfish industry. The proposed ELISA method is a fast and easy to perform method with ready to use reagents i.e. analyst only needs to extract shellfish sample or dilute water sample before analysis. The proposed ELISA also provides a quantitative and/or semi-quantitative screening for shellfish extracts and/or water samples. This assay is part of Abraxis platform for marine toxin testing and complements the company's other offering for NSP, DSP, and ASP testing. The proposed ELISA can be used on-site (boat, dock) or established analytical laboratories.

Public Health Significance:

Cost Information (if available): As low as $15 per sample.

Action by 2009 Laboratory Methods Review Committee Recommended no action on Proposal 09-107. Rationale: Insufficient data.

Action by 2009 Task Force I Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 09-107.

Action by USFDA 02/16/2010 Concurred with Conference action on Proposal 09-107 with the following comments and recommendations for ISSC consideration.

The Laboratory Methods Review Committee determined that Proposal 09-107 was accompanied by insufficient data necessary for the Committee to make a determination regarding the efficacy of the proposed saxitoxin test method for use under the NSSP. As a result the General Assembly voted "No Action" on the proposed analytical method. It has been FDA's observation and experience that the proposed ELISA method for saxitoxins presents itself as a reliable screening method to supplement existing NSSP tools for managing Paralytic Shellfish Poisoning (PSP). Therefore, FDA Recommended the Conference pursue submission of additional data from Abraxis, LLC via the Proposal submission process to advance a thorough examination of this method for saxitoxin screening.

Action by ISSC Executive Board March 2010 The Executive Office will send a letter to the submitter of Proposal 09-107 to resubmit Proposal 09-107 Saxitoxin (PSP) Elisa Kit with additional information.

Action by 2011 Laboratory Methods Review Committee	Recommended approval of Proposal 09-107 as an emerging method. NOTE: This approval is limited to the Abraxis Shipboard ELISA Method used in conjunction with the Extraction Method approved in Proposal 05-111.
Action by 2011 Task Force I	Recommended adoption of the Laboratory Methods Review Committee recommendation on Proposal 09-107 as an emerging method.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 09-107.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 09-107.

ISSC Method Application and Single Lab Validation Checklist For Acceptance of a Method for Use in the NSSP

The purpose of single laboratory validation in the National Shellfish Sanitation Program (NSSP) is to ensure that the analytical method under consideration for adoption by the NSSP is fit for its intended use in the Program. A Checklist has been developed which explores and articulates the need for the method in the NSSP; provides an itemized list of method documentation requirements; and, sets forth the performance characteristics to be tested as part of the overall process of single laboratory validation. For ease in application, the performance characteristics listed under validation criteria on the Checklist have been defined and accompany the Checklist as part of the process of single laboratory validation. Further a generic protocol has been developed that provides the basic framework for integrating the requirements for the single laboratory validation of all analytical methods intended for adoption by the NSSP. Methods submitted to the Interstate Shellfish Sanitation Conference (ISSC) Laboratory Methods Review (LMR) Committee for acceptance will require, at a minimum, six (6) months for review from the date of submission.

Name of the New Method	Saxitoxin (PSP) ELISA Kit	
Name of the Method Developer	Abraxis LLC	
Developer Contact Information	Fernando Rubio 54 Steamwhistle Drive Warminster, PA 18974 Phone: (215) 357-3911 FAX: (215) 357-5232	
Checklist	**Y/N**	**Submitter Comments**
A. Need for the New Method		
1. Clearly define the need for which the method has been developed.		Shellfish are filter feeders that pump large quantities of water through their bodies when actively feeding. During this process, shellfish can concentrate toxigenic micro-algae and other substances from the water column when they are present. The ability of shellfish to concentrate chemical pollutants from water can lead to accumulation of these toxins to levels that constitute a public health hazard. Dinoflagellates producing Saxitoxin have caused mortality events in fish, and sea mammals. In humans, Saxitoxin (PSP) poisoning causes neurological symptoms that can lead to respiratory paralysis and even death. Some of the currently available methods used for the detection and monitoring of saxitoxin in water and shellfish are not conducive for the quick on-site or real time, dockside or ship board monitoring of this toxin. For example: 1) the mouse bioassay is labor intensive, requires the use and destruction of many vertebrate animals, analyses is only performed in a few laboratories with a low turn around time, 2) a lateral flow ELISA developed by Jellet Rapid Testing Ltd., however, this assay seems to produce a high degree of false positives. Therefore, faster, easier and/or more reliable methods are needed to satisfy the needs of the regulatory community and shellfish industry. The proposed ELISA method is a fast and easy to perform method with ready to use reagents i.e. analyst only needs to extract shellfish sample or dilute water sample before analysis. The proposed ELISA also provides a quantitative and/or semi-quantitative screening for shellfish extracts and/or water samples.

		This assay is part of Abraxis platform for marine toxin testing and complements the company's other offering for NSP, DSP, and ASP testing.
2. What is the intended purpose of the method?		The fast analysis of Saxitoxin (PSP) in shellfish extracts and/or water quality monitoring. The proposed ELISA can be used on-site (boat, dock) or established analytical laboratories.
3. Is there an acknowledged need for this method in the NSSP?		Yes. NSSP Guidance Documents, Chapter II Constitution by-laws and procedures of the Interstate Shellfish Sanitation Conference. Procedure XVI. Procedure for acceptance and approval of analytical methods for the NSSP. And: National Shellfish Sanitation Program 2003 Model Ordinance III. Laboratory @ 02 Methods C. Biotoxin. Methods for the analysis of shellfish and shellfish harvest waters shall be: 1) The current AOAC and APHA methods used in bioassay for paralytic shellfish poisoning toxins
4. What type of method? i.e. chemical, molecular, culture, etc.		Immunochemical Method.
B. Method Documentation		
1. Method documentation includes the following information:		
Method Title		Abraxis ELISA Kit for the Screening of Saxitoxin in Shellfish Extract and/or Harvest Waters.
Method Scope		A Method for the screening out negative saxitoxin samples in shellfish regulatory labs, to determine if shellfish are safe to harvest and or distribute. A method for water classification for saxitoxin around harvest areas and to screen for toxic phytoplankton in seawater to provide early warning. A method that provides multiple simultaneous results (depending on chosen cut-off values). This can be easily done because the assay is run with multiple STX concentrations.
References		Etheridge, S., Deeds, J, Easy, D., Laycok, M., Caulfield, C., Deardorff, D., Church, J., PSP & TTX Kits: Regulatory Perspectives. Satellite Workshop to the Gordon Conference on Mycotoxins and Phycotoxins 2007, Maine, USA, E. Hignutt, S.W. Longan, Environmental Health Laboratory, State of Alaska, Anchorage, AK; Comparison of HILIC/Tandem Mass Spectrometry, Abraxis ELISA and Mouse Bioassay for Determination of PSP in Shellfish. To be presented at the 2008 AOAC Annual Meeting, Dallas, Texas.
Principle		**The test is a direct competitive ELISA based on the recognition of Saxitoxin by specific antibodies. Saxitoxin, when present in a sample and a saxitoxin-enzyme-conjugate compete for the binding sites of rabbit anti-saxitoxin antibodies in solution. The**

		saxitoxin antibodies are then bound by a second antibody (sheep anti-rabbit) immobilized on the plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of the Saxitoxin present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.
Any Proprietary Aspects		Immunoreagents and sample diluent.
Equipment Required		Pipettes and plate reader. Blender for shellfish extraction.
Reagents Required		Reagents provided in the ELISA kit. In addition diluted hydrochloric acid or vinegar and rubbing alcohol (depending on extraction procedure chosen by analyst).
Sample Collection, Preservation and Storage Requirements		Water samples need to be collected in glass vials and preserved according to users guide (attached). Diluted shellfish extracts should be stored in glass vials. All dilution should be done using provided sample diluent. If not analyzed promptly, samples should be stored refrigerated for up 2 days or frozen if longer periods are required.
Safety Requirements		As with any laboratory procedure, gloves and goggles should be used during the processing and analysis of samples.
Clear and Easy to Follow Step-by-Step Procedure		User's guide and an easy to follow flow chart are provided with each kit (attached).
Quality Control Steps Specific for this Method		As with any analytical procedure laboratory controls (positive and negative) are recommended.
C. Validation Criteria		
1. Accuracy / Trueness		Provided as an attachment.
2. Measurement Uncertainty		@ 0.046 ng/mL in water SD 0.004 CV 8.7% @ 0.087 ng/mL in water SD 0.004 CV 4.6% @ 0.227 ng/mL in water SD 0.008 CV 3.5%
3. Precision Characteristics (repeatability and reproducibility)		< 15%
4. Recovery		Average water recovery 112%; shellfish extract 96%.
5. Specificity		Saxitoxin (STX) 100% (per definition) Decarbamoyl STX 29% GTX 2 & 3 23% GTX-5B 23% Sulfo GTX 1 & 2 2.0% Decarbamoyl GTX 2 & 3 1.4% Neosaxitoxin 1.3% Decarbamoyl Neo STX 0.6% GTX 1 & 4 <0.2%
6. Working and Linear Ranges		0.02-0.4 ng/mL water or 20-400 ng/gm in shellfish extract or higher depending on dilution.
7. Limit of Detection		0.015 ng/mL
8. Limit of Quantitation / Sensitivity		0.02 ng/mL in water; 20 ng/gm in shellfish extract
9. Ruggedness		Since and analytical curve is run with each assay and the samples are compared to the standard curve, the proposed ELISA is rugged.
10. Matrix Effects		If used according to instructions (dilutions), none detected

11. Comparability (if intended as a substitute for an established method accepted by the NSSP)		Method is intended as a screening method to complement other accepted NSPP methods: i.e. mouse bioassay. Some comparison data is provided as an attachment.
D. Other Information		
1. Cost of the Method		As low as $15 per sample
2. Special Technical Skills Required to Perform the Method		Some technical skills are required. Familiarity with laboratory setting is adequate. Kit Manufacturer's on-site training is available.
3. Special Equipment Required and Associated Cost		As low as $1,800. Strip reader and pipette
4. Abbreviations and Acronyms Defined		ELISA: Enzyme linked immuno sorbent assay PSP: paralytic shellfish poisoning
5. Details of Turn Around Times (time involved to complete the method)		40 samples can be run in duplicate in approximately 2 hours. Shellfish sample extraction requires approximately 15 minutes
6. Provide Brief Overview of the Quality Systems Used in the Lab		The ELISA kits are manufactured following GMP and GLP procedures.
Submitters Signature	Date:	
Submission of Validation Data and Draft Method to Committee	Date:	
Reviewing Members	Date:	
Accepted	Date:	
Recommendations for Further Work	Date:	
Comments:		

DEFINITIONS

1. Accuracy/Trueness - Closeness of agreement between a test result and the accepted reference value.
2. Analyte/measurand - The specific organism or chemical substance sought or determined in a sample.
3. Blank - Sample material containing no detectable level of the analyte or measurand of interest that is subjected to the analytical process and monitors contamination during analysis.
4. Comparability - The acceptability of a new or modified method as a substitute for an established method in the NSSP. Comparability must be demonstrated for each substrate or tissue type by season and geographic area if applicable.
5. Fit for purpose - The analytical method is appropriate to the purpose for which the results are likely to be used.
6. HORRAT value - HORRAT values give a measure of the acceptability of the precision characteristics of a method.[4]
7. Limit of Detection - the minimum concentration at which the analyte or measurand can be identified. Limit of detection is matrix and analyte/measurand dependent.[4]
8. Limit of Quantitation/Sensitivity - the minimum concentration of the analyte or measurand that can be quantified with an acceptable level of precision and accuracy under the conditions of the test.
9. Linear Range - the range within the working range where the results are proportional to the concentration of the analyte or measurand present in the sample.
10. Measurement Uncertainty - A single parameter (usually a standard deviation or confidence interval) expressing the possible range of values around the measured result within which the true value is expected to be with a stated degree of probability. It takes into account all recognized effects operating on the result including: overall precision of the complete method, the method and laboratory bias and matrix effects.
11. Matrix - The component or substrate of a test sample.
12. Method Validation - The process of verifying that a method is fit for purpose.[1]
13. Precision - the closeness of agreement between independent test results obtained under stipulated conditions.[1, 2] There are two components of precision:
    a. Repeatability - the measure of agreement of replicate tests carried out on the same sample in the same laboratory by the same analyst within short intervals of time.
    b. Reproducibility - the measure of agreement between tests carried out in different laboratories. In single laboratory validation studies reproducibility is the closeness of agreement between results obtained with the same method on replicate analytical portions with different analysts or with the same analyst on different days.
14. Quality System - The laboratory's quality system is the process by which the laboratory conducts its activities so as to provide data of known and documented quality with which to demonstrate regulatory compliance and for other decision-making purposes. This system includes a process by which appropriate analytical methods are selected, their capability is evaluated, and their performance is documented. The quality system shall be documented in the laboratory's quality manual.
15. Recovery - The fraction or percentage of an analyte or measurand recovered following sample analysis.
16. Ruggedness - the ability of a particular method to withstand relatively minor changes in analytical technique, reagents, or environmental factors likely to arise in different test environments.[4]
17. Specificity - the ability of a method to measure only what it is intended to measure.[1]
18. Working Range - the range of analyte or measurand concentration over which the method is applied.

REFERENCES:

1. Eurachem Guide, 1998. The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics. LGC Ltd. Teddington, Middlesex, United Kingdom.
2. IUPAC Technical Report, 2002. Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis, Pure Appl. Chem., Vol. 74, (5): 835-855.
3. Joint FAO/IAEA Expert Consultation, 1999. Guidelines for Single-Laboratory Validation of Anilytical Methods for Trace-Level Concentrations of Organic Chemicals.
4. MAF Food Assurance Authority, 2002. A Guide for the Validation and Approval of New Marine Biotoxin Test Methods. Wellington, New Zealand.
5. National Environmental Laboratory Accreditation. , 2003. Standards. June 5.
6. EPA. 2004. EPA Microbiological Alternate Procedure Test Procedure (ATP) Protocol for Drinking Water, Ambient Water, and Wastewater Monitoring Methods: Guidance. U.S. Environmental Protection Agency (EPA), Office of Water Engineering and Analysis Division, 1200 Pennsylvania Avenue, NW, (4303T), Washington, DC 20460. April.

ACCURACY OF PSP ELISA METHOD

PSP Analysis of Shellfish

Dockside Samples Analysis by: Jellet, Abraxis, Mouse Bioassay

Coordinates	SeaWatch #	Depth	Species	MBA result	Lab #	Jellett Result	Abraxis Result* (@40 ug/100g)	Abraxis Result ** (@80 ug/100g)
41 35 80 68 23 33	1	73	SC	40 ug/100g	22	Pos	Pos	Neg
41 34 77 68 23 43	2	105	SC	41	23	Pos	Pos	Neg
41 33 56 68 22 57	3	123	SC	45	24	Pos	Pos	Neg
41 32 65 68 21 19	4	110	SC	39	25	Pos	Pos	Neg
41 35 13 67 58 05	16	117	SC	<39	26	Pos	Neg	Neg
41 08 54 68 33 74	20	98	SC	<40	27	Pos	Neg	Neg
41 37 84 68 10 79	23	86	SC	<41	28	Pos	Neg	Neg
41 36 46 68 09 38	24	91	SC	<39	29	Pos	Neg	Neg
41 35 58 68 09 38	25	80	SC	<39	30	Pos	Neg	Neg
41 47 02 67 45 90	29	102	SC	74	31	Pos	Pos	Pos
41 46 85 67 47 23	30	106	SC	79	32	Pos	Pos	Pos

Abraxis cut-off for positive = can be chosen at 40 or 80 ug/100g simultenously.
other multiple cut-off values can also be chosen.

Data provided by Wallace and Associates

Saxitoxin in Freshwater
Sample Preparation

1. Intended Use
For the detection of Saxitoxin in freshwater samples: groundwater, surface water, drinking water, effluent.

2. Materials Required (Not Provided)
Pipettes capable of delivering 100 and 900µL
Glass sample collection vials with Teflon lined caps

3. Notes and Precautions
Immediately upon collection, freshwater samples should be preserved with 10X Concentrated Sample Diluent to prevent adsorptive loss of Saxitoxin from the sample. This step is necessary for freshwater samples only. Saltwater samples do not require additional reagents for preservation due to their naturally occurring salts.

4. Procedure
Add 100µL of 10X Concentrated Sample Diluent per 900µL of Sample. Cap container and invert several times to thoroughly mix.

The sample is now ready to analyze according to the procedure described in the Abraxis Saxitoxin Kit package insert.

5. Evaluation of Results
Results obtained with freshwater samples which have been preserved with 10X Concentrated Sample Diluent as described above must be multiplied by a factor of 1.1 to account for the initial dilution of samples with 10X Diluent.

6. Performance Data
Recovery
Four (4) freshwater samples were spiked with various levels of Saxitoxin, preserved as described above, and then assayed using the Saxitoxin Assay. The following results were obtained:

	Recovery				
Amount of Saxitoxin Added (ppb)	Mean (ppb)	48 Hours Mean (ppb)	1 Week Mean (ppb)	S.D. (ppb)	%
0.04	0.046	0.046	0.050	0.002	117.9
0.08	0.087	0.085	0.086	0.001	107.5
0.2	0.227	0.217	0.217	0.006	110.1
Average					111.8

7. Assistance

For ordering or technical assistance contact:
Abraxis LLC
54 Steamwhistle Drive
Warminster, PA 18974
Tel.: (215) 357-3911
Fax: (215) 357-5232
Email: info@abraxiskits.com
Web: www.abraxiskits.com

082708

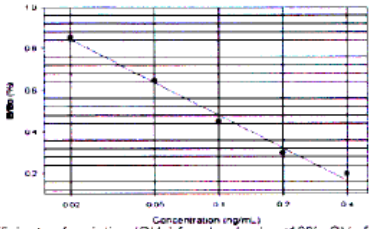
Importance of the Saxitoxin Determination
Saxitoxin is one of the "paralytic shellfish poisons" (PSP), produced by several marine dinoflagellates and fresh water algae. Contamination of shellfish with saxitoxin has been associated with harmful algal blooms throughout the world.

In man, PSP causes dose-dependent perioral numbness or tingling sensations and progressive muscular paralysis, which can result in death through respiratory arrest. The maximum tolerance levels established by the EU and FDA are 40-80 ug per 100 ug edible portion of fresh, frozen, or tinned shellfish.

The Saxitoxin ELISA allows the determination of 42 samples in duplicate determination. Only a few milliliters of sample are required. The test can be performed in about 1 hour.

Performance Data

Test sensitivity: The detection limit for Saxitoxin is 0.015 ng/mL (mean of 6 blank determinations minus 3 SD). The middle of the test (50% B/B_0) is at approximately 0.09 ng/mL. Determinations close to the middle of the tests gives the most accurate results.



Test reproducibility: Coefficients of variation (CVs) for standards: <10%, CVs for samples: <15%.

Selectivity: This ELISA recognizes Saxitoxin and other PSP toxins with varying degrees.

Cross-reactivities:		
	Saxitoxin (STX)	100% (per definition)
	Decarbamoyl STX	29%
	GTX 2 & 3	23%
	GTX-5B	23%
	Sulfo GTX 1 & 2	2.0%
	Decarbamoyl GTX 2 & 3	1.4%
	Neosaxitoxin	1.3%
	Decarbamoyl Neo STX	0.6%
	GTX 1 & 4	<0.2%

Cross-reactivities with other classes of algal toxins have not been observed.

Samples: Drinking water, ground water, and surface water were tested for matrix effects in the ELISA. No matrix effects were determined.

General Limited Warranty: Abraxis LLC warrants the products manufactured by the Company, against defects and workmanship when used in n accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose**

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WEB: WWW.abraxiskits.com

R082508

Saxitoxin (PSP) ELISA, Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Saxitoxin (PSP) in Water and Contaminated Samples

Product No. 52255B

1. General Description

The Saxitoxin ELISA is an immunoassay for the quantitative and sensitive detection of Saxitoxin. Saxitoxin is one of the toxins associated with paralytic shellfish poisoning (PSP). This test is suitable for the quantitative and/or qualitative detection of Saxitoxin in water samples as well as other contaminated samples. For shellfish samples a sample preparation is required. If necessary, positive samples can be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions in the test kit contain small amounts of Saxitoxin. In addition the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

3. Storage and Stability

The Saxitoxin ELISA should to be stored in the refrigerator (4–8°C). The solutions have to be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box.

4. Test Principle

The test is a direct competitive ELISA based on the recognition of Saxitoxin by specific antibodies. Saxitoxin, when present in a sample and a saxitoxin-enzyme-conjugate compete for the binding sites of rabbit anti-saxitoxin antibodies in solution. The saxitoxin antibodies are then bound by a second antibody (sheep anti-rabbit) immobilized on the plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of the Saxitoxin present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the Saxitoxin ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in water samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in water samples, test interferences caused by matrix effects can't be completely excluded. Mistakes in handling the test also can cause errors. Possible sources for such errors can be:

Inadequate storage conditions of the test kit, wrong pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

The Abraxis Saxitoxin ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.) positive samples requiring some action should be confirmed by an alternative method.

Working Instructions

A. Materials Provided

1. Microtiter plate coated with a second antibody (sheep anti-rabbit).
2. Standards (6): 0, 0.02, 0.05, 0.1, 0.2, 0.4 ng/mL
3. Antibody Solution (rabbit anti-saxitoxin), 6 mL
4. Saxitoxin-HRP Conjugate, 6 mL
5. Sample Diluent Concentrate (10X), 2 X 25 mL. Use to dilute samples
6. Wash Solution (5X) Concentrate, 100 mL
7. Color Solution (TMB), 12 mL
8. Stop Solution, 12 mL

B. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the antibody, the substrate solution and the stop solution in order to equalize the incubations periods of the standard solutions and the samples on the entire microtiter plate. Please use only the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microliter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The standard solutions, enzyme conjugate, substrate and stop solution are ready to use and do not require any further dilutions.
4. Dilute the Wash Buffer at a ratio of 1:5. If using the entire bottle (100 mL) then add to 400 mL of deionized or distilled water. Dilute the Sample Diluent at a ratio of 1:10 with deionized water.
5. The stop solution has to be handled with care as it contains diluted H_2SO_4.
6. Freshwater samples must be preserved immediately upon collection to prevent loss of saxitoxin from the samples. Please refer to the Saxitoxin in Freshwater Sample Preparation Bulletin for details.

C. Assay Procedure

1. Add 50 µL of the **standard solutions or the samples** (water) or sample extracts (shellfish) into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Add 50 µL of **enzyme conjugate** solution to the individual wells successively using a multi- channel pipette or a stepping pipette.
3. Add 50 µL of **antibody solution** to the individual wells successively using a multi- channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop for about 30 seconds. Be careful not to spill contents.
4. Incubate the strips for 30 min at room temperature.
5. Wash the strips four times using the washing buffer solution. Please use at least a volume of 300 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
6. Add 100 µL of **substrate solution** to the wells. The strips are incubated for 30 min at room temperature. Protect the strips from direct sunlight.
7. Add 100 µL of **stop solution** to the wells in the same sequence as for the substrate solution.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

D. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-Parameter (preferred) or Logit/Log). For a manual evaluation calculate the mean absorbance value for each of the standards. Calculate the %B/B_0 for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the %B/B_0 for each standard on a vertical linear (y) axis versus the corresponding Saxitoxin concentration on horizontal logarithmic (x) axis on graph paper. %B/B_0 for samples will then yield levels in ppb of Saxitoxin by interpolation using the standard curve.

The concentrations of the samples are determined using this standard curve. Samples showing lower concentration of Saxitoxin compared to standard 1 (0.02 ng/mL) are considered as negative. Samples showing a higher concentration than standard 6 (0.4 ng/mL) must be diluted further to obtain more accurate results.

E. Additional Materials (not delivered with the test kit)

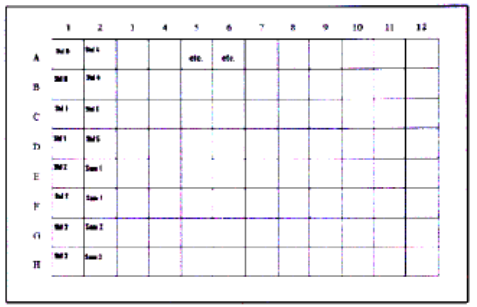
1. Micro-pipettes with disposable plastic tips (10-200, and 200-1000 µL)
2. Multi-channel pipette (10-300 µL) or stepper pipette with plastic tips (10-300 µL)
3. Microtiter plate washer
4. Microtiter plate reader (wave length 450 nm)
5. Shaker for microliter plates (optional)

F. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards have to be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std 0-Std 5: Standards
0; 0.02; 0.05; 0.10; 0.20; 0.40 ppb

Sam1, Sam2, etc.: Samples



G. Preparation of Sample (Mussels)

NOTE: If for regulatory purposes a 100 g sample is needed, extraction solution volume should be adjusted accordingly.

1. Mussels are removed from their shells, washed with deionized water and homogenized.
2. Mix 10 gm of homogenized mussels with 10 mL of 0.1M HCl and boil for 5 minutes while stirring.
3. Allow to cool and centrifuge for 10 minutes at approximately 3500 g.
4. Adjust pH to < pH 4.0 with 5 N HCl.
5. Remove 10 uL and dilute to 10 mL with Sample Dilution Buffer (1:1,000 dilution).
6. Run in the assay as sample (Assay Procedure step 1).

The STX concentration in the samples is determined by multiplying the concentration of the diluted extract by a factor of 2,000. Highly contaminated samples (outside the range of the curve), should be diluted further and re-analyzed, we recommend further dilutions of 1:10 with sample dilution buffer. The dilution factor will then be 20,000. Samples with low contamination of STX or samples that contain STX congeners with low cross-reactivity (see chart) can be detected in the assay by diluting samples 1:250 before analysis. The assay has low cross-reactivity against GTX 1 & 4, therefore food samples containing these congeners at low concentrations might be underestimated by this ELISA.

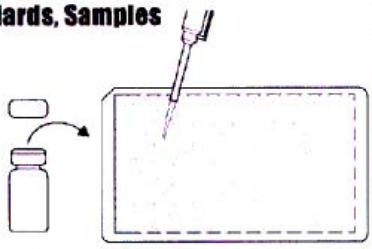
H. Alternative Sample Preparation

1. Mussels are removed from their shells, washed with deionized water, dried and homogenized using a Polytron or equivalent.
2. A 1.0 gm portion is then mixed with 6 mL methanol/DI water (80/20) using a Polytron or equivalent.
3. Centrifuge mixture for 10 minutes at 3000 g. Collect supernatant.
4. Add 2 mL methanol/deionized water (80/20) to the mussel tissue residue. Re-centrifuge the mixture for 10 minutes. Add supernatant to first portion.
5. Bring the volume of the collected supernatant to 10 mL with methanol deionized water (80/20). Filter extract through a 0.45 um filter (Millex HV, Millipore).
6. Remove 10 uL and dilute to 1.0 mL with sample Dilution Buffer (1:100 dilution), then analyze as samples (Assay Procedure, step 1). The STX concentration in the samples is determined by multiplying the concentration of the diluted extract by a factor of 1,000.

Saxitoxin (PSP) Plate, Detailed ELISA Procedure

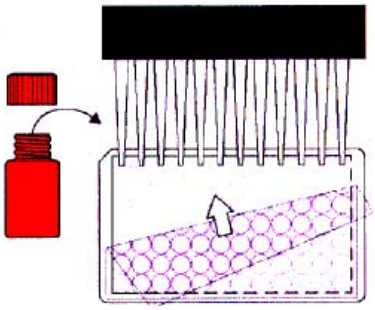
1. Addition of Standards, Samples

Add 50 uL of the standard solutions, control or samples into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.



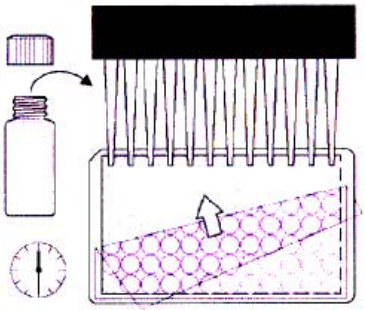
2. Addition of Enyzme Conjugate

Add 50 uL of the enzyme conjugate to the individual wells successively using a multi- channel pipette or a stepping pipette.



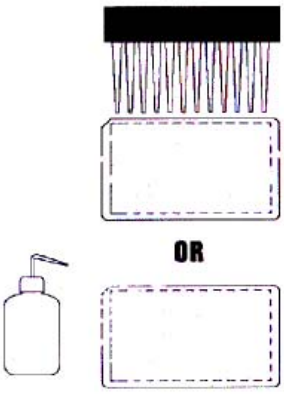
3. Addition of Antibody Solution

Add 50 uL of the Saxitoxin anitbody solution to the individual wells successively using a multi-channel pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 30 min at room temperature.



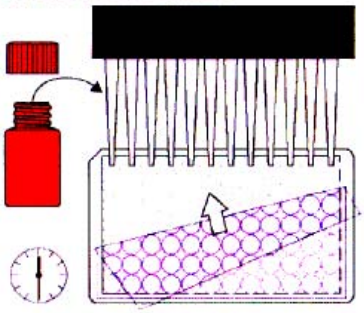
4. Washing of Plates

After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips three times with a multi-channel pipette or wash bottle using the diluted 1X washing buffer solution. Please use at least a volume of 300 uL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels. Repeat steps an additional three times.



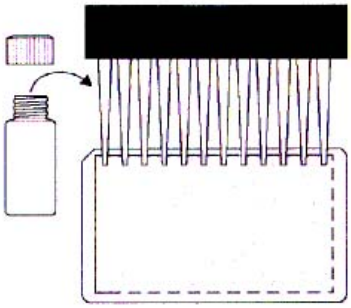
5. Addition of Substrate/Color Solution

Add 100 uL of substrate/color solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 30 min at room temperature.



6. Addition of Stopping Solution

Add 100 uL of stop solution to the wells in the same sequence as for the substrate solution using a multi- channel pipette or a stepping pipette.



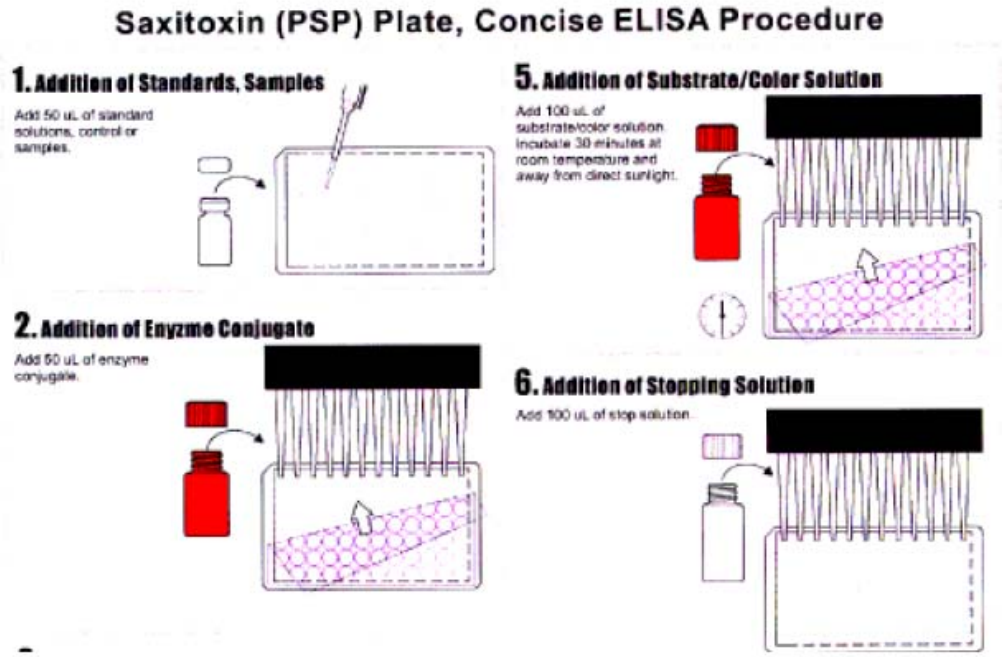
7. Measurement of Color

Read the absorbance at 450 nm using a microplate ELISA reader. Calculate results.

For Ordering or Technical Assistance Contact:
ABRAXIS, LLC
54 Steamwhistle Drive, Warminster, PA 18974
Phone: 215-357-3911 Fax: 215-357-5232
www.abraxiskits.com

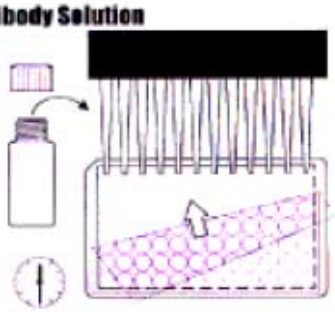
ABRAXIS

Saxitoxin (PSP) Plate Kit Part # 52255B


Saxitoxin (PSP) Plate, Concise ELISA Procedure
1. Addition of Standards, Samples
Add 50 uL of standard solutions, control or samples.
2. Addition of Enyzme Conjugate
Add 50 uL of enzyme conjugate.
5. Addition of Substrate/Color Solution
Add 100 uL of substrate/color solution. Incubate 30 minutes at room temperature and away from direct sunlight.
6. Addition of Stopping Solution
Add 100 uL of stop solution.

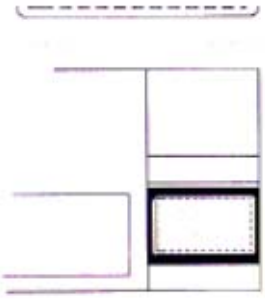
3. Addition of Antibody Solution

Add 50 uL of the antibody solution. Cover and mix for 30 seconds by rotating on benchtop. Incubate for 30 minutes at room temperature.



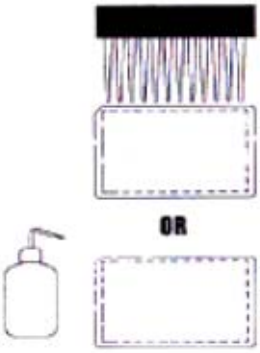
7. Measurement of Color

Measure color at 450 nm.
Calculate results.



4. Washing of Plates

Wash the plates four times with 300 uL of diluted 1X washing buffer.



OR

For Ordering or Technical Assistance Contact:
ABRAXIS, LLC
54 Steamwhistle Drive, Warminster, PA 18974
Phone: 215-357-3911 Fax: 215-357-5232
www.abraxiskits.com

ABRAXIS

Saxitoxin (PSP) Plate Kit Part # 522558

Proposal Subject: Post Harvest Processing

Specific NSSP Guide Reference: 2009 NSSP Section II Model Ordinance Chapter IV Shellstock Growing Areas @.03 Growing Area Classification D (1)(a)(ii)

Text of Proposal/ Requested Action

D. Restricted Classification.
(1) General

(a) A growing area may be classified as restricted when:

(i) A sanitary survey indicates a limited degree of pollution; and

(ii) Levels of fecal pollution, human pathogens, or poisonous or deleterious substances are at such levels that shellstock can be made safe for human consumption by either relaying, depuration or low acid-canned food processing or by other verifiable processes.

Public Health Significance: Including new technology for safe processing of shellstock from restricted areas will result in expanded industry access to resource, while maintaining public health standards.

Cost Information (if available): N/A

Action by 2011 Task Force I Recommended referral of Proposal 11-100 to the appropriate committee as determined by Conference Chairman.

Action by 2011 General Assembly Adopted recommendation of 2011 Task Force I on Proposal 11-100.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-100.

Proposal Subject: Re-opening Conditional Areas using Male-specific Coliphage after WTP Malfunction

Specific NSSP Guide Reference: NSSP 2009 Section II Model Ordinance Chapter IV Shellstock Growing Areas @ .03 Growing Area Classification A. (5) (c) (ii)

Text of Proposal/ Requested Action (ii) For emergency closures ~~(not applicable for conditional closures)~~ of harvest areas caused by the occurrence of raw untreated sewage or partially treated sewage discharged from a large community sewage collection system or wastewater treatment plant, the analytical sample results shall not exceed background levels or a level of 50 male-specific coliphage per 100 grams from shellfish samples collected no sooner than 7 days after contamination has ceased and from representative locations in each growing area potentially impacted; or

Public Health Significance: Male-specific Coliphage (MSC) is an RNA virus of E. coli present in high numbers in raw sewage (on the order of 10^5 PFU/100gm). MSC is similarly resistant to chlorine disinfection as are norovirus and hepatitis A viruses, which are the viral pathogens of primary concern in sewage. MSC is a good surrogate or marker for these enteric viruses. Raw or partially treated sewage accidentally discharged into a growing area by sewage by-pass from pump station failures, broken sewage lines, or malfunctions at the wastewater treatment facilities represent a serious public health risk and require emergency closure of adjacent conditional growing areas. These closures are typically 21 days after the wastewater treatment system returns to normal operation. Recent work has shown that persistence of viruses in the growing waters is much lower in the summer months than in the winter months. Likewise, bio-accumulation rates and retention of enteric viruses in molluscan shellfish is much lower in the summer months than the winter months. MSC can be a useful tool for state shellfish programs to mitigate the negative effect of prolonged conditional closures due to wastewater treatment system failures. This approach is most appropriate in the late-spring and summer months to shorten these closures from 21 to 7 days.

Cost Information (if available): The Male-specific Coliphage (MSC) Method is an inexpensive double-agar pour plate method that can be run in any state-certified microbiological laboratory. A refrigerated centrifuge capable of 9,000G is required which costs $10K to $12K (USD). Re-opening after 7 days using MSC method is optional for state shellfish control agencies.

Action by 2011 Task Force I Recommended referral of Proposal 11-101 to the appropriate committee as determined by the Conference Chairman. FDA is requested to prepare and provide MSC data from wastewater treatment plant sampling to the committee. FDA is further requested to involve the submitter in this proposal in analyzing that data.

Action by 2011 General Assembly Adopted recommendation of 2011 Task Force I on Proposal 11-101.

Action by FDA February 26, 2012 FDA concurred with Conference action on Propoposal 11-101 with the following recommendations.

FDA concurs with Conference action to refer Proposal 11-101 to an appropriate committee as determined by the Conference Chairperson. The intent of these Proposals is to expand the application of Male Specific Coliphage (MSC) for use in the management of conditional areas affected by raw or partially untreated sewage discharges from wastewater treatment plants (WWTP) or community sewage collection systems and for assessing the impact of WWTP discharges and/or sewerage collection system leaks in determining the size of adjacent areas for classification as conditionally

restricted or conditionally approved. Presently, however, there is insufficient data from which to make sound science based decisions regarding the use of MSC as a more comprehensive tool for growing area management.

Support for using MSC for conditional area management is based on uptake and elimination data for a single shellfish species, soft-shelled clams *(Mya arenaria),* impacted by effluent from a highly efficient WWTP at one geographic location over just one harvest season. Those data are not adequate to ensure the efficacy of MSC to safely manage other conditional areas for other species of shellfish, in other geographic regions, and over other seasons.

Careful consideration needs to be given to the fact that a WWTP malfunction is often a consequence of adverse weather conditions, most notably excessive rainfall over short periods. Such rainfall events usually cause excessive land based runoff, carrying non-point fecal pollution to conditional areas. While MSC are generally ubiquitous in municipal wastewater, that is not the case with smaller pollution sources. For this reason MSC are inappropriate for indexing smaller sources and do not lend themselves well to managing areas subject to pollution from both WWTPs and other sources. Shellfish associated norovirus (NoV) outbreaks investigated by FDA's Gulf Coast Seafood Laboratory (GCSL) in the past several years have, in nearly all instances, shown MSC levels in shellfish below the assay's sensitivity(< 10 pfu/lOOml), while testing positive for NoV. These results indicate that the source of NoV was not from a WWTP. Though MSC appear to have utility and promise in assessing potential viral contamination in shellfish, much remains to be learned about their prevalence and ability to reliably index fecal contamination from various sources of human sewage.

Several approaches for generating additional information and data needed to better define how MSC could potentially be used for growing area management and classification include:

- Continued studies to examine the uptake and elimination of NoV, enterovirus, and MSC by shellfish species other than soft-shelled clams. These investigations should be conducted in multiple geographic locations representative of the country and over all seasons.
- A SL V has been conducted and adopted by the ISSC for the method to enumerate SC in soft-shelled clams and oysters. A SL V is needed to demonstrate the efficacy of this or another method to enumerate MSC in other species of shellfish.
- Understanding the efficiency of various wastewater treatment systems to inactivate/remove enteric viruses prior to discharge.
- Continued studies to examine and compare MSC and enteric virus levels in wastewater influent and effluent, shellfish receiving waters, and shellfish.

As requested by Task Force I, information is currently being compiled by FDA regarding MSC data from WWTP sampling. Those data should be available to the ISSC in March, 2012.

Proposal Subject: Using Male-specific Coliphage as a Tool to Refine Determinations of the Size of the Areas to be Classified as Prohibited Adjacent to Each Outfall

Specific NSSP Guide Reference: NSSP 2009 Section II Model Ordinance Chapter IV Shellstock Growing Areas @.03 Growing Area Classification E. (5)

Text of Proposal/ Requested Action (c) An assessment of the combined impact of waste water treatment plant outfall and/or ex-filtration (leakage) from sewerage collection systems may be performed using male-specific coliphage assays on shellstock from adjacent growing areas. A male-specific coliphage standard of ≤ 50 PFU/100gm in shellfish meats may be used as the basis for the determination of the size of the adjacent area to be classified as conditionally restricted or approved.

Public Health Significance: Male-specific Coliphage (MSC) is a RNA virus of E. coli present in high numbers in raw sewage (on the order of 10^5 PFU/100gm). MSC is similarly resistant to chlorine disinfection as are norovirus and hepatitis A viruses, which are the viral pathogens of concern in sewage. MSC is a good surrogate or marker for these enteric viruses and is a powerful tool to assess the impact on a growing area of raw, partially treated and treated sewage on adjacent growing areas. US and EU studies show that during the summer months MSC and associated pathogenic enteric viruses are at seasonal lows. Conversely, the risk of viral disease transmission is significantly higher in the winter months as evidenced by epidemiological studies as well as studies conducted using MSC and molecular detection of target pathogens.

A better assessment of the risk of viral contamination at a particular location in an adjacent growing area at a particular time of year can be ascertained directly using MSC assays of the shellstock. Performing and evaluating dye studies on waste water treatment plant outfall evaluation is expensive and complicated. Difficulties assessing ex-filtration and leakage from the sewage collection system are well known. Few tools and less guidance are available to adequately assess the performance of a particular waste water treatment plant design and its operation with respect to virus removal. The advantages of using this specialty viral indicator to assess the overall impact of a municipal wastewater treatment system on a particular growing area are many. In growing areas impacted by waste water treatment systems, positive norovirus detected by molecular methods at significant levels in the shellfish are accompanied by corresponding high levels of MSC. MSC assays are a direct and straightforward method to determine the viral risk or validate traditional assessment techniques.

Cost Information (if available): The Male-specific Coliphage (MSC) method is an inexpensive double-agar pour plate method, which can be run in any state-certified microbiological laboratory. A refrigerated centrifuge capable of 9,000G is required which costs $10K to $12K (USD). Cost savings and a higher level of public health protection may be realized using MSC assays of shellfish verses the level of effort needed to ascertain the viral risk indirectly through dye studies, 1000:1 dilution line determinations and performance evaluations.

Action by 2011 Task Force I Recommended referral of Proposal 11-102 to the appropriate committee as determined by the Conference Chairman. FDA is requested to prepare and provide MSC data from wastewater treatment plant sampling to the committee. FDA is further requested to involve the submitter in this proposal in analyzing that data.

Action by 2011 General Assembly Adopted recommendation of 2011 Task Force I on Proposal 11-102.

Action by FDA
February 26, 2012

FDA concurred with Conference action on Propoposal 11-102 with the following recommendations.

FDA concurs with Conference action to refer Proposal 11-102 to an appropriate committee as determined by the Conference Chairperson. The intent of these Proposals is to expand the application of Male Specific Coliphage (MSC) for use in the management of conditional areas affected by raw or partially untreated sewage discharges from wastewater treatment plants (WWTP) or community sewage collection systems and for assessing the impact of WWTP discharges and/or sewerage collection system leaks in determining the size of adjacent areas for classification as conditionally restricted or conditionally approved. Presently, however, there is insufficient data from which to make sound science based decisions regarding the use of MSC as a more comprehensive tool for growing area management.

Support for using MSC for conditional area management is based on uptake and elimination data for a single shellfish species, soft-shelled clams *(Mya arenaria),* impacted by effluent from a highly efficient WWTP at one geographic location over just one harvest season. Those data are not adequate to ensure the efficacy of MSC to safely manage other conditional areas for other species of shellfish, in other geographic regions, and over other seasons.

Careful consideration needs to be given to the fact that a WWTP malfunction is often a consequence of adverse weather conditions, most notably excessive rainfall over short periods. Such rainfall events usually cause excessive land based runoff, carrying non-point fecal pollution to conditional areas. While MSC are generally ubiquitous in municipal wastewater, that is not the case with smaller pollution sources. For this reason MSC are inappropriate for indexing smaller sources and do not lend themselves well to managing areas subject to pollution from both WWTPs and other sources. Shellfish associated norovirus (NoV) outbreaks investigated by FDA's Gulf Coast Seafood Laboratory (GCSL) in the past several years have, in nearly all instances, shown MSC levels in shellfish below the assay's sensitivity(< 10 pfu/lOOml), while testing positive for NoV. These results indicate that the source of NoV was not from a WWTP. Though MSC appear to have utility and promise in assessing potential viral contamination in shellfish, much remains to be learned about their prevalence and ability to reliably index fecal contamination from various sources of human sewage.

Several approaches for generating additional information and data needed to better define how MSC could potentially be used for growing area management and classification include:

- Continued studies to examine the uptake and elimination of NoV, enterovirus, and MSC by shellfish species other than soft-shelled clams. These investigations should be conducted in multiple geographic locations representative of the country and over all seasons.
- A SL V has been conducted and adopted by the ISSC for the method to enumerate SC in soft-shelled clams and oysters. A SL V is needed to demonstrate the efficacy of this or another method to enumerate MSC in other species of shellfish.
- Understanding the efficiency of various wastewater treatment systems to inactivate/remove enteric viruses prior to discharge.

- Continued studies to examine and compare MSC and enteric virus levels in wastewater influent and effluent, shellfish receiving waters, and shellfish.

As requested by Task Force I, information is currently being compiled by FDA regarding MSC data from WWTP sampling. Those data should be available to the ISSC in March, 2012.

Proposal Subject:	Alternative Male-specific Coliphage Meat Standard for Restricted Classification of Growing Areas Impacted by wastewater treatment plant outfall.
Specific NSSP Guide Reference:	NSSP 2009 Section II Model Ordinance Chapter IV Shellstock Growing Area @ .02 Bacteriological Standards G. – add new section (4)
Text of Proposal/ Requested Action	(4) Exception. If the Male-specific Coliphage indicator is used for supplemental process verification using an end-point meat standard of < 50PFU/100gm and existing fecal coliform testing requirements in Chapter XV .03 J. are used, then FC water quality monitoring is not required for the restricted classification of growing areas affected by point sources such as wastewater treatment plant outfall.
Public Health Significance:	Under shellfish relay, water quality requirements are not needed for the restricted classification when a contaminant reduction study is conducted and a minimum time period of two weeks is used. For depuration, the restricted classification requires water quality monitoring and standards. The reason for these upper FC limits is that FC meat indicator does not adequately reflect the viral risk and/or viral depuration kinetics. Male-specific coliphage is a viral indicator organism to be used in growing areas impacted by point source sewage contamination. MSC demonstrates significant advantages over FC alone for both the assessment of viral contamination and assessment of viral depuration kinetics. Upper FC limits were put into the NSSP to prevent shellfish with higher levels of viruses from being depurated. Several studies clearly show that conventional depuration using FC for process validation is not adequate to protect public health with respect to virus contamination in growing areas with significant wastewater treatment plant and sewage impact. Studies have also shown that viral levels in shellfish impacted by sewage and partially treated sewage detected using MSC and molecular techniques are much lower in the summer months than the winter months. Additionally, the viral depuration rate is higher in the summer with process waters >18°C. Recent studies have also shown that MSC is an appropriate viral indicator to assess viral depuration. Therefore, seasonal viral depuration using male-specific coliphage as well as FC for process verification is a superior approach to taking water samples using FC in a growing area adjacent to wastewater treatment plant outfall. Combining the bacterial indicator of FC and the viral indicator MSC for mitigation strategies that use meat scores is far more direct and effective than water quality sampling in this context.
Cost Information (if available):	The Male-specific Coliphage (MSC) method is an inexpensive double-agar pour plate method that can be run in any state-certified microbiological laboratory. A refrigerated centrifuge capable of 9,000G is required which costs $10K to $12K (USD). Significant cost savings and a higher level of public health protection may be realized using strategies such as seasonal coliphage depuration process validated using MSC and seasonal coliphage relay using MSC in contaminant reduction studies than requiring water quality limits using FC.
Action by 2011 Task Force I	Recommended referral of Proposal 11-103 to the appropriate committee as determined by the Conference Chairman.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 11-103.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 11-103.

Proposal Subject: Use of Analytical Methods Other than NSSP Methods

Specific NSSP Guide Reference: 2009 NSSP Section II Model Ordinance Chapter III Laboratory @ .02 Methods, Paragraphs A, C, D (1) and (2)

Text of Proposal/ Requested Action Revise Chapter III @.02 Methods, Paragraphs A, C and D as follows.

Chapter III @ .02 Methods

A. Microbiological. Methods~~, practices, and procedures~~ for the analyses of shellfish and shellfish growing or harvest waters shall be:

(1) the Approved NSSP M~~m~~ethods validated for use in the National Shellfish Sanitation Program under Procedure XVI of the Constitution, Bylaws and Procedures of the ISSC and / or cited in the Guidance Documents, Chapter II. Growing Areas .10 Approved National Shellfish Sanitation Program Laboratory Tests;

(2) When there is an immediate or ongoing critical need for a method and no Approved NSSP Method exists, the following may be used:

(a) A validated AOAC, BAM, or EPA method;

(b) An Emergency Use Method pursuant to .02 D. (1) and (2) below.

B. Chemical and Physical.

(1) Methods for the analysis of shellfish and shellfish growing or harvest waters shall:

(a) Be the current AOAC or APHA method for all physical and chemical measurements; and

(b) Express results of all chemical and physical measurements in standard units, and not instrument readings.

(2) When there is an immediate or ongoing critical need for a Method and no Approved NSSP Method exist, the following may be used:

(a) A Validated AOAC, BAM, or EPA method;

(b) An Emergency Use Method pursuant to .02 D. (1) and (2) below.

~~(2) When an AOAC or APHA method is not available, EPA methods may be used.~~

~~(3) If a method is not approved or validated by AOAC, APHA, or EPA then the method shall be validated in accordance with Procedure XVI of the Constitution, Bylaws and Procedures of the ISSC.~~

C. Biotoxin. Methods for the analyses of shellfish and shellfish harvest waters shall be:

(1) The current AOAC and APHA methods used in the bioassay for paralytic shellfish poisoning toxins : and

(2) The current APHA method used in the bioassay for *Karenia brevis* toxins; or

(3) Approved NSSP Methods validated for use ~~in the National Shellfish Sanitation Program~~ under Procedure XVI of the Constitution, Bylaws and Procedures of the ISSC and / or cited in the Guidance Documents, Chapter II. Growing Areas .10 Approved National Shellfish Sanitation Program Laboratory Tests.

(4) When there is an immediate or ongoing critical need for a method and no Approved NSSP Method exists, the following may be used:

(a) A validated AOAC, BAM, or EPA method;

(b) An Emergency Use Method pursuant to .02 D. (1) and (2) below.

D. Emergency Use ~~Emerging~~ Methods.

(1) When there is an immediate or critical need and no Approved NSSP ~~approved m~~ Method~~s~~ exists, ~~and~~ the ISSC Executive Board may grant interim approval to ~~considers allowing~~ an unapproved or non-validated method to be used for a specific purpose.~~,~~ ~~t~~ The following minimum requirements ~~as the Lab Method Review Committee Advisory for Emerging Methods~~ will be provided to the Executive Board prior to granted interim approval ~~and shall contain the following criteria~~:

(a) Name of Method
(b) Date of Submission
(c) Specific purpose or intent of the method for use in the NSSP
(d) Step by step procedure including equipment, reagents and safety requirements necessary to run the method
(e) Data generated in the development and/or trials of the method and/or comparing to approved methods if applicable
(f) Any peer reviewed articles detailing the method
(g) Name of developer(s)~~/~~ or SSCA submitter~~s~~
(h) Developer/submitter contact information

(2) Within two years of Executive Board interim approval ~~the initial allowed use of~~ the Emergency Use M~~m~~ethod, the entire Single Lab Validation Protocol should be submitted. The Lab Methods Review Committee will report to the Executive Board on the status of the Single Lab Validation Protocol data submission.

Public Health Significance:

Cost Information (if available): None

Action by 2011 Laboratory Methods Review Committee Recommended adoption of Proposal 11-104 as amended.

B. Microbiological. Methods for the analyses of shellfish and shellfish growing or harvest waters shall be:

(1) the Approved NSSP Methods validated for use in the National Shellfish Sanitation Program under Procedure XVI of the Constitution, Bylaws and Procedures of the ISSC and / or cited in the Guidance Documents, Chapter II. Growing Areas .10 Approved National Shellfish Sanitation Program Laboratory Tests;

(2) When there is an immediate or ongoing critical need for a method and no Approved NSSP Method exists, the following may be used:

(a) A validated AOAC, BAM, or EPA method;
(b) An Emergency Use Method pursuant to .02 D. (1) and (2) below.

B. Chemical and Physical.

(1) Methods for the analysis of shellfish and shellfish growing or harvest waters shall:

(a) Be the current AOAC or APHA method for all physical and chemical measurements; and
(b) Express results of all chemical and physical measurements in standard units, and not instrument readings.

(2) When there is an immediate or ongoing critical need for a Method and no Approved NSSP Method exist, the following may be used:

(a) A Validated AOAC, BAM, or EPA method;

(b) An Emergency Use Method pursuant to .02 D. (1) and (2) below.

C. Biotoxin. Methods for the analyses of shellfish and shellfish harvest waters shall be:

(1) The current AOAC and APHA methods used in the bioassay for paralytic shellfish poisoning toxins : and

(2) The current APHA method used in the bioassay for *Karenia brevis* toxins; or

(3) Approved NSSP Methods validated for use under Procedure XVI of the Constitution, Bylaws and Procedures of the ISSC and / or cited in the Guidance Documents, Chapter II. Growing Areas .10 Approved National Shellfish Sanitation Program Laboratory Tests.

(4) When there is an immediate or ongoing critical need for a method and no Approved NSSP Method exists, the following may be used:

(a) A validated AOAC, BAM, or EPA method;

(b) An Emergency Use Method pursuant to .02 D. (1) and (2) below.

D. Emergency Use Methods.

(1) When there is an immediate or critical need and no Approved NSSP Method exists, ~~the ISSC Executive Board may grant interim approval to~~ an unapproved or non-validated method ~~to~~may be used for a specific purpose provided that~~. The following minimum requirements will be provided to the Executive Board prior to granted interim approval~~ :

(a) The appropriate FDA Regional Office is notified within a reasonable period of time regarding the method employed; and

(b) The ISSC Executive Board is notified within a reasonable period of time regarding the method employed.

(2) When it is necessary to continue the use of the emergency method employed under D. (1) beyond the initial critical need, then the following minimum criteria shall be provided to the ISSC Executive Board for interim approval:

(a) Name of Method

(b) Date of Submission

(c) Specific purpose or intent of the method for use in the NSSP

(d) Step by step procedure including equipment, reagents and safety requirements necessary to run the method

(e) Data generated in the development and/or trials of the method and/or comparing to approved methods if applicable

(f) Any peer reviewed articles detailing the method

(g) Name of developer(s) or SSCA submitter~~s~~

(h) Developer/submitter contact information

(~~2~~3) Within two years of Executive Board interim approval of the Emergency Use Method, the entire Single Lab Validation Protocol should be submitted. The Lab Methods Review Committee will report to the Executive Board on the status of the Single Lab Validation Protocol data submission.

Action by 2011 Task Force III Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 11-104.

Action by 2011 General Assembly Adopted the recommendation of Task Force III on Proposal 11-104.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-104.

Proposal Subject: Use of Analytical Methods Other than NSSP Methods

Specific NSSP Guide Reference: 2009 NSSP Section II Model Ordinance Chapter III Laboratory @ .02 Methods, Paragraphs A, C, D (1) and (2)

Text of Proposal/ Requested Action Revise Chapter III @.02 Methods, Paragraphs A, C and D as follows.
Chapter III @ .02 Methods

A. Microbiological. Methods for the analyses of shellfish and shellfish harvest waters shall be:
   (1) The methods validated for use in the National Shellfish Sanitation Program under Procedure XVI of the Constitution, Bylaws and Procedures of the ISSC and listed in the Guidance Documents, Chpater II. Growing Areas .10 Approved National Shellfish Sanitation Program Laboratory Tests.
   (2) When there is an immediate need for a method of analysis and no NSSP approved analytical method exists, a validated AOAC, BAM or EPA method may be used.
   (3) When there is an ongoing critical need for a method of analysis and no NSSP approved analytical method exista an emergent method may be used pursuant to .02 D (1) and (2) below.

B. Chemical and Physical
   (1) Methods for the analysis ……

      (a) Be the current ……..

      (b) Express results of all ……

   (2) When an AOAC……..

   (3) If a method is not ……..

C. Biotoxin. Methods for the analyses of shellfish shall be:

   (1) The current AOAC and APHA ……

   (2) The current APHA method ……

   (3) Methods validated for use in the National Shellfish Sanitation Program under Procedure XVI of the Constitution, Bylaws and Procedures of the ISSC and~~/~~ listed in the Guidance Documents, Chapter II. Growing Areas .10 Approved National Shellfish Sanitation Program Laboratory Tests.

   (4) When there is an immediate need for a method of analysis and no NSSP approved method exists, a validated AOAC method may be used.

   (5) When there is an ongoing critical need for a method of analysis and no NSSP approved method exists, an emergent method may be used pursuant to .02 D (1) and (2) below.

D. Emerging Methods.

   (1) When there is an ongoing critical need for a method of analysis

and no NSSP approved method exists, the ISSC Executive Board may consider allowing an unapproved or non-validated method to be used for a specific purpose. The minimum requirements as defined in the Laboratory Methods Review Committee Advisory for Emerging Methods will be provided to the Executive Board and shall contain the following:

Name of Method;

Date of Submission;

Specific purpose or intent of the method for use in the NSSP;

Step by step procedure including equipment, reagents and safety requirements necessary to run the method;

Data generated in support of the efficacy of the method if available;

Any peer reviewed articles detailing the method and its efficacy;

Name of the developer(s) or SSCA submitter;

Developer/submitter contact information.

(2) Within two years of the initial allowed use of the emerging method, the entire Single Lab Validation Protocol should be completed and submitted to the ISSC for consideration as an approved method. The Laboratory Methods Review Committee will review the submission and report to the Executive Board on its Status.

Public Health Significance:

Cost Information (if available): None

Action by 2011 Laboratory Methods Review Committee Recommended no action on Proposal 11-105. Rationale – Proposal 11-105 is addressed by action on Proposal 11-104.

Action by 2011 Task Force III Recommended no action on Proposal 11-105. Rationale-Proposal 11-105 was resolved by Task Force action on Proposal 11-104.

Action by 2011 General Assembly Adopted the recommendation of Task Force III on Proposal 11-105.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-105.

Proposal Subject: Definitions for Types I, II, III and IV Methods

Specific NSSP Guide Reference: 2009 NSSP Section II - Model Ordinance - Purpose and Definitions

Text of Proposal/ Requested Action Add the following definitions:

(115) **Type I Methods** mean the core methods of analysis used to support established Program requirements within the NSSP. Type I methods have been evaluated and the performance characteristics for specific applications in the NSSP have been determined and found fit for purpose.

(116) **Type II Methods** mean permanent methods of analysis used widely within the NSSP as alternative methods to improve turnaround time, cost effectiveness or to develop analytical capacity beyond what is achieved by the core methods. Type II methods are NSSP validated and the performance characteristics for specific applications within the NSSP have been determined and found fit for purpose.

(117) **Type III Methods** mean interim methods of analysis used to fill an ongoing NSSP Program need. Type III methods are NSSP validated and the performance characteristics for specific applications within the NSSP have been determined and found fit for purpose. Type III methods are designated for periodic review and assessment by the Laboratory Methods Review Committee for continued use, redesignation or deletion.

(118) **Type IV Methods** mean provisional methods of analysis developed to fill an ongoing NSSP Program need. Type IV methods are newly accepted for use in the NSSP and/or not yet used for Program support outside the laboratory in which the method was developed and/or validated. Type IV methods are NSSP validated and the performance characteristics for specific applications within the NSSP have been determined and found fit for purpose. Type IV methods are designated for periodic review and assessment by the Laboratory Methods Review Committee for continued use, redesignation or deletion.

(119) **Wet storage** means …….

Public Health Significance: These definitions help clarify the various categories of analytical methods accepted for use in the NSSP.

Cost Information (if available): None

Action by 2011 Laboratory Methods Review Committee Recommended no action on Proposal 11-106. Rationale – Proposal 11-106 is addressed by action on Proposal 11-104 and Proposal 11-307.

Action by 2011 Task Force III Recommended no action on Proposal 11-106. Rationale-Proposal 11-106 was resolved by Task Force action on Proposal 11-104 and Proposal 11-307.

Action by 2011 General Assembly Adopted the recommendation of Task Force III on Proposal 11-106.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-106 with the following comments.

FDA concurs with the "No Action" taken on Proposal 11-106. However, the Summary of

Actions incorrectly states that the reason for "No Action" was that Proposal 11-106 was addressed by action taken on Proposal 11-104. The Summary of Actions should be edited to correctly reflect that Proposal 11-106 was addressed by action taken on Proposal 11-104 and Proposal 11-307.

Proposal Subject: Reveal ASP (Domoic Acid) Test Kit

Specific NSSP Guide Reference: 2009 NSSP Section IV Guidance Documents Chapter II. Growing Areas .10 Approved NSSP Laboratory Tests, Table 4 - Type III and Type IV Marine Biotoxin Test Methods

Text of Proposal/ Requested Action We request review of the validation study submission for the Reveal ASP (domoic acid) test kit and consideration of the method for approval as a Type IV marine biotoxin screening method for qualitative determination of domoic acid in shellfish. Add Reveal ASP (domoic acid) test to list of approved Type III and Type IV marine biotoxin methods.

Public Health Significance: Amnesic shellfish poisoning is caused by the toxin domoic acid, produced by phytoplankton of the genus Pseudonitzschia. It is associated with eating contaminated oysters, clams, mussels, and other shellfish. There have been numerous outbreaks of ASP, and there is evidence that the occurrence of the phytoplankton responsible for ASP is widespread. Current methods for detection of domoic acid consist primarily of instrumental chemistry methods, which are laborious and time-consuming. Methods for rapid screening for domoic acid, in field and laboratory settings, are needed and will assist the industry and public health authorities in responding to this health concern. The Reveal ASP test is a lateral flow immunoassay designed for qualitative determination of domoic acid in shellfish at levels of 10 ppm (mg/kg) and above. The test uses minimal equipment and simple reagents, does not require specialized training, and can provide results in 20 minutes from sample receipt, including sample preparation.

Cost Information (if available): Approximately $17.00 per test.

Action by 2011 Laboratory Methods Review Committee Recommended referral of Proposal 11-107 to the appropriate committee as determined by the Conference Chairman and further recommended the following guidance on the data needed from the submitter:

- Analysis of samples with naturally incurred residues over a range of toxin concentrations.
- Evaluate extraction recovery by comparison with HPLC.
- Additional replicates of spiked samples of shellfish species.

Eliminate theoretical data regarding dose response curve.

Action by 2011 Task Force I Recommended adoption of Laboratory Methods Review Committee recommendations on Proposal 11-107.

Action by 2011 General Assembly Adopted recommendation of 2011 Task Force I on Proposal 11-107.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-107.

ISSC Method Application and Single Lab Validation Checklist For Acceptance of a Method for Use in the NSSP

The purpose of single laboratory validation in the National Shellfish Sanitation Program (NSSP) is to ensure that the analytical method under consideration for adoption by the NSSP is fit for its intended use in the Program. A Checklist has been developed which explores and articulates the need for the method in the NSSP; provides an itemized list of method documentation requirements; and, sets forth the performance characteristics to be tested as part of the overall process of single laboratory validation. For ease in application, the performance characteristics listed under validation criteria on the Checklist have been defined and accompany the Checklist as part of the process of single laboratory validation. Further a generic protocol has been developed that provides the basic framework for integrating the requirements for the single laboratory validation of all analytical methods intended for adoption by the NSSP. Methods submitted to the Interstate Shellfish Sanitation Conference (ISSC) Laboratory Methods Review (LMR) Committee for acceptance will require, at a minimum, six (6) months for review from the date of submission.

Name of the New Method	Reveal ASP (Domoic Acid)
Name of the Method Developer	Neogen Corporation
Developer Contact Information	Mark Mozola, 517-372-9200, mmozola@neogen.com

Checklist	Y/N	Submitter Comments
A. Need for the New Method		
1. Clearly define the need for which the method has been developed.		There is a need for a simple, rapid screening method for domoic acid in shellfish, one that can be used in the field as well as in a laboratory setting.
2. What is the intended purpose of the method?		The method is designed for rapid qualitative screening of shellfish for domoic acid.
3. Is there an acknowledged need for this method in the NSSP?		Simply assays that provide rapid results are needed.
4. What type of method? i.e. chemical, molecular, culture, etc.		Lateral flow immunoassay in dipstick format.
B. Method Documentation		
1. Method documentation includes the following information:		
Method Title		Reveal ASP (Domoic Acid)
Method Scope		Qualitative detection of domoic acid in oysters, clams, and mussels.
References		Study report and kit insert included in this submission.
Principle		Competitive lateral flow immunoassay in dipstick format. Water extraction of analyte from homogenized shellfish tissue.
Any Proprietary Aspects		Yes, commercial test kit.
Equipment Required		Extraction containers with lids (40 mL capacity), timer, bag roller, sample cup rack, pipettes (0.1 mL), result interpretation card.
Reagents Required		Reveal ASP test devices, extraction bags with mesh filter, sample cups, distilled water.
Sample Collection, Preservation and Storage Requirements		Shellfish should be collected according to standard industry practices and stored at 2-8^{o}C before testing.
Safety Requirements		Used test devices, extraction bags, sample cups, and pipettes should be treated as if contaminated with domoic acid and handled accordingly. Gloves and lab coats should be worn while performing the test.
Clear and Easy to Follow Step-by-Step Procedure		Step-by-step procedure in kit insert and study report.
Quality Control Steps Specific for this Method		Test device contains an internal positive control that confirms that it is functioning properly. A domoic acid solution in buffer at a concentration $\geq$ 10 mg/kg can be used as an external positive control if desired.

C. Validation Criteria		
1. Accuracy / Trueness		95.9% overall for oysters, clams, and mussels
2. Measurement Uncertainty		Not applicable.
3. Precision Characteristics (repeatability and reproducibility)		Not applicable.
4. Recovery		Not applicable.
5. Specificity		100%. No impact on test results by potentially interfering compounds – okadaic acid, glutamic acid, glutamine, saxitoxin. No false-positive results on unpiked samples.
6. Working and Linear Ranges		Not applicable.
7. Limit of Detection		≥ 10 ppm
8. Limit of Quantitation / Sensitivity		Not applicable.
9. Ruggedness		No statistically significant differences in results using 2 kit lots and +/- 2 min. variation in test incubation time.
10. Matrix Effects		None observed.
11. Comparability (if intended as a substitute for an established method accepted by the NSSP)		Agreement with LC-UV reference method in testing of mussel tissue samples with incurred domoic acid.
D. Other Information		
1. Cost of the Method		Approx. $17.00 per test.
2. Special Technical Skills Required to Perform the Method		None
3. Special Equipment Required and Associated Cost		None
4. Abbreviations and Acronyms Defined		ppm = parts per million, equivalent to mg/kg
5. Details of Turn Around Times (time involved to complete the method)		The test can be performed in approximately 20 minutes including sample preparation.
6. Provide Brief Overview of the Quality Systems Used in the Lab		

Submitters Signature 	Date: June 3, 2011
Submission of Validation Data and Draft Method to Committee	Date:
Reviewing Members	Date:
Accepted	Date:
Recommendations for Further Work	Date:
Comments:	

DEFINITIONS

1. Accuracy/Trueness - Closeness of agreement between a test result and the accepted reference value.
2. Analyte/measurand - The specific organism or chemical substance sought or determined in a sample.
3. Blank - Sample material containing no detectable level of the analyte or measurand of interest that is subjected to the analytical process and monitors contamination during analysis.
4. Comparability – The acceptability of a new or modified method as a substitute for an established method in the NSSP. Comparability must be demonstrated for each substrate or tissue type by season and geographic area if applicable.
5. Fit for purpose – The analytical method is appropriate to the purpose for which the results are likely to be used.
6. HORRAT value – HORRAT values give a measure of the acceptability of the precision characteristics of a method.[4]
7. Limit of Detection – the minimum concentration at which the analyte or measurand can be identified. Limit of detection is matrix and analyte/measurand dependent.[4]
8. Limit of Quantitation/Sensitivity – the minimum concentration of the analyte or measurand that can be quantified with an acceptable level of precision and accuracy under the conditions of the test.
9. Linear Range – the range within the working range where the results are proportional to the concentration of the analyte or measurand present in the sample.
10. Measurement Uncertainty – A single parameter (usually a standard deviation or confidence interval) expressing the possible range of values around the measured result within which the true value is expected to be with a stated degree of probability. It takes into account all recognized effects operating on the result including: overall precision of the complete method, the method and laboratory bias and matrix effects.
11. Matrix – The component or substrate of a test sample.
12. Method Validation – The process of verifying that a method is fit for purpose.[1]
13. Precision – the closeness of agreement between independent test results obtained under stipulated conditions.[1, 2] There are two components of precision:
    a. Repeatability – the measure of agreement of replicate tests carried out on the same sample in the same laboratory by the same analyst within short intervals of time.
    b. Reproducibility – the measure of agreement between tests carried out in different laboratories. In single laboratory validation studies reproducibility is the closeness of agreement between results obtained with the same method on replicate analytical portions with different analysts or with the same analyst on different days.
14. Quality System - The laboratory's quality system is the process by which the laboratory conducts its activities so as to provide data of known and documented quality with which to demonstrate regulatory compliance and for other decision-making purposes. This system includes a process by which appropriate analytical methods are selected, their capability is evaluated, and their performance is documented. The quality system shall be documented in the laboratory's quality manual.
15. Recovery - The fraction or percentage of an analyte or measurand recovered following sample analysis.
16. Ruggedness – the ability of a particular method to withstand relatively minor changes in analytical technique, reagents, or environmental factors likely to arise in different test environments.[4]
17. Specificity – the ability of a method to measure only what it is intended to measure.[1]
18. Working Range – the range of analyte or measurand concentration over which the method is applied.

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7. Eurachem Guide, 1998. The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics. LGC Ltd. Teddington, Middlesex, United Kingdom.
8. IUPAC Technical Report, 2002. Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis, Pure Appl. Chem., Vol. 74, (5): 835-855.
9. Joint FAO/IAEA Expert Consultation, 1999. Guidelines for Single-Laboratory Validation of Anilytical Methods for Trace-Level Concentrations of Organic Chemicals.
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11. National Environmental Laboratory Accreditation. , 2003. Standards. June 5.
12. EPA. 2004. EPA Microbiological Alternate Procedure Test Procedure (ATP) Protocol for Drinking Water, Ambient Water, and Wastewater Monitoring Methods: Guidance. U.S. Environmental Protection Agency (EPA), Office of Water Engineering and Analysis Division, 1200 Pennsylvania Avenue, NW, (4303T), Washington, DC 20460. April.

Validation Study of the Reveal® ASP Test for the Qualitative Detection of Domoic Acid in Shellfish

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Introduction

Domoic acid, produced by certain species of the diatom *Pseudonitzschia*, is the primary toxin responsible for amnesic shellfish poisoning (ASP) associated with consumption of contaminated shellfish including oysters, clams, and mussels. Current methodologies for detection of domoic acid in shellfish are laborious and time-consuming, consisting primarily of LC-UV, LC-MS, and immunoassay procedures. LC-UV methods [1, 2] have been accepted as quantitative reference methods in many parts of the world. Assays facilitating more rapid determination of domoic acid with simplified procedures are needed by the shellfish industry and regulatory authorities.

In this report, we describe results of a validation study of the Reveal® ASP test for qualitative detection of domoic acid in shellfish. Reveal ASP is a lateral flow immunoassay designed for rapid determination of domoic acid at a level of approximately 10 ppm or greater (one-half the regulatory limit in many countries). The test is easy to use and results can be obtained in less than 20 minutes, including sample preparation.

Principle of the Method

Reveal ASP is a single-step, lateral flow immunochromatographic assay based on the principle of competitive immunoassay. Following a simple distilled water extraction of domoic acid from homogenized shellfish tissue, the dipstick-format Reveal device is placed into the extract. The extract is wicked through a reagent zone containing antibodies specific for domoic acid conjugated to colloidal gold particles. If domoic acid is present, it will be captured by the labeled antibody. Migration of the sample continues through a membrane, which contains a zone of domoic acid conjugated to a protein carrier. This zone captures any unbound antibody- gold conjugate, resulting in a visible line. With increasing amounts of domoic acid in the test sample, less unbound conjugate is available for binding to the test line. Thus, intensity of the test line is inversely proportional to the amount of domoic acid in the sample. The test device also incorporates a control conjugate and which binds to a second line. The control line will form regardless of the amount of domoic acid present in the sample, ensuring that the test device is functioning properly. Test results are interpreted as positive or negative by scoring the intensity of the test line using an interpretation card supplied with the test kit.

Intended Use

For the qualitative detection (at greater than or equal to 10 ppm [mg/kg]) of domoic acid in shellfish, including oysters, clams, and mussels.

Reveal ASP Method

The kit insert is included as Appendix I.

Materials Provided

Starter Kit (Neogen #9563), contains:

- Sample cup rack
- Roller

Reveal ASP kit (Neogen #9560), contains:

- 25 lateral flow test strips
- 25 sample cups
- 25 filter extraction bags
- 50 100 µL disposable pipettes
- Interpretation card

Materials Required but not Supplied (available from Neogen Corp. and other sources)

- Blender and blender jar
- Scale, capable of weighing 0.5-400 g $\pm$ 0.1 g
- Timer
- 50-mL graduated cylinder or bottle-top liquid dispenser
- Distilled water
- Leakproof container with lid, 40 mL capacity

Storage Requirements

Store Reveal ASP kit components at controlled room temperature (18-30^{o}C, 64-86^{o}F). Do not freeze.
Test strips should remain in their original sample tubes until use to maintain shelf life and ensure optimal performance.

Precautions

Do not use test kits beyond their expiration date.
Treat all liquids, including sample extract, and used components as if contaminated with toxin. Gloves and other protective apparel should be worn at all times.
To avoid cross-contamination, use clean pipettes, extraction bags, and fresh extraction solution for each sample.
A Material Safety Data Sheet (MSDS) is available from Neogen Corp.

Sample Preparation and Extraction

Samples should be collected according to accepted sampling techniques.

1. Obtain a representative sample and shell the sample.
2. Thoroughly rinse with cold water.
3. Homogenize in a high-speed blender.
4. Weigh 1.0 $\pm$ 0.1 g of homogenized sample, preferably in a leak-proof container capable of holding 40 mL of liquid.
5. Add 20 mL distilled water to the container with sample.
6. Shake the container vigorously by hand for 30 seconds until all shellfish tissue is in solution (a cloudy appearance and/or bubbles are normal).
7. Number one side of the extraction bag “1” and the other side “2”.
8. Pour the sample extract into side 1 of the extraction bag. The extraction bag contains a mesh filter which allows for partial filtration of the sample extract.

9. Seal the extraction bag by positioning the green straw approximately 2-3 inches down from the top of the bag, fold the upper edge of the bag so that it covers the green straw, and firmly clip on the white bag clip. This prevents leakage of the sample extract.

10. Press the roller firmly on the extraction bag, pushing the roller back and forth for 30 seconds to aid in obtaining a homogenous sample extract.

11. Slide out the green straw and remove the white bag clip.

12. Pinch the top of the bag and carefully pour all the bag contents from side 2 back into the original sample container (there may be small pieces of shellfish remaining on side 1 of the bag). Discard the used extraction bag.

13. Shake container vigorously by hand for 30 seconds.

14. Remove 100 µL of the sample extract using the disposable pipette* provided (alternatively by use of a standard pipette), and add to a fresh container containing 20 mL distilled water.

    * To use the disposable pipettes provided, firmly press the top bulb of the pipette, insert the tip into the sample extract, and slowly release the top bulb to draw up the sample extract. Excess volume (above 100 µL) will overflow into the lower bulb, ensuring that 100 µL is available to dispense. Press the top bulb firmly and slowly release the top bulb to dispense the liquid into the container with distilled water. Discard the used pipette.

Assay Procedure

All steps should be performed at controlled room temperature (18-30^{o}C, 64-86^{o}C).

1. Remove the appropriate number of sample cups and place in the sample cup rack.

2. Shake the extracted sample prepared above vigorously by hand for 30 seconds.

3. Remove 100 uL using a fresh pipette and add 100 uL to the sample cup.

4. Remove the required number of Reveal ASP test strips from the container and immediately close the container.

5. Place the Reveal test strip with the sample end down (Neogen logo on top) into the sample cup.

6. Allow the test strip to develop in the sample cup for 10 minutes.

7. Remove the test strip and interpret the results as described below.

Interpretation of Results

Test strips should be interpreted immediately following completion of the 10 minute incubation.
Using the interpretation card provided, score the test line intensity to determine if the sample contains less than 10 ppm or greater than or equal to 10 ppm domoic acid.
Note: The control line should always be present and will be darker than the test line. If no control line is visible, this indicates an invalid result and the sample should be retested using another Reveal device.

Single-Laboratory Validation Study

A single-laboratory validation study was conducted to measure accuracy/trueness, specificity, and ruggedness of the Reveal ASP method, as well as effects of potential interfering compounds. In addition, Reveal ASP results were compared to those of an accepted LC-UV reference method [1]. Matrices tested were oysters, clams, and mussels.

I. Accuracy/trueness and specificity

Methods
Fresh oysters, clams, and mussels were obtained from a local retail market that receives fresh shellfish by air shipment daily. Shellfish were held at 2-8°C before use. Shellfish were shucked and approximately 12-15 animals were combined and homogenized in a blender to produce a bulk sample. The bulk samples were separated into 10 portions of 1 g each. Five served as unspiked controls. One each of the remaining 5 samples was spiked separately at 5, 10, 15, 20 and 40 ppm domoic acid. Certified reference material (CRM-DA-f), obtained from the National Research Council, Canada- Institute for Marine Biosciences (NRC- IMB), was used as the spiking material. The CRM consisted of 101.8 µg/mL domoic acid extracted from contaminated cultured blue mussels and dissolved in a solution of 5% acetonitrile/95% water.
Each sample was then prepared according to the procedures in **Sample Preparation and Extraction** above, and tested with the Reveal ASP assay. Ten replicates of each extracted spiked sample and three replicates of each extracted unspiked sample were tested with the Reveal ASP assay.

Accuracy rates were calculated for each shellfish matrix separately and in combination. A dose-response curve was constructed using the combined data.

Results
Results of the accuracy study are shown in Table 1. Accuracy is defined as the level of agreement between the assay and the expected test results based on the domoic acid spike level.
For oysters, accuracy of the Reveal ASP method was 95.4%. Seven of ten tests at 10 ppm domoic acid were positive. All tests at higher levels of domoic acid were positive. All tests at 5 ppm were negative. There were no false-positive results on unspiked control samples.
For clams, accuracy of the assay was 92.3%. All tests at 10 ppm domoic acid and higher were positive. Five of ten tests at 5 ppm domoic acid were also positive. There were no false positive results on unspiked control samples.
For mussels, accuracy of the assay was 100%. All tests at 10 ppm domoic acid and higher were positive. All tests at 5 ppm were negative. There were no false-positive results on unspiked control samples.

Overall accuracy of the Reveal ASP test was 95.9%. A dose-response curve was constructed using combined data from all three shellfish matrices and is shown in Fig. 1. Based on the dose-response curve, performance of the Reveal ASP test can be characterized as follows:

Zone 1	Positive < 5% of the time	< 2 ppm domoic acid
Zone 2	Positive 5-50% of the time	2-7 ppm domoic acid
Zone 3	Positive 51-95% of the time	8-11 ppm domoic acid
Zone 4	Positive > 95% of the time	> 11 ppm domoic acid

II. Interfering compounds

Methods
Fresh oysters, clams, and mussels were obtained as described above. Approximately 12-15 animals were combined and homogenized in a blender a produce a bulk sample. The bulk samples were separated into 12 portions of 1 g each. The 12 portions were separated into 4 groups each containing three 1-g samples. Samples in each group were spiked individually with one of the following potentially interfering compounds: okadaic acid, 10 ppm; glutamic acid, 100 ppm; glutamine, 100 ppm; or saxitoxin, 5 ppm. One sample in each group was spiked with 10 ppm domoic acid, one sample was spiked with 40 ppm domoic acid, and one sample was left unspiked. All interfering compounds were obtained from Sigma, except saxitoxin which was obtained from NRC-IMB. Domoic acid CRM, described above, was used as the spiking material.
Sample preparation and testing were performed as described above. Five replicates of each extracted sample were tested with the Reveal ASP assay.

Results

Results of testing for effects of potentially interfering compounds on performance of the Reveal ASP assay are shown in Table 2. There was no evidence of interference by okadaic acid, glutamic acid, glutamine, or saxitoxin on assay performance in any of the three shellfish types. All tests produced expected results at levels of 0, 10, and 40 ppm domoic acid.

III. Ruggedness

Methods
Fresh oysters, clams, and mussels were obtained as described above. Approximately 12-15 animals were combined and homogenized in a blender a produce a bulk sample. The bulk samples were separated into 3 portions of 1 g each. One portion was spiked at 10 ppm, one at 40 ppm, and the remaining sample left unspiked. Domoic acid CRM, described above, was used as the spiking material.

Sample preparation and testing were performed as described above. Ten replicates of each extracted sample were tested with the Reveal ASP assay. Each replicate was tested using devices from two different test kit lots (LFD-001 and LFD-002). The devices were interpreted after 8, 10 and 12 minutes to measure potential differences in results at different test incubation times. For each shellfish type, this trial was performed twice, on separate days, by two operators each day.
For each shellfish matrix, results from the two days of testing were pooled. Chi-square analysis (McNemar's test, [3]) was performed to determine if results were significantly different for the two kit lots or three test incubation times evaluated.

Results
Results of assay ruggedness trials with respect to Reveal ASP kit lot and assay incubation period are shown in Tables 3 and 4, respectively. In the trials measuring the effect of kit lot, there were no significant differences in the number of positives obtained with kit lots 1 and 2 at any spike level in any shellfish matrix, as determined by chi-square analysis at $p < 0.05$ (Table 3). Similarly, in the trials measuring the effect of variation in test incubation time, there were no significant differences in the number of positives obtained at incubation times of 8, 10 and 12 minutes at any spike level in any shellfish matrix (Table 4).

IV. Comparison with Reference Method

Methods
Fresh mussels were obtained as described above. Approximately 12-15 animals were combined and homogenized in a blender a produce a bulk sample. Incurred CRM consisting of a thermally stabilized homogenate of mussel tissue containing domoic acid at a concentration of 41 µg/g (ppm) was purchased from NRC-IMB (CRM-ASP-Mus-c). The incurred material was blended 1:1 with clean mussel tissue to obtain a domoic acid level of approximately 20 ppm. From the blended material, 20 samples of 1 g each were prepared. Ten samples were retained and tested in triplicate using the Reveal ASP test. The remaining 10 samples were sent to NRC-IMB for testing by the LC-UV method.

Results
Results of testing of samples of mussel tissue with incurred domoic acid by both the Reveal ASP assay and a reference LC-UV quantitative method are shown in Table 5. All 10 samples tested with the Reveal ASP method produced positive results. Results obtained with the LC-UV method were also positive for all 10 samples, ranging from 11.9 to 16.4 ppm.

Quality Control Testing
Quality control testing of manufactured lots of the Reveal ASP assay is performed at both in-process and finished product stages. In-process testing consists of balancing the antibody-colloidal gold conjugate for optimal test and control line intensity, and testing the device membrane for proper test and control line placement by running negative samples.

For finished product testing, samples are produced by diluting domoic acid (certified reference material CRM-DA-f, NRC-IMB) to concentrations of 2, 10, and 40 ppm in buffer. An unspiked sample is also prepared. Ten Reveal devices, randomly selected from the lot, are run at each concentration. For acceptance of the lot, all tests at 0 and 2 ppm must be negative and all tests at 10 and 40 ppm must be positive.

Discussion

Results of the validation study showed that the Reveal ASP test is an effective procedure for qualitative determination of domoic acid in oysters, clams, and mussels. In the accuracy study, all tests at the accepted action level of 20 ppm were positive. There were no false-positive results on unspiked control samples. The dose-response curve indicates that the test produces a positive result greater than 95% of the time at a concentration above 11 ppm, 51-95% of the time at a concentration of 8-11 ppm, and less frequently at levels below 8 ppm.

Four compounds, okadaic acid, glutamic acid, glutamine, and saxitoxin, were tested for potential interference with the Reveal ASP assay. None was noted, as all samples produced the expected results at 0, 10, and 40 ppm domoic acid.

Results of ruggedness trials indicated that there was no statistically significant difference in performance between two Reveal ASP kit lots, nor was there any significant difference in performance in assays conducted with variation of +/- 2 minutes around the specified incubation time of 10 minutes.

Results of testing of mussel tissue samples containing incurred domoic acid showed agreement between the Reveal ASP and reference LC-UV methods, with all 10 samples testing positive by Reveal and LC-UV producing results in the range of 11.9-16.4 ppm.

Reveal ASP can be used as an accurate screening test for the rapid determination of domoic acid in shellfish. The test requires little equipment, uses water for sample extraction, and can be performed by personnel with minimal training. The test can be used in a field or laboratory setting, with results available within 20 minutes of sample receipt.

It is recommended that the Reveal ASP test be approved by the Interstate Shellfish Sanitation Conference as a screening method for qualitative determination of domoic acid in oysters, clams, and mussels.

References

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Acknowledgements

We thank Dr. Michael Quilliam and Kelley Reeves of the National Research Council, Institute for Marine Biosciences, Halifax, Nova Scotia, Canada for performing the LC-UV analyses.

Table 1. Results of accuracy study of the Reveal ASP test.

Sample Type	Level Domoic Acid (ppm)	Number Tests	Number Positive
Oysters	0	15	0
	5	10	0
	10	10	7
	15	10	10
	20	10	10
	40	10	10
Clams	0	15	0
	5	10	5
	10	10	10
	15	10	10
	20	10	10
	40	10	10
Mussels	0	15	0
	5	10	0
	10	10	10
	15	10	10
	20	10	10
	40	10	10
All Data	0	45	0
	5	30	5
	10	30	27
	15	30	30
	20	30	30
	40	30	30

Table 2. Results of interference study for the Reveal ASP test.

Sample Type	Interfering Compound and Level	Level Domoic Acid (ppm)	Number Tests	Number Positive
Oysters	Okadaic acid 10 ppm	0	5	0
		10	5	5
		40	5	5
	Glutamic acid 100 ppm	0	5	0
		10	5	5
		40	5	5
	Glutamine 100 ppm	0	5	0
		10	5	5
		40	5	5
	Saxitoxin 5 ppm	0	5	0
		10	5	5
		40	5	5
Clams	Okadaic acid 10 ppm	0	5	0
		10	5	5
		40	5	5
	Glutamic acid 100 ppm	0	5	0
		10	5	5
		40	5	5
	Glutamine 100 ppm	0	5	0
		10	5	5
		40	5	5
	Saxitoxin 5 ppm	0	5	0
		10	5	5
		40	5	5
Mussels	Okadaic acid 10 ppm	0	5	0
		10	5	5
		40	5	5
	Glutamic acid 100 ppm	0	5	0
		10	5	5
		40	5	5
	Glutamine 100 ppm	0	5	0
		10	5	5
		40	5	5
	Saxitoxin 5 ppm	0	5	0
		10	5	5
		40	5	5

Table 3. Results of assay ruggedness trials for the Reveal ASP test – effect of kit lot.

Sample Type	Level domoic acid (ppm)	Number Tests	Number Positive Lot 1	Number Positive Lot 2	χ^{2a}
Oysters	0	20	0	0	-[b]
	10	20	15	15	-
	40	20	20	20	-
Clams	0	20	0	0	-
	10	20	18	20	0.50
	40	20	20	20	-
Mussels	0	20	0	0	-
	10	20	15	14	0.00
	40	20	20	20	-
All Data	0	60	0	0	-
	10	60	48	49	0.00
	40	60	60	60	-

[a] $\chi^2 > 3.84$ indicates a significant difference at $\rho < 0.05$.
[b] χ^2 not applicable since all results were in agreement.

Table 4. Results of assay ruggedness trials for the Reveal ASP test – effect of incubation time.

Sample Type	Level domoic acid (ppm)	Number Tests	Number Positive 8 min.	Number Positive 10 min.	Number Positive 12 min.	χ^{2a} 8 vs. 10 min.	χ^2 12 vs. 10 min.
Oysters	0	40	0	0	0	-[b]	-
	10	40	36	35	35	0.00	-
	40	40	40	40	40	-	-
Clams	0	40	0	0	0	-	-
	10	40	34	37	38	0.44	0.00
	40	40	40	40	40	-	-
Mussels	0	40	0	0	0	-	-
	10	40	30	29	29	0.00	-
	40	40	40	40	40	-	-
All Data	0	120	0	0	0	-	-
	10	120	100	101	102	0.00	0.00
	40	120	120	120	120	-	-

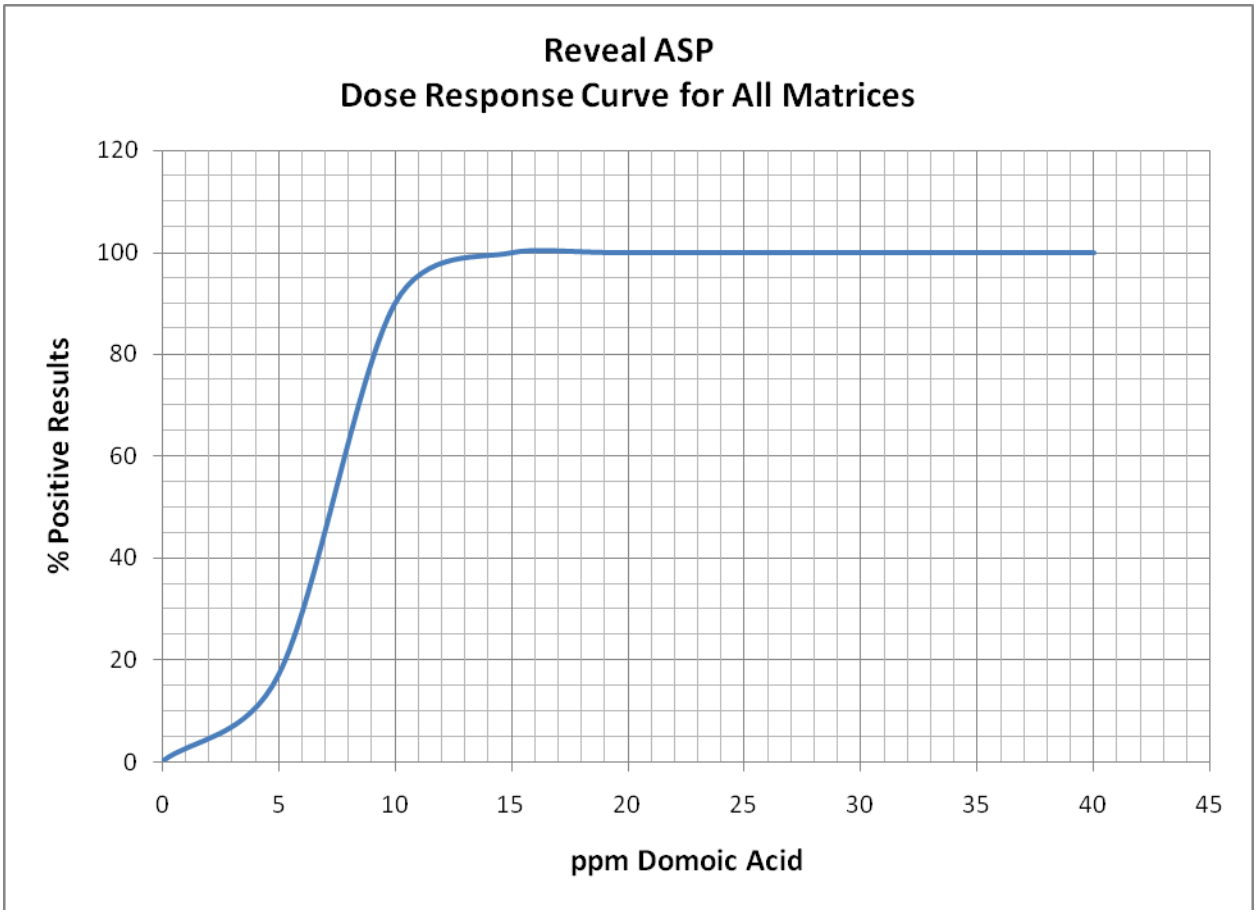
[a] $\chi^2 > 3.84$ indicates a significant difference at $\rho < 0.05$.
[b] χ^2 not applicable since all results were in agreement.

Table 5. Results of testing of domoic acid containing mussel tissue with Reveal ASP and a LC-UV reference method.

Sample No.	LC-UV Method Result ppm Domoic Acid (mean ± SD)[a]	Reveal ASP Result
1	16.4 ± 0.3	Positive
2	15.4 ± 0.1	Positive
3	14.5 ± 0.1	Positive
4	15.7 ± 0.2	Positive
5	15.06 ± 0.04	Positive
6	14.60 ± 0.03	Positive
7	13.65 ± 0.06	Positive
8	15.17 ± 0.08	Positive
9	14.0 ± 0.1	Positive
10	11.92 ± 0.05	Positive

[a] Mean of 3 determinations.

Fig. 1. Dose-response curve for the Reveal ASP test.



Appendix I. Reveal ASP kit insert

Product #9560

Read instructions carefully before starting test

Reveal®
ASP
(Domoic acid)

THE TOXIN

Amnesic shellfish poisoning (ASP) causing toxins are produced mainly by the toxigenic diatom strain *Pseudo-nitzchia multiseries*. The ASP toxins include primarily domoic acid (DA).

In addition to contamination of seafood, these marine biotoxins can result in human and marine wildlife mortality. The clinical toxicological effects attributed to DA can include: permanent loss of short-term memory, nausea, vomiting, headache, disorientation, and loss of balance.

Action limits for DA were established soon after the 1987 domoic acid/mussel crisis in Canada in which over 150 people became ill and four deaths resulted. Many countries have currently established a maximum permitted level of 20 mg DA per kg in whole shellfish (20 ppm).

INTENDED USE/USER

Reveal for ASP is intended for the qualitative screening of shellfish for DA, with the cut-off value for a positive result at approximately 10 ppm (i.e., half of the regulatory limit in many countries). The test kit is designed for use by personnel with an interest in the rapid screening of shellfish samples (further to obtaining a sample homogenate, it takes less than 20 minutes to carry out the sample extraction and obtain the result).

ASSAY PRINCIPLES

Reveal for ASP is a single-step lateral flow immunochromatographic assay based on a competitive immunoassay format. The extract is wicked through a reagent zone, which contains antibodies specific for DA conjugated to coloured particles. These DA-antibody-particle complexes result in the visible signal. If DA is present, it will be captured by the particle-antibody complex. The particle-antibody complex is then wicked onto a membrane, which contains a zone of DA conjugated to a protein carrier. This zone captures any uncomplexed DA antibody, allowing particles to concentrate and form a visible line. As the level of DA in the sample increases, free DA will complex with the particle-antibody complex. This, in turn, allows less particle-antibody complexes to be captured in the test zone. Therefore, as the concentration of DA in the sample increases, the test line decreases. The membrane also contains a control zone where an immune complex present in the reagent zone is captured by the antibody, forming a visible line. The control line will always form regardless of the level of DA, ensuring the strip is functioning properly.

STORAGE REQUIREMENTS

Store kit components at room temperature (18-30°C, 64–86°F) to assure full shelf life. Test strips should remain capped in their original sample tubes until used to assure optimal performance.

PRECAUTIONS

1. The test strips must remain inside the stay-dry tube before use.
2. Store test kit at room temperature (18–30°C, 64–86°F) when not in use, do not freeze.
3. Do not use kit contents beyond expiration date.
4. Treat all liquids, including sample extract, and used components as if contaminated with toxin. Gloves and other protective apparel should be worn at all times.
5. To avoid cross-contamination- use clean pipettors, extraction bags and fresh extraction solutions for each sample.

MATERIALS PROVIDED

9563 - Starter Kit - Reveal ASP (Domoic Acid)	**9560 - Reveal ASP (Domoic Acid)**
1 Reveal sample cup rack	25 ASP lateral flow test strips
1 Roller	25 sample cups
1 Bag-clip (white clip and green straw)	25 filter extraction bags
	50 exact volume pipettors

MATERIALS RECOMMENDED BUT NOT PROVIDED

1. Distilled water
2. 40 mL leakproof container including lids
3. Blender, Oster (Neogen item #9493)
4. Blender Jar, MINI, with blade & cap, 250 mL (Neogen item #9477)
5. Blender Jar, Stainless Steel 1 L (Neogen item #9495)
4. Scale capable of weighing 0.5–400 g ± 0.1 g (Neogen item #9427)
5. Timer (Neogen item #9452)
6. 50 mL Graduated cylinder (Neogen item #9367)
7. Bottle-top dispenser (Neogen item #9448)

EXTRACTION SOLUTION PREPARATION

The required extraction solution for the test is distilled water. A total volume of 40 ml of the solution is required per each sample tested. Fill 2 bottles with 20 mL each of distilled water, and label as SOLUTION 1 and SOLUTION 2, respectively.

Please note: *The solution containers should be capable of holding a volume of 40 mL to effectively carry out the procedure.*

SAMPLE PREPARATION AND EXTRACTION

The sample to be tested should be collected according to accepted sampling techniques.

1. Obtain a representative sample. Shell the samples.
2. Thoroughly rinse with cold water.
3. Homogenise† the shellfish in a high-speed blender.
   IMPORTANT: A good homogenate is essential in order to obtain an accurate result.
4. Weigh 1g (± 0.1g) of homogenized sample, ideally in leak-proof bottle capable of holding ~40 mL of liquid.
5. Pour entire contents of one bottle containing 20 mL of SOLUTION 1 into bottle containing one sample.
6. Shake the sample bottle vigorously by hand for 30 seconds, until all shellfish tissue is in solution (a cloudy appearance or bubbles may form, which do not affect the running of the test).
7. Number both sides of the extraction bag using a marker, so that there is a side labeled "1" and the other side labeled "2". Pour solution/sample mixture into one extraction bag (side 1). The extraction bag contains a mesh filter which allows for partial filtration of the sample.

8. To seal the bag, position and hold the green straw approximately 2–3 inches down from the top of the bag, fold the upper edge of the bag so that it covers the green straw and firmly clip on the white bag-clip. This prevents leakage of the sample.
9. Press the roller firmly on the sample extraction bag, pushing the roller back and forth for 30 seconds to aid in obtaining a homogenous sample extract.
10. Slide out the green straw and remove the white bag-clip.
11. Pinch the top of the bag and carefully pour all the bag contents from side 2 back into the original sample container (there may be small pieces of shellfish remaining on side "1" of the bag). **Discard the used extraction bag** (***Note:*** *pinching the top of the bag to create a sharp edge allows easy pouring of the sample, preventing any spillage*).
12. Shake bottle with sample extract vigorously by hand for 30 seconds.
13. Remove 100ul of the sample extract using the exact-volume pipettors* provided (alternatively by use of a standard pipettor), and add into a fresh bottle containing SOLUTION 2.

    **To use the exact-volume pipettors, firmly press the top bulb of the pipettor, insert the tip into the sample, slowly release the top bulb to draw up the sample extract. Excess volume (i.e. above 100 µL) will overflow into the lower bulb, ensuring 100µl is ready to dispense. Press the top bulb firmly and slowly release the top bulb to dispense 100 µL into the bottle containing SOLUTION 2. Discard the used pipettor.*

    †Homegenise – to blend or to puree

TEST PROCEDURE

1. Remove the appropriate number of sample cups and place in the sample cup tray.
2. Shake the SOLUTION 2 bottle (which contains 100 µL of the sample extract) vigorously by hand for 30 seconds.
3. Remove 100 µL from the SOLUTION 2 bottle using a fresh pipettor and add 100 µL per sample cup.
4. Remove the required number of strips from the lateral flow device container and immediately close the container.
5. Place the new ASP strip with the sample end down (Neogen logo on top) into the sample cup.
6. Allow the strip to develop in the sample cup for 10 minutes.
7. Remove strip and interpret the results (as described below).

INTERPRETATION OF RESULTS (VISUAL)

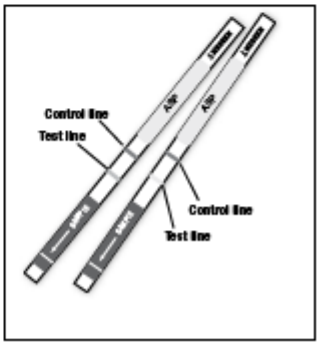
Test strips should be interpreted directly following completion of the 10 minute incubation.

Use the provided interpretation card to determine the level of the toxin present in the sample.

Note: *The control line should always be present and will always be darker than the test line. If there is no control line development, this indicates an invalid result and the sample should be retested using another device.*

MSDS INFORMATION AVAILABLE

Material safety data sheets (MSDS) are available for this test kit, and all of Neogen's Food Safety test kits, at www.neogeneurope.com.



VALIDATED MATRICES

Mussels, scallops, oysters, clams and cockles. Contact your Neogen representative concerning additional commodities.

WARRANTY

Neogen Corporation makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. If any materials are defective, Neogen will provide a replacement product. Buyer assumes all risk and liability resulting from the use of this product. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. Neogen shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.

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 NE430-0111

Proposal Subject: Update Microbiology Laboratory Evaluation Checklist

Specific NSSP Guide Reference: 2009 NSSP Section IV. Guidance Documents Chapter II. Growing Areas
.11 Evaluation of Laboratories By State Shellfish Laboratory Evaluation Officers Including Laboratory Evaluation Checklists Laboratory Evaluation Checklist – Microbiology

Text of Proposal/ Requested Action Update Microbiology Laboratory Evaluation Checklist. Please find the updated Microbiology Laboratory Checklist attached - word document titled "Revised Microbiology Checklist 11-08-2010.doc".
A summary of the changes is:

- Renumbered checklist items to accommodate proposed additions and deletions and to better identify each checklist item.
- Added, deleted or changed language for checklist items to be consistent with the PSP laboratory evaluation checklist.
- Deleted the requirement for metals testing on reagent water and the inhibitory residue test for washed labware and increased the requirements for the bromothymol blue test.
- Clarified and defined requirements for laboratory equipment, reagents including the bacterial quality control requirements for media productivity and method process control testing.
- Update thermometer requirements to accommodate state bans on the use of mercury thermometers.
- Updated the sterility check requirements for both in lab sterilized items and purchased pre-sterilized items.

Public Health Significance: The current microbiology laboratory checklist was last revised in 2009 when the male specific coliphage method was approved and added to the checklist. Deficiencies have been identified while using the microbiology checklist in evaluation of laboratories and the microbiology checklist is inconsistent with some requirements in the PSP checklist. It is important that the checklist items and quality assurance requirements are clear and understandable. It is important that quality assurance requirements among the different laboratory evaluation checklists remain as consistent as possible since many monitoring laboratories perform multiple types of tests and are evaluated using multiple NSSP checklists; inconsistencies among the checklist cause confusion, extra expense and work for the laboratories.

Cost Information (if available): None

Action by 2011 Laboratory Methods Review Committee Recommended referral of Proposal 11-108 to the appropriate committee as determined by the Conference Chairman.

Action by 2011 Task Force I Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 11-108.

Action by 2011 General Assembly Adopted recommendation of 2011 Task Force I on Proposal 11-108.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-108.

PUBLIC HEALTH SERVICE
U.S. FOOD AND DRUG ADMINISTRATION
~~**SHELLFISH PROGRAM IMPLEMENTATION BRANCH**~~ **OFFICE OF FOOD SAFETY**
~~**SHELLFISH SAFETY TEAM**~~ **SHELLFISH AND AQUACULTURE POLICY BRANCH**
5100 PAINT BRANCH PARKWAY
COLLEGE PARK, MD 20740-3835
TEL. ~~301~~240-~~436~~ 402-2151/~~2147~~2055 FAX ~~301~~240-~~436~~ 402-~~2601~~2672

SHELLFISH LABORATORY EVALUATION CHECKLIST

LABORATORY:		
ADDRESS:		
TELEPHONE:	**FAX:**	
EMAIL:		
DATE OF EVALUATION:	**DATE OF REPORT:**	**LAST EVALUATION:**

LABORATORY REPRESENTED BY:	**TITLE:**
LABORATORY EVALUATION OFFICER:	**SHELLFISH SPECIALIST:** **REGION:**
OTHER OFFICIALS PRESENT:	**TITLE:**

Items which do not conform are noted by:
C- Critical K - Key O - Other NA- Not Applicable Conformity is noted by a "√"

Check the applicable analytical methods:

☐	Multiple Tube Fermentation Technique for Seawater (APHA)[PART II]
☐	Multiple Tube Fermentation Technique for Seawater using MA-1 [PART II]
☐	Membrane Filtration Technique for Seawater using mTEC [PART II]
☐	Multiple Tube Fermentation Technique for Shellfish Meats (APHA)[PART III]
☐	Standard Plate Count for Shellfish Meats [PART III]
☐	Elevated Temperature Coliform Plate Method for Shellfish Meats [PART III]
☐	Male Specific Coliphage for Soft-shelled Clams and American Oysters [PART III]

PART 1 - QUALITY ASSURANCE

CODE	REF.	ITEM
K	8, 11	1.1 **Quality Assurance (QA) Plan**

			~~1.~~ 1.1.1 Written Plan (Check those items which apply.)
		☐	a. Organization of the laboratory.
		☐	b. Staff training requirements.
		☐	c. Standard operating procedures.
		☐	d. Internal quality control measures for equipment, their calibration, maintenance, repair, ~~and for~~ performance ~~checks~~ and rejection criteria established
		☐	e. Laboratory safety.
		☐	f. Internal performance assessment.
		☐	g. External performance assessment.
C	**8**	☐	~~6.~~ **1.1.2 QA Plan Implemented**
K	11	☐	~~7.~~ 1.1.3 The Laboratory participates in a proficiency testing program annually. Specify Program(s)_____
		1.2 Educational/Experience Requirements	
C	State's Human Resources Department	☐	~~2.~~ **1.2.1 In state/county laboratories, the supervisor meets the state/county educational and experience requirements for managing a public health laboratory**
K	State's Human Resources Department	☐	~~3.~~ 1.2.2 In state/county laboratories, the analyst(s) meets the state/county educational and experience requirements for processing samples in a public health laboratory.
C	USDA Microbiology & EELAP	☐	~~4.~~ **1.2.3 In ~~private~~ commercial laboratories, the supervisor must have at least a bachelor's degree in microbiology, biology, or equivalent discipline with at least two years of laboratory experience.**
K	USDA Microbiology & EELAP	☐	~~5.~~ 1.2.4 In ~~private~~ commercial laboratories, the analyst(s) must have at least a high school diploma and shall have at least three months of experience in laboratory sciences.
		1.3 Work Area	
O	8,11	☐	~~1.~~ 1.3.1 Adequate for workload and storage.
K	11	☐	~~2.~~ 1.3.2 Clean, well lighted.
K	11	☐	~~3.~~ 1.3.3 Adequate temperature control.
O	11	☐	~~4.~~ 1.3.4 All work surfaces are nonporous, easily cleaned and disinfected.
K	11	☐	~~5. Microbiological quality and density of air is < 15 colonies/plate in a 15 minute exposure determined monthly and results recorded.~~ 1.3.5 Microbiological quality of the air contains fewer than 15 colonies for a 15 minute exposure and determined monthly. The results are recorded and records maintained.
O	11	☐	~~6. Pipette aid used, mouth pipetting not permitted.~~ Moved to equipment 1.4.25
		1.4 Laboratory Equipment	
O	9	☐	~~1.~~ 1.4.1 To determine the pH of prepared media, the pH meter has a standard accuracy of 0.1 units.
O	14	☐	~~2.~~ 1.4.2 pH electrodes consisting of pH half cell and reference half cell or equivalent combination electrode/triode (free from silver/silver chloride (Ag/AgCl) or contains an ion exchange barrier ~~preventing passage of Ag ions into the medium which may effect the accuracy of the pH reading~~) to prevent the passage of silver (Ag) ions into the substance being measured.
K	11	☐	~~3.~~ 1.4.3 The effect of temperature on the pH is compensated for by an ATC probe or by manual adjustment.
K	8	☐	~~4.~~ 1.4.4 pH meter is calibrated daily or with each use ~~and records are maintained.~~ Results are recorded and records maintained.
K	11	☐	~~5.~~ 1.4.5 A minimum of two standard buffer solutions is used to calibrate the pH meter.

			The first must be near the electrode isopotential point (pH 7). The second near the expected sample pH (i.e., pH 4 or pH 10). Standard buffer solutions are used once daily and discarded.
O	8,15	☐	~~6. Electrode effectiveness is determined daily or with each use.~~ ~~Method of determination _____.~~ 1.4.6 Electrode acceptability is determined daily or with each use by the millivolt procedure or through determination of the slope. (*Circle the method used.*)
K	9	☐	~~7.~~ 1.4.7 Balance provides a sensitivity of at least 0.1 g at ~~a load of 150 g.~~ weights of use.
K	11,13	☐	~~8. Balance checked monthly using NIST Class S or ASTM Class 1 or 2 weights or equivalent and records are maintained.~~ 1.4.8 Balance calibrations are checked monthly according to manufacturer's specifications using NIST Class S or ASTM Class 1 or 2 weights or equivalent. The accuracy of the balance is verified at the weight range of use. Results are recorded and records maintained.
K	11	☐	~~9.~~ 1.4.9. Refrigerator temperature (s) monitored at least once daily on workdays ~~and recorded.~~ Results are recorded and records maintained
K	1	☐	~~10.~~ 1.4.10 Refrigerator temperature maintained at 0° to 4°C.
C	**9**	☐	**~~11.~~ 1.4.11 The temperature of the incubator is maintained at 35 ± 0.5°C.**
C	**11**	☐	**~~12.~~ 1.4.12 Thermometers used in the air incubator(s) are graduated at no greater than ~~0.5°~~ 0.1° C increments.**
K	9	☐	~~13.~~ 1.4.13 Working thermometers are located on top and bottom shelves of use in the air incubator(s).
C	**11**	☐	**~~14.~~ 1.4.14 Temperature of the waterbath is maintained at 44.5 ± 0.2°C under ~~any~~ all loading ~~capacity~~ conditions.**
C	**9**	☐	**~~15.~~ 1.4.15 The thermometers used in the waterbath are graduated in 0.1°C increments.**
~~O~~ C	**13**	☐	**~~16.~~ 1.4.16 The waterbath has adequate capacity for workload.**
K	9	☐	~~17.~~ 1.4.17 The level of water in the waterbath covers the level of liquid in the incubating tubes.
K	8, 11	☐	~~18.~~ 1.4.18 Air incubator/waterbath temperatures are taken twice daily ~~and recorded~~ on workdays. The results are recorded and records maintained.
~~K~~	~~13~~	~~☐~~	~~19. Working thermometers are tagged with identification, date of calibration, calibrated temperature and correction factor.~~
~~K~~ C	**4**	☐	**~~20.~~ 1.4.19 All working thermometers are appropriately immersed.**
C	**29**	☐	**1.4.20 Either mercury-in-glass thermometers or non-mercury-in-glass thermometers having the accuracy (uncertainty), tolerance and response time of mercury are used as working thermometers. In the case of the waterbath, low drift electronic resistance thermometers with an accuracy of +0.05°C may also be used.**
~~K~~ C	**11**	☐	~~21. A standards thermometer has been calibrated by NIST or one of equivalent accuracy at the points 0°, 35° and 44.5° C (45.5° C for ETCP). Calibration records maintained.~~ **1.4.21 A standards thermometer has been calibrated by a qualified calibration laboratory using a primary standard traceable to NIST or an equivalent authority at the points 0°, 35° and 44.5°C (45.5°C for ETCP). These calibration records are maintained.**
K	9	☐	~~22.~~ 1.4.22 Standards thermometers ~~is~~ are checked annually for accuracy by ice point determination. Results recorded and maintained. Date of most recent determination _____.
C	**29**	☐	**1.4.23 Either mercury-in-glass thermometers, non-mercury-in-glass thermometers having the accuracy (uncertainty), tolerance and response time of mercury or**

			low drift electronic resistance thermometers with an accuracy of < ±0.05°C are used as the laboratory standards thermometer. (*Circle the thermometer type used.*)
K	13	☐	~~23.~~ 1.4.24 Incubator and waterbath working thermometers are checked annually against the standards thermometer at the temperatures at which they are used. Results are recorded and records maintained.
O	11	☐	1.4.25 Appropriate pipet aids are available and used to inoculate samples. Mouth pipetting is not permitted.
		1.5 Labware and Glassware Washing	
O	9	☐	~~1.~~ 1.5.1 Utensils and containers are clean borosilicate glass, stainless steel or other noncorroding materials
K	9	☐	~~2.~~ 1.5.2 Culture tubes are of a suitable size to accommodate the volume for nutritive ingredients and samples
K	9	☐	~~3.~~ 1.5.3 Sample containers are made of glass or some other inert material ~~(ie polypropylene)~~.
O	9	☐	~~4.~~ 1.5.4 Dilution bottles and tubes are made of borosilicate glass or plastic and closed with rubber stoppers, caps or screw caps with nontoxic liners.
K	9	☐	~~5.~~ 1.5.5 Graduations are indelibly marked on dilution bottles and tubes or an acceptable alternative method is used to ensure appropriate volumes.
~~K~~ C	**9**	☐	~~6.~~ **1.5.6 Pipettes used to inoculate the sample deliver accurate aliquots, have unbroken tips and are appropriately graduated. Pipettes larger than 10 mL are not used to deliver 1mL aliquots; nor, are pipets larger than 1mL used to deliver 0.1 mL aliquots.**
K	9	☐	~~7.~~ 1.5.7 Reusable sample containers are capable of being properly washed and sterilized.
K	9	☐	~~8.~~ 1.5.8 In washing reusable pipits, a succession of at least three fresh water rinses plus a final rinse of distilled/deionized water is used to thoroughly rinse off all the detergent.
~~C~~	~~9~~	☐	~~9. In washing reusable sample containers, glassware and plasticware, the effectiveness of the rinsing procedure is established annually and when detergent (brand or lot) is changed by the Inhibitory Residue Test as described in the current edition of Standard Methods for the Examination of Water and Wastewater. Records are kept.~~ ~~Date of most recent testing_____~~ ~~Average difference between Groups A and B_____~~ ~~Average difference between Groups B and D_____~~ ~~Detergent Brand_____ Lot #_____~~
C	2	☐	**1.5.9 An alkaline or acidic detergent is used for washing glassware/labware.**
~~K~~ C	**11**	☐	~~10. Once during each day of washing several pieces of glassware (pipettes, sample bottles, etc.) from one batch are tested for residual acid or alkali w/aqueous 0.04% bromthymol blue. Records are maintained.~~ **1.5.10 With each load of labware/glassware washed the contact surface of several dry pieces from each load are tested for residual detergent (acid or alkali) with aqueous 0.04% bromothymol blue. Results are recorded and records maintained.**
		1.6 Sterilization and Decontamination	
~~O~~ K	9	☐	~~1.~~ 1.6.1 Autoclave(s) are of sufficient size to accommodate the workload.
O	8	☐	~~2.~~ 1.6.2 Routine autoclave maintenance performed ~~(e.g. pressure relief valves, exhaust trap, chamber drain)~~ and the records maintained.
~~O~~	~~8~~	☐	~~3. Autoclave(s) and/or steam generators serviced annually or as needed by qualified technician and records maintained.~~

C	**11, 30**	☐	~~4. Autoclave(s) provides a sterilizing temperature of 121° C (tolerance 121 ± 2° C) as determined weekly using a calibrated working maximum registering thermometer or equivalent (thermocouples, platinum resistance thermometers).~~ **1.6.3 The autoclave provides a sterilizing temperature of 121°C (tolerance 121 + 2°C) as determined for each load using a working maximum registering thermometer concluded to be within temperature tolerance specifications. As an alternative, an appropriate temperature monitoring device is used in place of the maximum registering thermometer when these are unavailable due to the ban on mercury.**
K	11	☐	~~5. An autoclave standards thermometer has been calibrated by the National Institute of Standards and Technology (NIST) or its equivalent at 121° C.~~ 1.6.4 An autoclave standards thermometer has been calibrated by a qualified calibration laboratory using a primary standard traceable to NIST or an equivalent authority at 121°C. Calibration at 100°C, the steam point, is also recommended but not required.
K	16	☐	~~6. The autoclave standards thermometer is checked every five years for accuracy at either 121° C or at the steam point.~~ 1.6.5 The autoclave standards thermometer is checked every five years for accuracy at either 121°C or at 100°C, the steam point, if the thermometer has been previously calibrated at this temperature. Date of most recent determination_____
K	1	☐	~~7.~~ 1.6.6 Working autoclave thermometers are checked against the autoclave standards thermometer at 121°C yearly. Date of last check _____ Method _____
K	11	☐	~~8.~~ 1.6.7 Spore strips/suspensions appropriate for use in an autoclave are used monthly according to manufacturer's instructions to evaluate the effectiveness of the ~~autoclave~~ sterilization process. Results are recorded and the records maintained.
O	11	☐	~~9.~~ 1.6.8 Heat sensitive tape is used with each autoclave batch.
K	11, 13	☐	~~10.~~ 1.6.9 Autoclave sterilization records including length of sterilization, total heat exposure time and chamber temperature are maintained. Type of record: Autoclave log, computer printout or chart recorder tracings. (*Circle appropriate type or types.)*
K	11	☐	~~11.~~ 1.6.10 For dry heat sterilized material, the hot-air sterilizing oven provides heating and sterilizing temperatures in the range of 160° to 180°C.
K	9	☐	~~12.~~ 1.6.11 A thermometer capable of determining temperatures accurately in the range of 160 to 180°C is used to monitor the operation of the hot-air sterilizing oven ~~when in use.~~
K	13	☐	~~13.~~ 1.6.12 Records of temperatures and exposure times are maintained for the operation of the hot-air sterilizing oven during use.
K	11	☐	~~14.~~ 1.6.13 Spore strips/suspensions are used quarterly to evaluate the effectiveness of the sterilization process in the hot-air oven. Records are maintained.
K	11	☐	~~15.~~ 1.6.14 Reusable sample containers are sterilized for 60 minutes at 170°C in a hot-air oven or autoclaved for 15 minutes at 121°C.
~~O~~ C	**1**	☐	~~16. The sterility of reusable/disposable sample containers is determined for each batch/lot.~~ **1.6.15 The sterility of reusable sample containers is determined for each load sterilized. The results are recorded and the records maintained.**
C	1	☐	**1.6.16 The sterility of pre-sterilized disposable sample containers is determined for each lot received. Results are recorded and the records maintained.**
K	9	☐	~~17.~~ 1.6.17 Reusable pipettes are stored and sterilized in aluminum or stainless steel canisters ~~or equivalent alternative~~ .

K	9	☐	~~18.~~ 1.6.18 Reusable pipettes (in canisters) are sterilized in a hot-air oven at 170°C for 2 hours.
~~O~~ C	2	☐	~~19. The sterility of reusable/disposable pipettes is determined with each batch/lot. Results are recorded and maintained.~~ **1.6.19 The sterility of reusable pipettes is determined with each load sterilized. Results are recorded and records maintained.**
C	2	☐	**1.6.20 The sterility of pre-sterilized disposable pipets is determined with each lot received. Results are recorded and the records maintained.**
K	18	☐	~~20.~~ 1.6.21 Hardwood applicator transfer sticks are properly sterilized. Method of sterilization _____
C	2	☐	**1.6.22 The sterility of the hardwood transfer sticks is checked routinely. Results are recorded and the records maintained.**
O	13	☐	~~21. Spent broth cultures and agar plates are decontaminated by autoclaving for at least 30 minutes before conventional disposal.~~ 1.6.23 Spent broth cultures and agar plates are decontaminated before disposal. Method _____.
		1.7 Media Preparation	
K	3, 5	☐	~~1.~~ 1.7.1 Media is commercially dehydrated except in the case of medium A-1 which ~~is~~ must be prepared from the individual components and modified MacConkey agar which may be prepared from its components.
O	11	☐	~~2.~~ 1.7.2 Dehydrated media and media components properly stored in cool, clean, dry place.
O	11	☐	~~3.~~ 1.7.3 Dehydrated media are labeled with the analyst's initials date of receipt and date opened.
C	12	☐	~~4.~~ **1.7.4 Caked or expired media or media components are discarded.**
C	11	☐	~~5. Make-up water is distilled or deionized *(circle one)* and exceeds 0.5 megohm resistance or is less than 2µ Siemens/cm conductivity at 25° C to be tested and recorded monthly for resistance or conductivity *(circle the appropriate)*~~ **1.7.5 Reagent water is distilled or deionized (circle appropriate choice), tested monthly and exceeds 0.5 megohm-cm resistance (2 megohms-cm in-line) or is less than 2.0 µSiemens/cm conductivity at 25°C. (*Circle the appropriate water quality descriptor determined.*) Results are recorded and the records maintained.**
C	11	☐	~~6.~~ **1.7.6** ~~Makeup~~ **Reagent water is analyzed for residual chlorine monthly and is at a non-detectable level (< 0.1 ppm). Results are recorded and the records ~~are~~ maintained.** **Specify method of determination _____.**
~~K~~	~~11~~	☐	~~7. Make-up water is free from trace (<0.05mg/L) dissolved metals, specifically Cd, Cr, Cu, Ni, Pb, and Zn as determined annually with total heavy metal content < or equal to 1.0mg/L and records are maintained.~~
K	11	☐	~~8.~~ 1.7.7 ~~Make up~~ Reagent water contains ~~<1000~~ <100 CFU/mL as determined monthly using the heterotrophic plate count method. Results are recorded and the records maintained.
K	11	☐	~~9.~~ 1.7.8 Commercially prepared dehydrated media are sterilized according to the manufacturer's instructions.
K	9	☐	~~10.~~ 1.7.9 The volume and concentration of media in the tube are suitable for the amount of sample inoculated.
C	11	☐	~~11.~~ **1.7.10 Total time of exposure of sugar broths to autoclave temperatures does not exceed 45 minutes.**
C	1	☐	~~12. Media sterility and positive and negative controls are run with each lot of commercially prepared media or are run with each batch of media prepared from its components as a check of media productivity. Results recorded and records~~

			~~maintained.~~ **1.7.11 Media sterility is determined for each load sterilized. Results are recorded and the records maintained.**
C	1	☐	**1.7.12 Media productivity is determined using media appropriate, properly diluted positive and negative control cultures for each lot of dehydrated media received or with each batch of media prepared from the individual components. When an alternative visual temperature monitoring device is used in place of the maximum registering autoclave thermometer, media productivity is determined using media appropriate, properly diluted positive and negative control cultures with each batch of media prepared.**
O	9	☐	~~13.~~ 1.7.13 Sterile phosphate buffered dilution water is used as the sample diluent.
K	11	☐	~~14.~~ 1.7.14 The pH of the prepared media is determined after sterilization to ensure that it is consistent with manufacturer's requirements. Results are recorded and records are maintained.
		1.8 Storage of Prepared Culture Media	
~~O~~ K	9	☐	~~1.~~ 1.8.1 Prepared culture media are stored in a cool, clean, dry space where excessive evaporation and the danger of contamination are minimized.
K	5,11	☐	~~2.~~ 1.8.2 Brilliant green bile 2% broth and A-1 media are stored in the dark.
K	13	☐	~~3.~~ 1.8.3 Stored media are labeled with the storage expiration date or the sterilization date.
O	9	☐	~~4.~~ 1.8.4 Storage of prepared culture media at room temperature does not exceed 7 days.
O	2	☐	~~5.~~ 1.8.5 Storage under refrigeration of prepared broth media with loose fitting closures shall not exceed 1 month.
O	11	☐	~~6.~~ 1.8.6 Storage under refrigeration of prepared culture media with screw-cap closures does not exceed 3 months.
K	17	☐	~~7.~~ 1.8.7 All prepared ~~media~~ MPN broth stored under refrigeration are held at room temperature overnight prior to use. Culture tubes containing any type of precipitate or Durham tubes containing air bubbles are discarded.
PART II - SEAWATER SAMPLES			
		2.1 Collection and Transportation of Samples	
C	**11**	☐	**~~1.~~ 2.1.1 Sample containers are of a suitable size to contain at least ~~100~~ 110 mL of sample and to allow adequate headspace for proper shaking. Seawater samples are collected in clean, sterile, watertight, properly labeled sample containers.**
K	1	☐	~~2.~~ 2.1.2 Samples are identified with collectors name, harvest area, sampling station, time and date of collection.
C	**9**	☐	~~3. After collection, seawater samples shall be kept at a temperature between 0 and 10° C until examined.~~ **2.1.3 Immediately after collection, seawater samples are placed in dry storage (ice chest or equivalent) which is maintained between 0° and 10°C with ice or cold packs for transport to the laboratory. Once received, the samples are placed in the refrigerator unless processed immediately.**
~~K~~ O	1	☐	~~4.~~ 2.1.4 A temperature blank is used to determine the temperature of samples upon receipt at the laboratory. Results are recorded and maintained.
C	**9**	☐	~~5. Examination of the sample is initiated as soon as possible after collection. However, seawater samples are not tested if they are held beyond 30 hours of refrigeration.~~ **2.1.5 Analysis of the sample is initiated as soon as possible after collection. Seawater samples are not tested if they have been held for more than 30 hours from the time of collection.**
		2.2 Bacteriological Examination of Seawater by the APHA MPN	
C	**9**	☐	**~~1.~~ 2.2.1 Lactose broth or lauryl tryptose broth is used as the presumptive medium.** *(Circle appropriate one.)*

C	2	☐	2. 2.2 The media productivity controls utilized are properly diluted and appropriate for the presumptive medium being used. The results are recorded and the records maintained. Positive productivity control _____ Negative productivity control _____
C	9	☐	2. 2.2.3 **Sample and dilutions of sample are shaken ~~mixed~~ vigorously (25 times in a 12" arc in 7 seconds) before inoculation.**
C	9	☐	3. 2.2.4 **In a multiple dilution series not less than 3 tubes per dilution are used (5 tubes are recommended).**
C	6	☐	4. 2.2.5 **In a single dilution series not less than 12 tubes are used (for depuration at least 5 tubes are used).**
~~K~~ C	6	☐	5. 2.2.6 **In a single dilution series, the volumes analyzed ~~examined~~ are adequate to meet the needs of routine monitoring.** **Sample volume inoculated _____** **Range of MPN_____** **Strength of media used_____**
K	9	☐	6. 2.2.72.2.7 Inoculated ~~media~~ tubes are ~~placed in an air incubator~~incubated in air at 35~~+~~± 0.5°C ~~for up to 48 ± 3 hours~~.
~~K~~ C	2	☐	7. Positive and negative control cultures accompany samples throughout the procedure. Records are maintained. 2.2.8 Appropriately diluted process control cultures accompany the samples *throughout both the presumptive and confirmed phases of incubation.* Results are recorded and the records maintained. **Positive process control _____ Negative process control _____**
K	9	☐	8. Inoculated media are read after 24 ± 2 hours and 48 ± 3 hours of incubation and transferred at both intervals if positive for gas. 2.2.9 Inoculated tubes are read after 24+ 2 hours and 48+ 3 hours of incubation and transferred at both time intervals if positive for growth (the presence of turbidity) and gas or effervescence in the culture tube. These tubes are considered presumptive positive requiring further confirmatory testing
		2.3 **Confirmed Test for Seawater by APHA MPN**	
C	9	☐	1. 2.3.1 **Brilliant green bile 2% broth (BGB) is used as the confirmatory medium for total coliforms.**
C	9	☐	2. 2.3.2 **EC medium is used as the confirmatory medium for fecal coliforms.**
C	2	☐	2.3.3 The media productivity controls utilized are properly diluted and appropriate for the confirmed medium being used. The results are recorded and the records maintained. Positive productivity control _____ Negative productivity control _____
K	9, 11	☐	3. 2.3.4 Transfers are made to BGB/EC by either sterile loop or sterile hardwood transfer stick from positive presumptive~~s~~ tubes incubated for 24 and 48 hours as appropriate . *(Circle the method of transfer.)*
~~K~~	~~2~~	☐	4. When the inoculation of both EC and BGB broths is performed using the same loop or transfer stick, the order of inoculation is EC first, followed by BGB.
C	9	☐	5. 2.3.5 **BGB tubes are incubated at 35 ± 0.5°C.**
K	9	☐	6. 2.3.6 BGB tubes are read after 48 ± 3 hours of incubation.
C	9	☐	7. 2.3.7 **EC tubes are incubated in a circulating waterbath maintained at 44.5 ± 0.2°C ~~for 24 ± 2 hours~~.**
C	9	☐	2.3.8 EC tubes are read after 24 ± 2 hours of incubation.
C	9	☐	8. 2.3.9 **The presence of turbidity and any amount of gas or effervescence in the culture tube constitutes a positive test.**

		2.4 Computation of Results – APHA MPN	
K	9	☐	~~1.~~ 2.4.1 Results of multiple dilution tests are read from tables in *Recommended Procedures for the Examination of Sea Water and Shellfish*,Fourth ~~4~~th Edition.
K	7	☐	~~2.~~ 2.4.2 Results from single dilution series are calculated from Hoskins' equation or interpolated from Figure 1, Public Health Report 1621 entitled "Most Probable Numbers for Evaluation of Coli aerogenes Tests by Fermentation Tube Method".
~~K~~C	7, 9	☐	**~~3.~~ 2.4.3 Results are reported as MPN/100 mL of sample.**
		2.5 Bacteriological Examination of Seawater by the MA-1 Method	
C	5	☐	**2.5.1 A-1 medium complete is used in the analysis.**
C	2, 31	☐	**2.5.2 A-1 medium without salicin is used in the analysis. Comparability testing with medium A-1 complete has been undertaken and the results justify exclusion of the salicin from the formulation of medium A-1.**
C	5	☐	**~~1.~~ 2.5.3 A-1 medium sterilized for 10 minutes at 121°C.**
C	2	☐	**2.5.4 The media productivity controls used are properly diluted and appropriate for use with A-1 medium. The results are recorded and the results maintained.** **Positive productivity control _____ Negative productivity control _____**
C	9	☐	**~~2.~~ 2.5.5 Sample and dilutions of sample are shaken ~~mixed~~ vigorously (25 times in a 12" arc in 7 seconds) before inoculation.**
C	9	☐	**~~3.~~ 2.5.6 In a multiple dilution series not less than 3 tubes per dilution are used (5 tubes are recommended).**
C	6	☐	**~~4.~~ 2.5.7 In a single dilution series at least 12 tubes are used.**
~~K~~C	6	☐	**~~5.~~ 2.5.8 In a single dilution series, the volumes analyzed ~~examined~~ are adequate to meet the needs of routine monitoring.** **Sample volume inoculated _____** **Range of MPN _____** **Strength of media used _____**
~~K~~C	2	☐	~~6. Positive and negative control cultures accompany samples throughout the procedure. Records are maintained.~~ **2.5.9 Appropriately diluted process control cultures accompany the samples *throughout both resuscitation and waterbath incubation* Results are recorded and the records maintained.** **Positive process control _____ Negative process control _____**
C	2,5	☐	**~~7.~~ 2.5.10 Inoculated ~~media~~ tubes are placed in an air incubator at 35 ± 0.5°C for 3 ± 0.5 hours of resuscitation.**
C	5	☐	**~~8.~~ 2.5.11 After 3 ± 0.5 hours resuscitation at 35°C, inoculated tubes ~~media~~ are incubated at 44.5 ± 0.2° C in a circulating waterbath for the remainder of the 24 ± 2 hours.**
C	5	☐	**~~9.~~ 2.5.12 The presence of turbidity and any amount of gas or effervescence in the culture tube constitutes a positive test.**
		2.6 Computation of Results - MPN	
K	9	☐	~~1.~~ 2.6.1 Results of multiple dilution tests are read from tables in *Recommended Procedures for the Examination of Sea Water and Shellfish*, 4th Edition.
K	7	☐	~~2.~~ 2.6.2 Results from single dilution series are calculated from Hoskins' equation or interpolated from Figure 1, Public Health Report 1621 entitled "Most Probable Numbers for Evaluation of Coli aerogenes Tests by Fermentation Tube Method".
~~K~~ C	7, 9	☐	**~~3.~~ 2.6.3 Results are reported as MPN/100 mL of sample.**
		2.7 Bacteriological ~~Examination~~ Analysis of Seawater by Membrane Filtration	

			(MF) using mTEC Agar -Materials and Equipment
C	**23, 24**	☐	**1. 2.7.1 When used for elevated temperature incubation in conjunction with ethafoam resuscitation, the temperature of the hot air incubator is maintained at 44.5 ± 0.5°C under any loading capacity.**
C	**23**	☐	**2. 2.7.2 When using a waterbath for elevated temperature incubation, the level of the water completely covers the plates.**
C	**23**	☐	**3. 2.7.3 Pre-sterilized plastic or sterile glass culture plates that are clear, flat bottomed, free of bubbles and scratches with tight fitting lids are used.**
C	**2**	☐	**2.7.4 The sterility of pre-sterilized culture plates is determined for each lot received. Results are recorded and the records maintained.**
K	11	☐	4. 2.7.5 Colonies are counted with the aid of magnification.
C	**11, 23**	☐	**5. 2.7.6 Membrane filters are made from cellulose ester material, white, grid marked, 47 mm in diameter with a pore size of 0.45 µm and certified by the manufacturer for fecal coliform analyses.**
O C	**2**	☐	**6. 2.7.7 Lot number, date of receipt and if provided the expiration date of the membrane filters are recorded and records maintained.**
C	**2**	☐	**2.7.8 When initiating monitoring by mTEC or switching brands or types of membrane filters used and no previous lots of filters are available for comparing acceptable performance, an appropriate method for determining the suitability of the lot is developed and the comparison testing implemented. The results are recorded and this record is maintained.**
K	2, 11	☐	7. 2.7.9 New lots of membrane filters are checked by comparing recovery of fecal coliform organisms against membrane filters from previously acceptable lots.
C	**2**	☐	**8. 2.7.10 The sterility of each lot or autoclave batch of membrane filters are checked before use.**
K	2	☐	9. 2.7.11 Membrane filters which are beyond their expiration date are not used.
O	11	☐	10. 2.7.12 Forceps tips are clean.
O	11	☐	11. 2.7.13 Forceps tips are smooth without pitting or corrugations to damage the filters being manipulated.
K	11	☐	12. 2.7.14 Forceps are dipped in alcohol and flame sterilized between sample filters.
K	11	☐	13. 2.7.15 If indelible graduation marks are used on clear glass or plastic funnels to measure sample volumes, their accuracy is checked gravimetrically or with a Class A graduated cylinder before use and periodically rechecked. Funnels having a tolerance greater than 2.5% are not used. Checks are recorded and records maintained
K	11	☐	14. 2.7.16 Membrane filtration units are made of stainless steel, glass or autoclavable plastic free of scratches, corrosion and leaks.
C	**11**	☐	**15. 2.7.17 Membrane filter assemblies are autoclave sterilized for 15 minutes at 121°C prior to the start of a filtration series.**
O	11, 23, 26	☐	16. 2.7.18 A UV sterilization unit is used to disinfect filter assemblies between sample and filtration runs.
K	11	☐	17. 2.7.19 ~~If used,~~ The effectiveness of the UV sterilization unit is determined by biological testing monthly. Results are recorded and records maintained.
K	2	☐	2.7.20 Maintenance of the UV sterilization unit is performed as needed. This maintenance is documented and the records maintained.
			2.8 Media Preparation and Storage – MF using mTEC Agar
K	11	☐	1. 2.8.1 Phosphate buffered saline is used as the sample diluent and filter funnel rinse.
C	**11**	☐	**2. 2.8.2 The phosphate buffered saline is properly sterilized.**
K	23	☐	3. 2.8.3 A sufficient amount of medium (4-5 mL) is used in each plate.
O	11	☐	4. 2.8.4 Refrigerated prepared plates are stored for no more than 2 weeks in sealed plastic bags or containers to minimize evaporation.
			2.9 Sample Analyses -MF using mTEC Agar
C	**24**	☐	**1. 2.9.1 mTEC agar is used.**

C	2	☐	2.9.2 The media productivity controls used are properly diluted and appropriate for use with mTEC medium. The results are recorded and the results maintained. Positive productivity control _____ Negative productivity control _____
C	23	☐	2. 2.9.3 **The sample is ~~mixed~~ shaken vigorously (25 times in a 12″ arc in 7 seconds) before filtration.**
C	23	☐	3. 2.9.4 **The membrane is placed grid side up within the sterile filter apparatus.**
C	23, 25	☐	4. 2.9.5 **Sample volumes tested are consistent with the sampling regime employed (i.e., half log or other appropriate dilutions are used with systematic random sampling).**
C	23	☐	5. 2.9.6 **Sample volumes are filtered under vacuum.**
K	26	☐	6. 2.9.7 The pressure of the vacuum pump does not exceed 15 psi.
C	23, 26	☐	7. 2.9.8 **The sides of the filter funnel are rinsed at least twice with 20-30 mL of sterile phosphate buffered saline after sample filtration.**
C	23	☐	8. 2.9.9 **The membrane filter is removed from the filtering apparatus with sterile forceps and rolled onto mTEC agar so that no bubbles form between the filter and the agar.**
C	11	☐	9. 2.9.10 **Blanks are run at the beginning of filtration, after every 10th aliquot and at the end of the filtration run to check the sterility of the testing system (phosphate buffered saline, filter funnel, forceps, membrane filter, media and culture plate).**
~~K~~C	2, 11	☐	10. Positive and negative control cultures accompany samples throughout the procedure. Records are maintained. 2.9.11 Appropriately diluted process control cultures accompany the samples throughout both resuscitation and elevated temperature incubation. Results are recorded and the records maintained. **Positive process control _____ Negative process control _____**
C	11, 23, 24	☐	11. 2.9.12 ~~Innoculated plates are placed inverted wither directly in an air incubator or in a watertight, tightly sealed container at 35 + 0.5°C for 2 hours of resuscitation prior to waterbath incubation or in Ethyfoam for incubation in air at 44.5 + 0.5°C.~~ Inoculated plates are placed inverted into a watertight, tightly sealed container prior to being placed in the air incubator and incubated at 35 + 0.5°C for 2 hours of resuscitation. Alternatively inoculated plates may be placed in ethafoam prior to air incubation at 44.5 + 0.5°C for 24 + 2 hours .
C	11, 23, 24	☐	12. 2.9.13 **After 2 hours of resuscitation at 35°C, the watertight, tightly sealed containers are transferred to a circulating waterbath at 44.5 + 0.2°C, submerged completely and incubated for 22-24 hours.** ~~Individual plates are transferred inverted to a watertight container, tightly sealed and submerged completely in a circulating waterbath at 44.5 + 0.2°C for 22-24 hours of incubation.~~
		2.10 **Computation of Results- MF using mTEC Agar**	
C	23	☐	1. 2.10.1 **All yellow, yellow-green or yellow-brown colonies are counted.**
C	23	☐	2. 2.10.2 **Only plates having 80 or fewer colonies are counted. If it is unavoidable ~~necessary~~ to use plates having more than 80 colonies, counts are given as >80 x 100/the volume of sample filtered.**
C	2, 11, 23	☐	2.10.3 When multiple dilutions are filtered, the laboratory has developed a procedure for assessing the contribution of all positive dilutions to the final count.
K	23, 11	☐	3. 2.10.4 The number of fecal coliforms is calculated by the following equation: Number of fecal coliforms per 100 mL = [number of colonies counted per plate

			used in the count / volume (s) of sample filtered in ml] x 100.
~~K~~C	23, 11	☐	~~4.~~ **2.10.5 Results are reported as CFU/100 mL of sample.**
PART III - SHELLFISH SAMPLES			
		3.1 Collection and Transportation of Samples	
C	9	☐	~~1.~~ **3.1.1 A representative sample of shellstock is collected.**
K	9	☐	~~2.~~ 3.1.2 Shellstock samples are ~~is~~ collected in clean, waterproof, puncture resistant containers loosely sealed .
K	9	☐	~~3.~~ 3.1.3 Shellstock samples are labeled with collector's name, type of shellstock, the source or harvest area, sampling station, time, date and place (if applicable ~~market sample~~) of collection.
C	9	☐	~~4. Shellstock samples are maintained in dry storage between 0 and 10° C until examined.~~ **3.1.4 Immediately after collection, shellfish samples are placed in dry storage (ice chest or equivalent) which is maintained between 0° and 10°C with ice or cold packs for transport to the laboratory. Once received, the samples are placed under refrigeration unless processed immediately.**
C	1	☐	~~5.~~ **3.1.5 ~~Examination~~ Analysis of the sample~~s~~ is initiated as soon as possible after collection. ~~However,~~ Shellfish samples are not tested ~~examined~~ if the time interval between collection and analysis ~~examination~~ exceeds 24 hours.**
		3.2 Preparation of Shellfish for Examination	
K	2,11	☐	~~1.~~ 3.2.1 Shucking knives, scrub brushes and blender jars are (autoclave) sterilized for 15 minutes prior to use.
O	2	☐	~~2.~~ 3.2.2 Blades of shucking knives are not corroded.
O	9	☐	~~3. Prior to scrubbing and rinsing debris off shellstock , the hands of the analyst are thoroughly washed with soap and water.~~ 3.2.3 The hands of the analyst are thoroughly washed with soap and water immediately prior to cleaning the shells of debris.
O	2	☐	~~4.~~ 3.2.4 The faucet used ~~to provide the potable water~~ for rinsing the shellstock does not contain an aerator.
K	9	☐	~~5.~~ 3.2.5 Shellstock are scrubbed with a stiff, sterile brush and rinsed under tap water of drinking water quality.
C	2	☐	**3.2.6 If a water supply is a non-chlorinated private well, the water is tested every six months for total coliforms. Results are recorded and maintained.**
O	9	☐	~~6.~~ 3.2.7 Shellstock are allowed to drain in a clean container or on clean towels prior to opening.
K	9	☐	~~7.~~ 3.2.8 Immediately prior to ~~opening~~ shucking, the hands (or gloved hands) of the analyst are thoroughly washed with soap and water and rinsed in 70% alcohol.
~~K~~C	9	☐	~~8.~~ **3.2.9 Shellstock are not shucked directly through the hinge.**
C	9	☐	~~9.~~ **3.2.10 Contents of shellstock (liquor and meat) are shucked into a sterile, tared blender jar or other sterile container.**
K	9	☐	~~10.~~ 3.2.11 At least 200 grams of shellfish meat or a quantity of meat sufficient to cover the blender blades is used for the analysis.
K	9	☐	3.2.12 A representative sample of at least 12 shellfish is used for the analysis.
K	2, 19	☐	~~11.~~ 3.2.13 The sample is weighed to the nearest 0.1 gram and an equal amount by weight of (tempered for ETCP) diluent is added.
O	9	☐	1~~2.~~ 3.2.14 Sterile phosphate buffered dilution water is used as the sample diluent.
~~K~~	~~3~~	☐	~~13. Sterile phosphate buffered saline is used as a sample diluent for the ETCP procedure.~~ Moved to ETCP section
C	9	☐	~~14.~~ **3.2.15 Samples are blended at high speed for 60 to 120 seconds until homogenous.**
K	9	☐	~~15. For other shellstock, APHA *Recommended Procedures* are followed for the examination of freshly shucked and frozen shellfish meats.~~

			3.2.16 APHA *Recommended Procedures for the Examination of Sea Water And Shellfish*, Fourth Edition is followed for the analysis of previously shucked and frozen shellfish meats.
		3.3 MPN Analysis for Fecal Coliform Organisms, Presumptive Test, APHA	
C	**9**	☐	~~1.~~ **3.3.1 Appropriate strength lactose or lauryl tryptose broth is used as presumptive media in the analysis.** ~~*(circle appropriate choice)*~~ ***(Circle the medium used.)***
C	**2**	☐	**3.3.2 The media productivity controls utilized are properly diluted and appropriate for the presumptive medium being used. The results are recorded and the records maintained.** **Positive productivity control _____ Negative productivity control _____**
K	9	☐	~~2.~~ 3.3.3 Immediately (within 2 minutes) after blending, the ground sample is diluted and inoculated into tubes of presumptive media.
C	**9**	☐	~~3.~~ **3.3.4 No fewer than 5 tubes per dilution are used in a multiple dilution MPN series.**
C	**9**	☐	~~4.~~ **3.3.5 Allowing for the initial 1:1 dilution of the sample, appropriate portions are inoculated (i.e., 2 ml of original 1:1 dilution for the 1 g portion) and diluted for subsequent inoculation (i.e., 22 ml of 1:1 diluted sample to 88 ml of diluent or the equivalent for 0.1 g portion). All successive dilutions are prepared conventionally.**
K	6	☐	~~5.~~ 3.3.6 In a single dilution series, the volumes examined are adequate to meet the needs of routine monitoring. Sample volume inoculated _____ Range of MPN_____ Strength of media used_____
C	**2**	☐	~~6. Positive and negative control cultures accompany samples throughout the procedure. Records are maintained.~~ **3.3.7 Appropriately diluted process control cultures accompany the samples throughout both the presumptive and confirmed phases of incubation. Results are recorded and the records maintained.** **Positive Process control _____ Negative Process control_____**
K	9	☐	~~7.~~ 3.3.8 Inoculated media are incubated at 35 ± 0.5°C.
K	10	☐	~~8. Presumptive tubes are read at 24 ± 2 hours of incubation and transferred if positive.~~ 3.3.9 Tubes are read after 24+2 hours of incubation and transferred if positive for growth (the presence of turbidity and gas or effervescence in the culture tube). These tubes are considered presumptive requiring further confirmatory testing.
		3.4 Confirmed Test for Fecal Coliforms - APHA	
C	**9**	☐	~~1.~~ **3.4.1 EC medium is used as the confirmatory medium.**
C	**2**	☐	**3.4.2 The media productivity controls utilized are properly diluted and appropriate for use with EC medium. The results are recorded and the records maintained.** **Positive productivity control _____ Negative productivity control _____**
K	9, 11	☐	~~2.~~ 3.4.3 Transfers are made to EC medium by either sterile loop or hardwood sterile ~~applicator~~ transfer sticks from positive presumptives ~~incubated for 24 hours~~. *(Circle the method of transfer.)*
C	**9**	☐	~~3.~~ **3.4.4 EC tubes are incubated in a circulating waterbath at 44.5 ± 0.2°C** ~~for 24 ± 2 hours.~~
K	9	☐	~~4.~~ 3.4.5 EC tubes are read for gas production after 24 ± 2 hours of incubation.
C	**9**	☐	~~5.~~ **3.4.6 The presence of turbidity and any amount of gas and/or effervescence in**

			the Durham tube constitutes a positive test.
		3.5 Computation of Results for MPN Analyses	
K	9	☐	~~1.~~ 3.5.1 Results of multiple dilution tests are read from tables in *Recommended Procedure for the Examination of Sea Water and Shellfish,* 4th Edition and multiplied by the appropriate dilution factor.
K	7	☐	~~2.~~ 3.5.2 Results from single dilution series are calculated from Hoskins' equation or interpolated from Figure 1, Public Health Report 1621 entitled "Most Probable Numbers for Evaluation of Coli aerogenes Tests by Fermentation Tube Method".
~~K~~ C	**9**	☐	**~~3.~~ 3.5.3 Results are reported as MPN/100 grams of sample.**
		3.6 Standard Plate Count Method	
O	20	☐	~~1.~~ 3.6.1 A standard plate count (SPC) analysis ~~is~~ may be performed in conjunction with the analysis for fecal coliform organisms.
K	9	☐	~~2.~~ 3.6.2 In the standard plate count procedure at least four plates are used, duplicates of two dilutions ~~are used to provide 30 to 300 colonies per plate~~. One of the dilutions should produce colonies of 30 to 300 per plate.
K	2	☐	~~3.~~ 3.6.3 Fifteen to 20 mL of tempered sterile plate count agar is used per plate.
~~K~~ C	**9**	☐	**~~4.~~ 3.6.4 Agar tempering bath maintains the agar at 44- 46°C.**
~~O~~ C	**9**	☐	~~5. Temperature control of the plate count agar is used in the tempering bath.~~ **3.6.5 An agar based temperature control having a similar volume and shape as the tempering plate count agar is used in the tempering bath.**
K	9	☐	~~6.~~ 3.6.6 Not more than 1 mL nor less than 0.1 mL of sample or sample dilution is plated.
C	**9**	☐	**~~7.~~ 3.6.7 Samples or sample dilutions to be plated are ~~mixed~~ shaken vigorously (25 times in a 12" arc in 7 seconds) before plating.**
K	11	☐	~~8.~~ 3.6.8 Control plates are used to check air quality and the sterility of the ~~air,~~ agar and the diluent.
K	9,21	☐	~~9.~~ 3.6.9 Solidified plates are incubated at 35 ± 0.5°C for 48 ± 3 hours inverted and stacked no more than four high.
K	9	☐	~~10.~~ 3.6.10 Quebec Colony Counter or its equivalent is used to provide the necessary magnification and visibility for counting plates.
K	1	☐	~~11.~~ 3.6.11 A hand tally or its equivalent is used for accuracy in counting.
		3.7 Computation of Results -SPC	
K	9	☐	~~1.~~ 3.7.1 Colony counts determined in accordance with Part III, A, Sections 4.31 through 4.33 in *Recommended Procedures for the Examination of Sea Water and Shellfish,*, 4th Fourth Edition.
C	**19**	☐	**~~2.~~ 3.7.2 Colony counts are reported as APC/g of sample.**
		3.8 Bacteriological ~~Examination~~ Analysis of Shellfish Using the ETCP	
C	2,3	☐	**3.8.1 Prepared modified MacConkey agar is used on the day that it is made.**
~~K~~	~~9~~	~~☐~~	~~**1. Sample homogenate is cultured within 2 minutes of blending.**~~
K	3	☐	~~2.~~ 3.8.2 Double strength modified MacConkey agar is used.
C	**3**	☐	~~3. Hydrated double strength Modified MacConkey Agar is heated to boiling, removed from the heat, and boiled again. This agar is never autoclaved.~~ **3.8.3 Prepared double strength modified MacConkey agar is heated to boiling, removed from the heat, and boiled again. This agar is never autoclaved.**
K	2, 3	☐	~~4.~~ 3.8.4 Twice boiled, double strength modified MacConkey agar ~~and sterile phosphate buffered saline~~ are maintained in a tempering bath at 45 to 50°C until used. ~~Prepared Modified MacConkey Agar is used on the day it is made.~~
K	2, 3	☐	3.8.5 Phosphate buffered saline is used as the sample diluent in the ETCP.
C	**2, 3**	☐	**3.8.6 The phosphate buffered saline is tempered at 45 - 50°C to prevent premature solidification of the agar.**
C	**9**	☐	**3.8.7 The sample homogenate is cultured within 2 minutes of blending.**

C	**2,3**	☐	~~5. The equivalent of 6 grams of the homogenate is placed into a sterile container and the contents brought up to 60 ml with tempered, sterile phosphate buffered saline.~~ **3.8.8 Six grams of shellfish (12 grams of homogenate if initially diluted 1:1) is placed into a sterile container and the contents brought up to 60 mL with sterile, tempered phosphate buffered saline.**
K	3	☐	~~6.~~ 3.8.9 Sixty (60) mL of tempered, twice boiled double strength Modified MacConkey Agar is added.
K	2,3, 22	☐	~~7.~~ 3.8.10 The container is gently swirled or ~~rotated~~ slowly inverted once to mix the contents, which are ~~then~~ subsequently distributed uniformly over ~~6 to 8~~ ~~petri~~ six plates.
C	**1**	☐	~~8.~~ **3.8.11 Media and diluent sterility are determined with each use. Results are recorded and the records maintained.**
C	**1**	☐	~~9. To determine media productivity, positive and negative control cultures are pour plated in an appropriate concentration to accompany samples throughout the procedure.~~ **3.8.12 Media productivity is determined using media appropriate properly diluted pour plated positive and negative control cultures for each batch of Modified MacConkey agar prepared.** **Positive control culture _____ Negative control culture _____**
C	**3, 13**	☐	~~10. Plates are incubated inverted within 3 hours of plating in air at 45.5 ± 0.5° C for 18 to 30 hours. Plates are stacked not more than four high.~~ **3.8.13 When solidified the plates are placed inverted into an air incubator at 45.5 ± 0.5°C for 18 to 30 hours of incubation.**
C	**2**	☐	**3.8.14 Plates are stacked no more than three high in the incubator.**
C	**2**	☐	**3.8.15 Appropriately diluted pour plated process control cultures *accompany each set of samples throughout incubation.* The results are recorded and the records maintained.** **Positive process control_____Negative process control_____**
C	**3**		~~11.~~ **3.8.16 Incubator temperature is maintained at 45.5 ± 0.5°C.**
		3.9 Computation ~~Expression~~ of Results - ETCP	
K	11	☐	~~1.~~ 3.9.1 Quebec Colony counter or its equivalent is used to provide the necessary magnification and visibility for counting.
O	1	☐	~~2.~~ 3.9.2 A hand tally or its equivalent is used to aid in counting.
C	**3, 6**	☐	~~3.~~ **3.9.3 All brick red colonies greater than 0.5 mm in diameter are totaled over all the plates and multiplied by a factor of 16.7** ~~to report results as CFU/100 grams of sample~~ **.**
C	**3**	☐	**3.9.4 Results are reported as CFU/100 grams of sample.**
		Bacteriological Examination of Soft-shelled Clams and American Oysters for Male Specific Coliphage (MSC)	
		3.10 MSC Equipment and Supplies	
K	30	☐	~~1.~~ 3.10.1 Sample containers used for the shucked sample are sterile, made of glass or some other inert material (i.e. polypropylene) and hold 100 – 125 mL.
C	**27, 28**	☐	~~2.~~ **3.10.2 The refrigerated centrifuge used must have the capacity to accommodate the amount of shellfish sample required for the procedure, perform at 9000 x g and maintain a temperature of 4°C.**
C	**27, 28**	☐	~~3.~~ **3.10.3 The tempering bath(s) must be able to maintain the temperature within 2°C of the set temperature.**
K	9	☐	~~4.~~ 3.10.4 The level of water in the tempering bath covers the level of liquid and agar in the container or culture tubes.
C	**27, 28**	☐	~~5.~~ **3.10.5 Sterile 0.22 µm pore size syringe filters and pre-sterilized plastic or sterile glass syringes are used to sterilize the antibiotic solutions.**

K	1	☐	~~6.~~ 3.10.6 The sterility of each lot of pre-sterilized syringes and syringe filters is determined. Results are recorded and records maintained.
K	1	☐	~~7.~~ 3.10.7 The sterility of each batch of reusable glass syringes is determined. Results are recorded and records maintained.
C	**27, 28**	☐	**~~8.~~ 3.10.8 The balance used provides a sensitivity of at least 10 mg.**
C	**27, 28**	☐	**~~9.~~ 3.10.9 The temperature of the incubator used is maintained between 35 – 37°C.**
C	**28**	☐	**~~10.~~ 3.10.10 Sterile disposable 50 mL centrifuge tubes are used and their sterility is determined with each lot. Results are recorded and records maintained.**
		3.11 MSC Media Preparation	
K	28	☐	~~1.~~ 3.11.1 Media preparation and sterilization is according to the validated method.
K	27, 28	☐	~~2.~~ 3.11.2 Bottom agar, double strength soft agar and growth broth are prepared from their individual components.
K	27, 28	☐	~~3.~~ 3.11.3 Soft agar is prepared double strength in volumes of 2.5 mL.
C	**27, 28**	☐	**~~4.~~ 3.11.4 The streptomycin and ampicillin solutions are added to tempered bottom agar.**
O	27, 28	☐	~~5.~~ 3.11.5 Storage of the bottom agar under refrigeration does not exceed 1 month.
K	27, 28	☐	~~6.~~ 3.11.6 Unsterilized soft agar is stored at -20°C for up to 3 months.
K	27, 28	☐	~~7.~~ 3.11.7 The soft agar is removed from the freezer and sterilized for 15 minutes at 121°C before use.
K	27, 28	☐	~~8.~~ 3.11.8 Storage of growth broth in the refrigerator in loosely capped tubes/bottles does not exceed 1 month and in screw capped tubes/bottles does not exceed 3 months.
K	27, 28	☐	~~9.~~ 3.11.9 Bottom agar plates are allowed to reach room temperature before use.
		3.12 Preparation of the Soft-Shelled Clams and American Oysters for MSC Analysis	
K	2,11	☐	~~1.~~ 3.12.1 Shucking knives, scrub brushes and blender jars are autoclave sterilized for 15 minutes prior to use.
O	2	☐	~~2.~~ 3.12.2 The blades of shucking knives are not corroded.
O	9	☐	~~3.~~ 3.12.3 The hands of the analyst are thoroughly washed with soap and water immediately prior to ~~scrubbing and rinsing~~ cleaning the shells of debris ~~off the shellfish~~.
O	2	☐	~~4.~~ 3.12.4 The faucet used for rinsing the shellfish does not contain an aerator.
K	9	☐	~~5.~~ 3.12.5 The shellfish are scrubbed with a stiff, sterile brush and rinsed under tap water of drinking water quality.
C	**2**	☐	**3.12.6 If a water supply is a non-chlorinated private well, the water is tested every six months for total coliforms. Results are recorded and maintained.**
O	9	☐	~~6.~~ 3.12.7 The shellfish are allowed to drain in a clean container or on clean towels unlayered prior to shucking.
K	9	☐	~~7.~~ 3.12.8 Immediately prior to shucking, the hands (or gloved hands) of the analyst are thoroughly washed with soap and water and rinsed in 70% alcohol.
C	**9**	☐	**~~8.~~ 3.12.9 Shellfish are not shucked through the hinge.**
C	**9**	☐	**~~9.~~ 3.12.10 The contents of shellfish (liquor and meat) are shucked into a sterile, tared blender jar or other sterile container.**
K	9	☐	~~10.~~ 3.12.11 A representative sample of at least 12 shellfish is used for the analysis.
~~C~~ K	2, 19	☐	~~11.~~ 3.12.12 The sample is weighed to the nearest 0.1 gram.
		3.13 MSC Sample Analysis	
C	**28**	☐	**~~1.~~ 3.13.1 *E.coli Famp* ATCC 700891 is the bacterial host strain used in this procedure.**
K	27, 28	☐	~~2.~~ 3.13.2 Host cell growth broth is tempered at 35 – 37°C and vortexed (or shaken) to aerate prior to inoculation with host cells.
K	27, 28	☐	~~3.~~ 3.13.3 Several host cell colonies are transferred to a tube of tempered, aerated growth broth and incubated at 35 – 37°C to provide host cells in log phase growth for sample analysis.

C	27, 28	☐	~~4.~~ 3.13.4 **Inoculated growth broth is incubated at 35 – 37°C for 4 to 6 hours to provide a host cell culture in log phase growth.**
C	27, 28	☐	~~5.~~ 3.13.5 **After inoculation, the host cell growth broth culture is not shaken.**
C	28	☐	~~6.~~ 3.13.6 **A 2:1 mixture of growth broth to shellfish tissue is used for eluting the MSC.**
C	28	☐	~~7.~~ 3.13.7 **The elution mixture is prepared w/v by weighing the sample and adding two equal portions of growth broth by volume to the shellfish tissue.**
C	28	☐	~~8.~~ 3.13.8 **The elution mixture is homogenized at high speed for 180 seconds.**
C	28	☐	~~9.~~ 3.13.9 **Immediately after blending, 33 grams of the homogenized elution mixture are weighed into centrifuge tubes.**
C	28	☐	~~10.~~ 3.13.10 **The homogenized elution mixture is centrifuged for 15 minutes at 9000 x g at 4°C.**
C	27, 28	☐	~~11.~~ 3.13.11 **The supernatant is pipetted off, weighed and the weight recorded.**
C	27, 28	☐	~~12.~~ 3.13.12 **The supernatant is allowed to warm to room temperature about 20 to 30 minutes.**
K	27, 28	☐	~~13.~~ 3.13.13 The autoclaved soft agar is tempered and held at 50 – 52°C throughout the period of sample analysis.
K	27, 28	☐	~~14.~~ 3.13.14 Two hundred microliters (0.2 mL) of log phase host strain *E coli* is added to the tempering soft agar immediately prior to adding the sample supernatant.
K	27, 28	☐	~~15.~~ 3.13.15 The sample supernatant is shaken or vortexed before being added to the tempering soft agar.
C	27, 28	☐	~~16.~~ 3.13.16 **2.5 mL of sample supernatant is added to each tube of tempering soft agar.**
C	27, 28	☐	~~17.~~ 3.13.17 **The soft agar/sample supernatant/host cell mixture is gently rolled between the palms of the hands to mix.**
C	27, 28	☐	~~18.~~ 3.13.18 **The soft agar/sample supernatant/host cell mixture is overlaid onto bottom agar plates and swirled gently to distribute the mixture evenly over the plate.**
C	28	☐	~~19.~~ 3.13.19 **Ten (10) plates are used, 2.5 mL per plate for a total of 25 mL of supernatant analyzed per sample.**
K	27, 28	☐	~~20.~~ 3.13.20 Negative and positive control plates are prepared and accompany each set of samples analyzed. The results are recorded and records maintained.
K	27, 28	☐	~~21.~~ 3.13.21 Growth broth is used as the negative control or blank.
K	27, 28	☐	~~22.~~ 3.13.22 Type strain MS2 (ATCC 15597) male specific bacteriophage appropriately diluted to provide countable low levels of phage is used as the positive control.
K		☐	~~23.~~ 3.13.23 A negative control plate is plated at the beginning and end of each set of samples analyzed.
K	27, 28	☐	~~24.~~ 3.13.24 The positive control is plated after all the samples are ~~analyzed~~ inoculated and immediately prior to the final negative control.
C	27, 28	☐	~~25.~~ 3.13.25 **All plates are incubated at 35 – 37°C for 16 to 20 hours.**
		3.14 Computation of Results - MSC	
C	27	☐	~~1.~~ 3.14.1 **Circular zones of clearing or plaques of any diameter in the lawn of host bacteria are counted.**
C	28	☐	~~2.~~ 3.14.2 **The working range of the method is 1 to 100 PFU per plate. When there are no plaques on all ten plates, the count is <6 PFU/100 grams for soft-shelled clams and <7 PFU/ 100 grams for American oysters. If the density exceeds 100 PFU per plate on all plates, the count is given as > 10,000 PFU/100 grams.**
K	28	☐	~~3.~~ 3.14.3 The formula used for determining the density of MSC in PFU/100 grams is: (0.364)(N)(Ws), where N = total number of plaques counted on all 10 plates and Ws = weight of the supernatant used.
O	9	☐	~~4.~~ 3.14.4 The MSC count is rounded off conventionally to give a whole number.

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SHELLFISH LABORATORY EVALUATION CHECKLIST

SUMMARY OF NONCONFORMITIES

Page	Item	Observation	Documentation Required

LABORATORY STATUS	
LABORATORY	**DATE**
LABORATORY REPRESENTATIVE:	
MICROBIOLOGICAL COMPONENT: (Part I-III)	
A. Results Total # of Critical (C) Nonconformities in Parts I-III Total # of Key (K) Nonconformities in Parts I-III Total # of Critical, Key and Other (O) Nonconformities in Parts I-III	 _____ _____ _____

B. Criteria for Determining Laboratory Status of the Microbiological Component:

1. **Does Not Conform Status**: The Microbiological component of this laboratory is not in conformity with NSSP requirements if:

   a. The total # of Critical nonconformities is **≥ 4 or**

   b. The total # of Key nonconformities is **≥ 13 or**

   c. The total # of Critical, Key and Other is **≥ 18**

2. **Provisionally Conforms Status**: The microbiological component of this laboratory is determined to be provisionally conforming to NSSP requirements if the number of critical nonconformities is **≥ 1 but ≤ 3**

C. Laboratory Status (*circle appropriate*)

Does Not Conform Provisionally Conforms Conforms

Acknowledgment by Laboratory Director/Supervisor:

All corrective Action will be implemented and verifying substantiating documentation received by the Laboratory Evaluation Officer on or before _____

Laboratory Signature:_____ Date:_____

LEO Signature:_____ Date:_____

NSSP Form LAB-100 Microbiology Rev. 2010-11-08

Proposal Subject: Update PSP Laboratory Evaluation Checklist

Specific NSSP Guide Reference: 2009 NSSP Section IV. Guidance Documents Chapter II. Growing Areas
.11 Evaluation of Laboratories By State Shellfish Laboratory Evaluation Officers Including Laboratory Evaluation Checklists-Laboratory Evaluation Checklist - PSP

Text of Proposal/ Requested Action Update PSP Laboratory Evaluation Checklist. Please find the updated PSP Laboratory Checklist attached - word document titled "Revised PSP Cecklist 11-08-2010.doc". A summary of the changes is:

- Added the checklist items for Jellett Rapid Test for PSP
- Renumbered checklist items to accommodate proposed additions and deletions and to better identify each checklist item.
- Added, deleted or changed language for checklist items to be consistent with the microbiology laboratory evaluation checklist including added laboratory education and experience requirements
- Deleted the requirement for metals testing on reagent water
- Clarified and defined requirements for laboratory equipment, reagents and the mouse bioassay method.

Public Health Significance: The current PSP laboratory checklist was last revised in 2005. Since that time the Jellett Rapid Test has received approval and is not in the checklist. Deficiencies have been identified while using the PSP checklist in evaluation of laboratories and the PSP checklist is inconsistent with some requirements in the microbiology checklist which has more recently been revised . It is important that the checklist items and quality assurance requirements are clear and understandable. It is important that quality assurance requirements among the different laboratory evaluation checklists remain as consistent as possible since many monitoring laboratories perform multiple types of tests and are evaluated using multiple checklists; inconsistencies among the checklist cause confusion, extra expense and work for the laboratories.

Cost Information (if available): None

Action by 2011 Laboratory Methods Review Committee Recommended referral of Proposal 11-109 to the appropriate committee as determined by the Conference Chairman.

Action by 2011 Task Force I Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 11-109.

Action by 2011 General Assembly Adopted recommendation of 2011 Task Force I on Proposal 11-109.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-109.

PUBLIC HEALTH SERVICE
U.S. FOOD AND DRUG ADMINISTRATION
OFFICE OF FOOD SAFETY
SHELLFISH AND AQUACULTURE POLICY BRANCH
5100 PAINT BRANCH PARKWAY
COLLEGE PARK, MD 20740-3835
TEL. 240-402-2151/2055 FAX 240-402-2601

SHELLFISH LABORATORY EVALUATION CHECKLIST

LABORATORY:		
ADDRESS:		
TELEPHONE:	**FAX:**	
EMAIL:		
DATE OF EVALUATION:	**DATE OF REPORT:**	**LAST EVALUATION:**
LABORATORY REPRESENTED BY:	**TITLE:**	
LABORATORY EVALUATION OFFICER:	**SHELLFISH SPECIALIST:** **REGION:**	
OTHER OFFICIALS PRESENT:	**TITLE:**	

Items which do not conform are noted by:

C- Critical K - Key O - Other NA - Not Applicable Conformity is noted by a "√"

Check the applicable assays performed:

	Mouse Bioassay (MBA)
	Jellett Rapid Test (JRT)

PART I – QUALITY ASSURANCE

ITEM		
CODE		
		1.1 Quality Assurance (QA) Plan
K	☐	1. 1.1 Written plan adequately covers all the following [check (√) those that apply]
		a. Organization of the laboratory.
		b. Staff training requirements.
		c. Standard operating procedures (SOPs).

		d. Internal quality control measures for equipment, calibration, maintenance repair ~~and~~, performance and rejection criteria established.
		e. Laboratory safety.
		f. ~~Quality assessment.~~ Internal performance assessment.
		g. ~~Proper animal care.~~External performance assessment.
		h. Animal care.
C	☐	**~~2.~~ 1.1.2 QA plan implemented.**
		1.2 Educational/Experience Requirements
C	☐	**1.2.1 In state/county laboratories, the supervisor meets the state/county educational and experience requirements for managing a public health laboratory.**
K	☐	1.2.2 In state/county laboratories, the analysts meet the state/county educational and experience requirements for processing samples in a public health laboratory.
C	☐	**1.2.3 In commercial laboratories, the supervisor must have at least a bachelor's degree in microbiology, biology or an equivalent discipline with at least two years of laboratory experience.**
K	☐	1.2.4 In commercial laboratories, the analysts must have at least a high school diploma and shall have at least three months of experience in laboratory science.
		1.~~2~~3 Work Area
O	☐	~~1.~~ 1.3.1 Adequate for workload and storage.
O	☐	~~2.~~ 1.3.2 Clean and well lighted.
O	☐	~~3.~~ 1.3.3 Adequate temperature control.
O	☐	~~4.~~ 1.3.4 All work surfaces are nonporous and easily cleaned.
C	☐	**~~5.~~ 1.3.5 A separate, quiet area with adequate temperature control for mice acclimation and injection is maintained.**
		1.~~3~~4 Laboratory Equipment
O	☐	~~1.~~1.4.1 The pH meter has a standard accuracy of 0.1 pH unit.
K	☐	~~1.4.2pH paper in the appropriate range (i.e. 1-4) is used with minimum accuracy of 0.5 pH units.~~ ~~2.~~ 1.4.2 pH paper in the appropriate range (i.e., pH <2 to >4.5) having a minimum accuracy of 0.5 units is used.
K	☐	~~3.~~1.4.3 The pH electrodes being used consist of a pH half cell and reference half cell or equivalent combination electrode/triode free from silver/silver chloride (Ag/AgCl) or contains an ion exchange barrier to prevent the passage of silver (Ag) ions into the ~~medium that may result in inaccurate pH readings~~ substance being measured.
K	☐	~~4.~~1.4.4 pH meter is calibrated daily or with each use. Results are recorded and records maintained.
K	☐	~~5.~~1.4.5 Effect of temperature has been compensated for by an ATC probe, use of a triode or by manual adjustment.
K	☐	~~6.~~1.4.6 A minimum of two standard buffer solutions (pH 2 & pH 7) is used to calibrate the pH meter. Standard buffer solutions are used once and discarded.
K	☐	~~7.~~1.4.7 Electrode ~~efficiency~~acceptability is determined daily or with each use ~~following either slope or~~ by the millivolt procedure or through determination of the slope. (circle the method used.)
K	☐	~~8. The balance provides a sensitivity of at least 0.1g at a load of 150 grams.~~ 1.4.8 The differing sensitivities in weight measurements required by the various steps in the assay are met by the balance/balances being used. a. To prepare the reference solution, the balance used must have a sensitivity of at least 0.1 gram at a load of 1 gram. b. For sample extraction, the balance used must have a sensitivity of at least 0.1 gram at a load of 100 grams. c. For gravimetric extract volume adjustment, the balance used must have a sensitivity of at least 0.1 gram at a load of 200 grams.

		d. To determine the weight of the mice, the balance must have a sensitivity of at least 0.1 gram at a load of 20 grams.
K	☐	~~9. The balance calibration is checked monthly using NIST Class S or ASTM Class 1or 2 weights or equivalent. Records maintained.~~ 1.4.9 Balance calibrations are checked monthly according to manufacturer's specifications using NIST Class S or ASTM Class 1 or 2 weights or equivalent. The accuracy of the balance is verified at the weight range of use. Results are recorded and records maintained.
K	☐	~~10.~~1.4.10 Refrigerator temperatures ~~is~~are maintained between 0 and 4°C.
O	☐	~~11~~1.4.11 Refrigerator temperatures ~~is~~are monitored at least once daily on workdays. Results are recorded and records maintained.
K	☐	~~12.~~1.4.12 Freezer temperatures ~~is~~ are maintained at ~~20°C~~or below -15°C.
O	☐	~~13.~~1.4.13 Freezer temperatures ~~is~~ are monitored at least once daily on workdays. Results are recorded and records maintained.
O	☐	~~14.~~1.4.14 All glassware is clean.
~~O~~ C	☐	~~15. Once during each day of washing, several pieces of glassware from each batch washed are tested for residual detergent with aqueous 0.04% bromthymol blue solution. Records are maintained.~~ **1.4.15 With each load of labware/glassware washed, the contact surface of several dry pieces from each load are tested for residual detergent (acid or alkali) with aqueous 0.04% bromthymol blue (BTB) solution. Results are recorded and records maintained.**
C	☐	**1.4.16 An alkaline or acid based detergent is used for washing glassware/labware**
		~~1.4~~1.5 Reagent and Reference Solution Preparation and Storage
C	☐	**1.5.1 Opened PSP ~~reference~~ standard solution (100µg/mL) is not stored.**
K	☐	~~2. PSP working standard solution (1 µg/ml) and all dilutions are prepared with dilute HCl, pH 3 water, using 'Class A' volumetric glassware (flasks and pipettes) or prepared gravimetrically.~~ 1.5.2 PSP reference solution (1µg/mL) is prepared by weight (grayimetrically) with dilute HCl, pH 3 water.
K	☐	~~3. Refrigerated storage of PSP working standard solution (1µg/ml) does not exceed 6 months and is checked gravimetrically for evaporation loss.~~ 1.5.3 Refrigerated storage of PSP reference solution (1µg/mL) in a sealed container is stored indefinitely as long as there is no evaporation loss as checked by weight. If evaporation is detected, the solution is discarded appropriately. Records are maintained.
C	☐	**1.5.4 Dilutions of the 1µg/mL reference solution are prepared by weight or volume using dilute HCl, pH 3 water.**
K	☐	~~4.~~1.5.5 PSP working dilutions(dilutions of the 1µg/mL reference solution) are discarded after use.
K	☐	~~5. Make up water is distilled or deionized (*circle one*) and exceeds 0.5 megohm resistance or is less than 2 µ Siemens/cm conductivity at 25°C to be tested and recorded monthly for resistance or conductivity *(circle the appropriate)*.~~ 1.5.6 Reagent water is distilled or deionized (*circle appropriate choice*), tested monthly and exceeds 0.5 megohm-cm resistance (2 megohms-cm in-line) or is less than 2.0 µSiemens/cm conductivity at 25°C (*circle the appropriate water quality descriptor determined*). Results are recorded and the records maintained.
O	☐	~~6.~~ 1.5.7 ~~Make up~~ Reagent water is analyzed for residual chlorine monthly and is at a nondetectable level (<0.1ppm). Results are recorded and records maintained.
~~K~~	☐	~~7. Make up water is free from trace (< 0.5 mg/l) dissolved metals specifically Cd, Cr, Cu, Ni, Pb, and Zn as determined annually with total heavy metal content ≤1.0 mg/l. Records maintained.~~
O	☐	~~8.~~1.5.8 ~~Makeup~~ Reagent water contains ~~<1000~~ <100 CFU/mL as determined monthly using the heterotrophic plate count method. Results are recorded and records maintained.
		1.~~5~~6 Collection and Transportation of Samples
O	☐	~~1. Shellstock are collected in clean, waterproof, puncture resistant containers.~~ 1.6.1 Shellfish are collected in clean, waterproof , loosely sealed, puncture

		resistant containers.
K	☐	~~2.~~1.6.2 Samples are appropriately labeled with the collector's name, harvest area, sampling station and time and date of collection.
K	☐	~~3. Immediately after collection, shellstock samples are placed in dry storage for transport (e.g. cooler) which is maintained between 0 and 10°C. Upon receipt at the lab, samples are placed under refrigeration.~~ 1.6.3 Immediately after collection, shellfish samples are placed in dry storage (ice chest or equivalent) which is maintained between 0 and 10°C with ice or cold packs for transport to the laboratory. Upon receipt at the laboratory, samples are placed under refrigeration.
K	☐	~~4.~~1.6.4 The time from collection to completion of the bioassay should not exceed 24 hours. However, if there are significant transportation delays, then shellstock samples are processed immediately as follows (*circle the appropriate choice*): a. Washed, shucked, drained, frozen until extracted. b. Washed , shucked, drained, homogenized and frozen. c. Washed, shucked, drained, extracted, the supernatant decanted and refrigerated (**best choice**) ; or d. The laboratory has an appropriate contingency plan in place to handle samples which can't be analyzed within 24 hours due to transportation issues.
~~K~~C	☐	~~5.~~**1.6.5 Frozen, shucked product or homogenates are allowed to thaw completely and all liquid is included as part of the sample before being processed further.**
Part II – ~~EXAMINATION~~ ANALYSIS OF SHELLFISH FOR PSP TOXINS		
		2.1 Preparation of the Sample
C	☐	**~~1.~~ 2.1.1 At least 12 animals (equivalent to at least 100 g of shellfish meat) are used per sample or the laboratory has a~~n~~ ~~appropriate~~ proven effective contingency plan for dealing with non-typical species of shellfish.**
O	☐	~~2.~~ 2.1.2. The outside of the shell is thoroughly cleaned with fresh water.
O	☐	~~3.~~ 2.1.3 Shellstock are opened by cutting adductor muscles.
O	☐	~~4.~~ 2.1.4 The inside of the shell is rinsed with fresh water to remove sand or other foreign material.
O	☐	~~5.~~ 2.1.5 Shellfish meats are removed from the shell by separating adductor muscles and tissue connecting at the hinge.
K	☐	~~6.~~ 2.1.6 Damage to the body of the mollusk is minimized in the process of opening.
O	☐	~~7.~~2.1.7 Shucked shellfish are drained on a #10 mesh sieve (or equivalent) without layering for 5 minutes.
K	☐	~~8.~~ 2.1.8 Pieces of shell and drainage are discarded.
C	☐	**~~9. Drained meats or thawed homogenates are blended at high speed until homogenous~~** ~~(60 – 120 seconds).~~ **2.1.9 Drained meats or previously cooled/refrigerated, shucked, drained meats and their drip-loss liquid or thawed, shucked meat with its freeze-thaw liquid or thawed homogenates with their freeze-thaw liquid are blended at high speed until homogenous (60 – 120 seconds).**
		2.2 Extraction
K	☐	~~1.~~ 2.2.1 100 grams of homogenized sample is weighed into a beaker.
K	☐	~~2.~~ 2.2.2 An equal amount of 0.1 N/0.18 N HCl is added to the homogenate and thoroughly mixed. (*circle the appropriate normality*).
C	☐	**~~3.~~ 2.2.3 The pH is checked and, if necessary adjusted to between pH 2.0 and 4.0.**
C	☐	**~~4.~~ 2.2.4 Adjustment of the pH is made by the dropwise addition of either (5 N HCl) or base (0.1 N NaOH) as appropriate while constantly stirring the mixture.**
C	☐	**~~5.~~ 2.2.5 The homogenate/acid mixture is promptly brought to a boil, 100 +1°C then gently boiled for 5 minutes.**
O	☐	~~6.~~ 2.2.6 The homogenate/ acid mixture is boiled under adequate ventilation (i.e., fume hood).

O	☐	~~7.~~ 2.2.7 The extract is cooled to room temperature.
C	☐	**~~8.~~ 2.2.8 The pH of the extract is determined and adjusted if necessary to between pH 2 and 4 preferably to pH 3 with the stirred dropwise addition of 5 N HCl to lower the pH or 0.1 N NaOH to raise the pH.**
K	☐	~~9.~~ 2.2.9 The extract volume~~(or mass)~~ is adjusted to 200 mL (or grams) with dilute HCl, pH 3.0 water.
K	☐	~~10.~~2.2.10 The extract is returned to the beaker, stirred to homogeneity and allowed to settle to remove particulates; or, if necessary, an aliquot of the stirred supernatant is centrifuged at 3,000 RPM for 5 minutes before ~~injection~~ being bioassayed.
K	☐	~~11. If mice cannot be injected immediately then the supernatant should be removed from the centrifuge tubes and refrigerated for up to 24 hours.~~ 2.2.11 If the extract cannot be bioassayed or the Jellett Rapid Test (JRT) for PSP cannot be performed immediately, then the supernatant is removed from the centrifuge tubes and sealed and refrigerated for up to 24 hours.
K	☐	~~12.~~ 2.2.12 Refrigerated extracts are allowed to reach ambient temperature before being bioassayed or tested by the JRT for PSP.
		2.3 Bioassay
O	☐	~~1.~~ 2.3.1 A 26-gauge hypodermic needle is used for injection.
~~K~~**C**	☐	~~2. Healthy mice in the weight range of 17 – 23 grams (19 – 21 grams is preferable) from a stock colony are used for routine assays. Mice are not reused for the bioassay.~~ ~~Stock strain used_____ Source of the mice _____~~ **2.3.2 Healthy mice in the weight range of 17 – 23 grams (19 – 21 grams is preferable) from a stock colony are used for routine assays. Mice are not reused for the bioassay.** **Stock strain used_____ Source of the mice_____**
C	☐	**~~3.~~ 2.3.3 Mice are allowed to acclimate for at least 24 hours prior to injection. In some cases up to 48 hours may be required.**
C	☐	**~~4.~~ 2.3.4 A conversion factor (CF) has been determined as _____. Month and year when current CF determined _____.**
C	☐	**~~5.~~ 2.3.5 CF value is checked weekly if assays are done on several days during the week, or, once each day that assays are performed if they are performed less than once per week.** **Date of most recent CF check_____** **CF verified~~/CF not verified~~; *yes / no*: (*circle the appropriate choice*).**
C	☐	**~~6.~~ 2.3.6 If the CF is not verified, 5 additional mice are injected with the dilution used in the CF check to complete a group of 10 mice. Ten additional mice are also injected with this dilution to produce a second group of 10 mice. The CF is calculated for each group of 10 mice and averaged to give the CF to be used in sample toxicity calculations for the day's or week's work only. All subsequent work must make use of the original laboratory CF value unless this value continues to fail to be verified by routine CF checks.**
C	☐	**~~7.~~ 2.3.7 If the CF fails to be verified, the cause is investigated and the situation corrected. If the cause cannot be determined with reasonable certainty and fails >3 times per year, the bioassay is restandardized.**
O	☐	~~8.~~ 2.3.8 Mice are weighed to the nearest ~~0.5 gram~~ 0.1 gram .
C	☐	**~~9.~~ 2.3.9 Mice are injected intrapertioneally with 1 mL of the acid extract.**
K	☐	~~10.~~2.3.10 For the CF check at least 5 mice are used.
C	☐	**~~11.~~ 2.3.11 At least 3 mice are used per sample in routine assays.**
C	☐	**~~12.~~2.3.12 Elapsed time is accurately determined and recorded.**
K	☐	~~13.~~ 2.3.13 If death occurs, the time of death to the nearest second is noted by the last gasping breath.

C	☐	**2.3.14 Mice are continually observed for up to 20 minutes after injection with periodic checks for a total of 60 minutes as appropriate.**
C	☐	**~~14.~~ 2.3.15 If the median death time~~(2 out of 3 mice injected die)~~ is <5 minutes, a dilution is made with dilute HCl, pH 3 water, to obtain a median death time in the range of 5 to 7minutes.**
		2.4 Calculation of Toxicity
C	☐	**~~1.~~ 2.4.1 The death time of each mouse is converted to mouse units (MU) using Sommer's Table (Table 6, *Recommended Procedures for the examination of Sea Water and Shellfish, Fourth*, ~~4th~~ Fourth Edition). The death time of mice surviving beyond 60 minutes is considered to be <0.875 MU.**
K	☐	~~2.~~ 2.4.2 A weight correction in MU is made for each mouse injected using Table 7 in *Recommended Procedures for the Examination of Sea Water and Shellfish*, Fourth 4th- Edition.
C	☐	**~~3.~~ 2.4.3 The death time of each mouse in MU is multiplied by a weight correction in MU to give the corrected mouse unit (CMU), the true death time for each mouse.**
C	☐	**~~4.~~ 2.4.4 The median value of the array of corrected mouse units (CMU) is determined to give the median corrected mouse unit (MCMU), median death time.**
C	☐	**~~5.~~ 2.4.5 The concentration of toxin is determined by the formula, MCMU x CF x Dilution Factor (DF) x 200.**
C	☐	**~~6.~~ 2.4.6 Any value greater than 80 µg/100 grams of meat is actionable.**
PART III – JELLETT RAPID TEST (JRT) FOR PSP		
		3.1 Procedure
K	☐	3.1.1 The batch/lot numbers of the test strips and buffers, their expiration dates, date received and date used are recorded.
K	☐	3.1.2 When placed into service, test strips and buffers (PSP & Matrix) are within their respective expiration dates.
C	☐	**3.1.3 When opened, the test strip desiccant pouch is blue in color indicating its suitability for use. Test strips emerging from desiccant pouches which are pink in color are never used.**
K	☐	3.1. 4 Test strips and buffer are stored according to the manufacturer's instructions.
C	☐	**3.1.5 Negative extracts are spiked at a low level concentration (40 – 60 µg/100 grams of sample) or equivalent (a bioassayed extract) and used as a positive control for testing both new batches/lots of kits and buffers. Results are recorded and records maintained.**
C	☐	**3.1.6 Micropippettors capable of accurately delivering volumes of 100 and 400 µL are used to transfer buffer and sample extracts and to inoculate test strips with diluted extract.**
K	☐	3.1.7 Volumes delivered by the micropipettor are checked for accuracy at 100 and 400 µL monthly while in service. Results are recorded and records maintained.
C	☐	**3.1.8 400 µL of the buffer supplied with the test kits is accurately transferred to a small tube.**
C	☐	**3.1.9 100 µL of the sample extract is added to the buffer.**
K	☐	3.1.10 The sample/extract is thoroughly mixed with buffer by inserting the tip of the micropipettor into the buffer/sample extract mixture and pipetting up and down at least three (3) times.
C	☐	**3.1.11 100 µL of the thoroughly mixed diluted sample extract is inoculated into the test strip sample well.**
K	☐	3.1.12 Micropippettor tips are not reused.
K	☐	3.1.13 Inoculated test strips are allowed to react with the sample extract for the period of time specified by the manufacturer.
C	☐	**3.1.14 The test is interpreted according to the manufacturer's instruction card which is specific to each batch/lot of test strips.**
K	☐	3.1.15 When invalid tests are repeated, the pH of the sample extract is checked and adjusted as necessary to between pH 2.0 and pH 4.0. An aliquot of Matrix

		buffer and a fresh test strip is used to reassay the sample.
C	☐	**3.1.16 When a repeated JRT test for PSP gives identical invalid results, the sample contains interfering substances which require the use of the mouse bioassay for testing.**
C	☐	**3.1.17 A positive JRT for PSP is actionable.**

Revised 11 – 08 2010

REFERENCES

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5. APHA/WEF/AWWA. 1992. *Standard Methods for the Examination of Water and Wastewater*, 18th Edition. APHA, Washington, D.C.
6. Title 21, Code of Federal Regulations, Part 58, Good Laboratory Practice for Nonclinical Laboratory Study. U.S. Government Printing Office, Washington, D.C.
7. National Research Council. 1996. *Guide for the Care and Use of Laboratory Animals*. National Academy Press. Washington, D.C.
8. Personal communication with USFDA Seafood Laboratory Branch, Office of Seafood, CFSAN, 1998-1999.
9. JRT Instruction Materials with specified batch/lot number instructions.
10. NELAP – National Environmental Laboratory Accreditation Conference. 2003. Chapter 252. ENVIRONMENTAL LABORATORY ACCREDITATION, 252.302. Qualifications of the Laboratory Supervisor, 252.304. Personnel Requirements.

Laboratory Evaluation Checklist – **PSP**

LABORATORY:			**DATE OF EVALUATION:**
SHELLFISH LABORATORY EVALUATION CHECKLIST **SUMMARY OF NONCONFORMITIES**			
Page	**Item**	**Observation**	**Documentation Required**

Revised 11 – 08 – 2010 Page _____ of _____

Laboratory Evaluation Checklist - **PSP**

LABORATORY STATUS	
LABORATORY:	**DATE:**
LABORATORY REPRESENTATIVE:	

PARALYTIC SHELLFISH TOXIN COMPONENT: PARTS I and II and III

A. Results:

Total # of Critical (C) Nonconformities _____
Total # of Key (K) Nonconformities _____
Total # of Other (O) Nonconformities _____
Total # of Critical, Key and Other Nonconformities _____

B. Criteria for Determining Laboratory Status of the PSP Component

1. **Does not Conform Status**. The PSP component of this Laboratory is not in conformity with NSSP requirements if :
   a. The total # of Critical Nonconformities is **>3 or**
   b. The total # of Key Nonconformities is **>6 or**
   c. The total # of Critical, Key and Other is **>10**

2. **Provisionally Conforms Status.** The PSP component of this Laboratory is determined to be provisionally conforming to NSSP requirements if the number of **Critical** Nonconformities is **< 3 and** the number of **Key** Nonconformities is **<6 and** the number of **Other** Nonconformities is **<4.**

3. **Conforming Status.** The PSP component of this Laboratory is determined to be conforming when it has **no Critical** Nonconformities **and < 6 Key** Nonconformities **and < 4 Other** Nonconformities.

C. Laboratory Status *(circle appropriate choice)*:
Does Not Conform - Provisionally Conforms - Conforms

Revised 11 - 08 – 2010

Proposal Subject:	Refinement of Fecal Colliform Sources
Specific NSSP Guide Reference:	2009 NSSP Guide for the Control of Molluscan Shellfish Section II Model Ordinance Chapter IV Shellstock Growing Areas @.02 Bacteriological Standards.
Text of Proposal/ Requested Action	Add the following statement to Note: "Where there is evidence that the fecal coliform strategy for sampling is effected by false positives from decaying vegetation or other bacteria (within 1000 feet of shoreline) that do not indicate a risk to consumer health, the authority is required to perform adequate source testing. The authority shall subtract these false positive results from the fecal coliform result to get an accurate reading of the actual bacteriological quality of the test station."
Public Health Significance:	None. This additional source testng is to refine the source of fecal in a non-point source remote site where there is no other evidence of human pathogens. There is substantial evidence that the bacteria that is involved in the decay of vegitation does test positive for the fecal coliform in the test that is currently the standard. Three documents are attached to provide adequate and sufficient rationale for this change to the NSSP.
Cost Information (if available):	Unkown. It is expected that cost of sampling will be reduced as more accurate sampling will result in less sampling required.
Action by 2011 Task Force I	Recommended no action on Proposal 11-110. Rationale: Adequately addressed in the Model Ordinance.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 11-110.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 11-110.

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Infectious Diseases Associated with Molluscan Shellfish Consumption

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INTRODUCTION

Raw and partially cooked molluscan shellfish (clams, oysters, and mussels) have a long history as vectors of infectious agents and marine biotoxins. Illnesses associated with these food sources originate principally from bacterial and viral pathogens and from toxin-producing dinoflagellates concentrated by shellfish during the filter-feeding process. Infectious disease outbreaks have been reported in the United States since the late 1800s; since then, more than 400 outbreaks and 14,000 cases have been reported (Table 1). These illnesses are attributed to bacterial and viral agents that are associated either with human wastes (delivered to estuarine and marine environments in sewage effluents that have received variable levels of treatment) or to bacterial pathogens indigenous to coastal marine environments (e.g., *Vibrio* spp.).

Before the 1950s, the most common illness associated with the consumption of raw molluscan shellfish was typhoid fever (Fig. 1). After several large outbreaks of typhoid in the mid-1920s (15), when more than 1,500 cases and 150 deaths were reported in several U.S. cities, the U.S. Public Health Service convened a committee to establish regulations for the sanitary control of shellfish. This committee, a forerunner of the National Shellfish Sanitation Program, made the following recommendations (7). (i) Shellfish should be marketed from growing areas that, on careful examination, are free from any suspicion of dangerous contamination with disease-producing organisms or from any deleterious or offensive substances. (ii) After their removal from the water, shellfish should be handled in a manner that would safeguard them from contamination with pathogenic microorganisms or nonpathogenic agents (e.g., toxins, heavy metals, and organics), deterioration, or alteration that would render them unfit for consumption, either hygienically or aesthetically. (iii) Epidemiological studies should be conducted for all outbreaks (epidemics) that implicate shellfish so that the sources of the shellfish can be promptly and accurately traced and measures can be initiated to prevent further infection.

It was understood at that time that the inappropriate disposal of raw and partially treated sewage was a principal reason for the increasing incidence of shellfish-borne illness, particularly typhoid fever. In addition, the process of "fattening" oysters, whereby the animals absorb water through osmosis when placed in tanks of low salinity, was also of significant public health concern. Under poor sanitary conditions, these tank waters (and shellfish) may have been contaminated with pathogenic microorganisms, including *Salmonella typhi*.

As the National Shellfish Sanitation Program recommendations gradually gained acceptance, the incidence of typhoid began to decline for at least two reasons. First, the technology for treating sewage wastes improved, particularly with regard to the removal of pathogen-associated particulates and disinfection. Second, a water quality standard was developed for classifying shellfish-growing areas on the basis of densities of the total coliform bacterial indicator group. This early classification system was used to determine whether or not shellfish could be harvested from given waters, depending on the levels of the indicator group found therein. The standard, as one aspect of the National Shellfish Sanitation Program, appears to have been effective, since no shellfish-associated typhoid cases have been reported in the United States in almost 40 years (Fig. 1).

In the past two decades, however, the nature of shellfish-vectored illness has changed. This report considers infectious diseases from a historical perspective, leading up to current public health issues associated with consumption of raw shellfish. It deals with problems that result from the contamination of molluscan shellfish resources by infectious agents from human and/or animal fecal wastes, treated and untreated wastewaters, and the marine environment.

Data are presented for outbreak (defined as two or more cases of illness resulting from a common exposure), incident (a report of infectious disease resulting from a given exposure, involving usually one person and an etiological agent of *Vibrio* spp.), and case reports primarily from the United States. Information used for this report was obtained from federal,

TABLE 1. Cases and outbreaks of infectious disease (all agents) resulting from the consumption of molluscan shellfish (1898–1990)

Decade	Outbreaks		Cases	
	No.	% of total	No.	% of total
1900	11	2.6	364	2.5
1910	7	1.7	208	1.4
1920	17	4.0	2,161	14.8
1930	31	7.4	567	3.9
1940	40	9.5	1,840	12.6
1950	6	1.4	134	0.9
1960	48	11.4	1,726	11.9
1970	44	10.5	871	6.0
1980	217	51.5	6,687	45.9

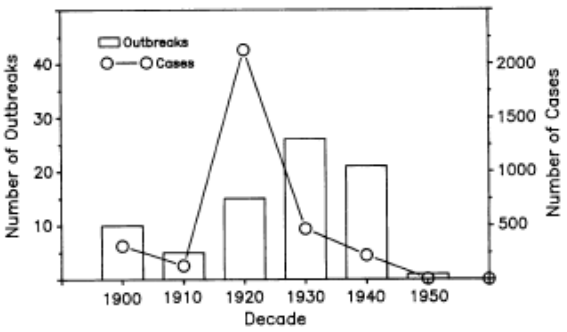

FIG. 1. Number of outbreaks and cases of shellfish-vectored typhoid fever reported since 1894.

state, and local government agencies, research reports, news accounts, and personal communications and does not represent an active, prospective investigation to identify cases of shellfish-associated disease. The data reported here probably represent only a small portion of the actual number of cases that occur annually (10). The true incidence of shellfish-vectored infectious disease may be underestimated as much as 20-fold or more (2). This is true for several reasons. First, because there are no mandatory federal requirements for reporting gastroenteritis of an unspecified nature (i.e., it is not a reportable illness), physicians and state health departments are generally under no obligation to forward case reports to federal authorities. Second, many reported illnesses are cases of relatively mild gastroenteritis; thus, few victims ever seek treatment by a physician. Those reported often describe outbreaks in which relatively large groups of people are affected (e.g., company picnics or gatherings at restaurants). Third, when only a limited number of people are infected, it is very difficult to ascribe the illness to one particular food source. For these reasons, the data may not accurately reflect the true magnitude of the social and economic consequences of illnesses that result in death, that require extended physician and/or hospital care, or, if moderately acute, that prevent individuals from pursuing normal daily activities (2).

ILLNESS ASSOCIATED WITH DISPOSAL OF FECAL WASTES AND SEWAGE INTO THE AQUATIC ENVIRONMENT

Wastewater Disposal Practices and Shellfish-Vectored Illnesses

The association of shellfish consumption and infectious disease has been known or suspected for many years. In 1816, more than 40 years before Pasteur advanced his germ theory of disease, the French physician Pasquier described typhoid fever in a group of people who had consumed oysters harvested from a coastal area contaminated by raw sewage (9, 18). In the United States, infectious bacterial disease associated with molluscan shellfish consumption was first reported in 1894 with two cases of typhoid fever described in Connecticut from shellfish harvested from its coastal waters. No documented cases of infectious disease were reported in the United States before that time, although other types of shellfish-associated illnesses (caused by marine biotoxins) were reported in the late 1700s (16). There are several reasons for this. The construction of storm water or sewerage systems, which began during the mid- to late 1800s in urban centers, resulted in the consolidation of human-derived wastes in collection systems and their eventual release into near coastal environments (8). This practice resulted in the progressive contamination of commercial and recreational shellfish-growing areas and outbreaks of enteric disease associated with shellfish harvested from them. Also, unlike the case for illnesses caused by marine biotoxins, the association between consumption of sewage-contaminated shellfish and infectious disease was not established until the late 1800s. Marine biotoxins, which are produced by dinoflagellates and are a naturally occurring and often highly visible phenomenon, are not associated with sewage contamination of coastal environments. In addition, the occurrence of algal blooms coupled with shellfish-associated human intoxications (generally occurring within several hours of ingestion) establishes an immediately evident relationship. With enteric infections, the relationship is not as clear, since there is no visible measure of water quality and the onset of illness after consumption of contaminated shellfish can be days to weeks.

Until the 1980s, the number of shellfish-associated infectious disease outbreaks was less than 50 outbreaks per decade. Outbreaks and cases by decade as a percentage of the total reported are presented in Table 1. More than 45% of the total historical cases were reported in the 1980s, although there are no obvious reasons for this dramatic increase. However, in the past decade, certain states have adopted aggressive procedures for identifying and describing shellfish-associated disease outbreaks. New York and Florida alone account for more than 50% of the total number of incidents reported nationwide (Table 2). This fact, coupled with increasing consumer awareness of health problems associated with seafood consumption,

TABLE 2. Incidents and cases of shellfish-associated disease (all agents) by principal reporting states

State	Incidents		Cases	
	No.	%[a]	No.	%[b]
Florida	197	26.5	735	5.0
New York	195	26.3	6,611	45.3
Louisiana	48	6.5	195	1.3
Massachusetts	41	5.5	665	4.6
Connecticut	37	5.0	517	3.5
Texas	31	4.2	452	3.1
California	26	3.5	323	2.2
New Jersey	22	3.0	1,989	13.6
Alabama	20	2.7	291	2.0
Georgia	12	1.6	37	0.3

[a] Percentage of the total number of incidents reported nationwide (see text for definitions).
[b] Percentage of the total number of cases reported nationwide.

TABLE 3. Shellfish-borne disease agents occurring in and transmitted by sewage and/or wastewater (1898–1990)

Agent	No. of cases	No. of incidents	No. of outbreaks
Unknown[a]	7,978	277	256
Typhoid	3,270	93	78
Hepatitis A	1,798	51	42
Norwalk virus	311	7	7
Salmonella spp.	130	8	3
Snow Mountain virus	116	4	4
Shigella spp.	111	9	4
Hepatitis[b]	47	5	2
Campylobacter spp.	27	12	1
Plesiomonas spp.	18	3	1
Aeromonas spp.	7	1	1
Staphylococcus aureus	5	1	1
Bacillus cereus	4	1	1
Escherichia coli	2	1	1

[a] No agent isolated or identified.
[b] Type unspecified.

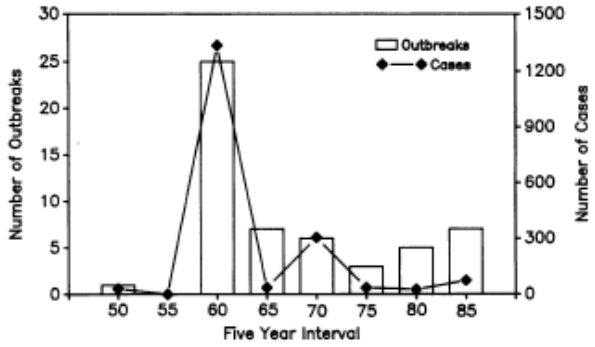

FIG. 2. Shellfish-vectored outbreaks and cases of hepatitis A from 1950 to 1989.

may partially explain the abrupt increase in outbreak and case reports. In addition, shellfish, and particularly oysters, are becoming an increasingly scarce resource as the total acreage of estuarine and marine environments approved or conditionally approved for harvest for direct human consumption decreases with increasing inputs of human-associated contaminants to those areas (14). Also, large areas of potentially productive shellfishing grounds remain closed because they have not been subjected to the sanitary survey work required for proper classification. As a result, there is a strong economic incentive for the illegal harvesting of shellfish from closed but productive growing areas where contaminant loads exceed a generally accepted safe level. This criminal activity is certainly a factor that affects public health, as sewage-contaminated shellfish enter the marketplace. Finally, the rise in case reports may be attributed to deficiencies in current sewage treatment practices (e.g., sewage treatment plants may exceed their design capacity or may have periodic breakdowns which result in inadequate particle removal or disinfection), coupled with the increasing volumes of wastes disposed of in our coastal waters. The use of chlorine to disinfect wastewater effluents is a particular problem in this regard. Certain human enteric viral pathogens (e.g., Norwalk virus) are resistant to the elevated chlorine levels (12) that effectively inactivate vegetative bacterial cells, including the total and fecal coliform indicator groups. Thus, the fecal coliform group, which is the principal indicator of the sanitary quality of most state shellfish-growing waters, may not reliably index the quality of waters that receive chlorine-disinfected effluents. Waters presently considered to be safe for the harvest of molluscan shellfish may, in fact, be contaminated with enteric viral pathogens, and shellfish harvested from those areas may pose an unacceptably high risk of viral illness.

There is no conclusive evidence of an association between contamination derived from animal fecal wastes and the occurrence of shellfish-vectored human illnesses (22). Current assumptions are that illnesses occur primarily from shell stock that accumulate waste from human-associated sources.

Agents of Viral and Bacterial Gastroenteritis

The etiological agents associated with the consumption of raw and lightly cooked molluscan shellfish are listed in Table 3. Most illness reports are ascribed to gastroenteritis, with no causative agent isolated or identified. However, in most cases, the symptoms of disease are very similar. Many reports describe a relatively "mild" gastroenteritis with a typical onset time of 24 to 48 h and a duration of about 2 days. Rarely is a physician's care required. The symptoms, onset, and duration are characteristic of viral gastroenteritis (Norwalk virus has often been implicated). However, since methods for identifying some of these viruses in stools have only recently been developed, and since a limited supply of antigen (obtained from fecal samples of infected individuals) has previously been available only for serological work, these viral pathogens have rarely been identified in shellfish-associated outbreaks. Moreover, there are presently no methods for isolating and culturing viruses from the Norwalk family of agents, including many of the small round viruses.

Bacterial illnesses associated with molluscan shellfish consumption have been infrequently reported since the last case of shellfish-vectored typhoid fever in 1954. Among these bacterial agents (e.g., *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Plesiomonas* spp., *Aeromonas* spp., and *Escherichia coli*), most are found in sewage wastes and are readily inactivated by chlorine disinfection. *Aeromonas* spp. and *Plesiomonas* spp. also occur naturally in freshwater and estuarine environments but appear to present a minimal public health hazard, especially compared with that associated with the environmental *Vibrio* spp. Illnesses caused by *Staphylococcus aureus* or *Bacillus cereus* are most likely a result of postharvest contamination. The recently recognized *E. coli* O157 biotype associated with outbreaks from improperly cooked beef has yet to be associated with a shellfish vector. However, its frequent occurrence in livestock indicates a potential public health problem with shellfish harvest areas affected by farm runoff.

Hepatitis A Infections

Hepatitis A is one of the most serious illnesses associated with shellfish-vectored disease, causing debilitating and chronic infection and even death. The first documented outbreak of shellfish-borne hepatitis occurred in Sweden (19) in 1956, when 629 cases associated with raw oyster consumption were reported. Subsequent to that, hepatitis A cases were reported in the United States (Fig. 2). In 1961, several large outbreaks were reported among consumers of raw oysters in Mississippi and Alabama and consumers of raw clams in New Jersey and Connecticut. In 1964, 20 outbreaks and 743 cases were reported among consumers of both oysters and hard clams and other, unspecified shellfish in several states. In most instances, shellfish harvest areas were not identified for several

reasons. First, the onset time of this illness is 2 to 8 weeks or more, and by then, the implicated shellfish were no longer in the distribution system. Second, the tagging systems used to identify original shellfish harvest sites were, and still are, often unreliable. (The tagging system involves labeling sacks of shellfish with specified information, including harvest area. Tags remain attached to the sacks throughout the distribution network until a specified time after their retail distribution.) There is no standard, nationally accepted tagging system for confidently determining the original harvest area of a given lot of shellfish. Third, the economic incentive for "bootlegging" shellfish (i.e., illegally harvesting animals from unapproved or prohibited areas) is quite compelling. The state patrol procedures needed to deter this illegal activity are often compromised by the lack of financial resources and manpower needed for active and suitable enforcement. In addition, the penalties for these offenses are often not a sufficient deterrent. Thus, shellfish that are not suitable for raw consumption can, and do, enter the marketplace. The magnitude of this problem is not known.

The percentage of hepatitis A virus outbreaks is lower than that caused by certain other infectious agents, and most outbreaks that are reported usually involve a large number of cases. Underreporting of a shellfish-vectored hepatitis A virus outbreak is due to the extended onset period following consumption of the contaminated food and the corresponding difficulty in determining a common food source when only a limited number of individuals are involved. Outbreaks of hepatitis A have been reported consistently since the early 1960s (Fig. 2), and the illness continues to be a public health concern today. Worldwide, the illness is reported frequently. The most disturbing recent incident occurred in China in 1988 (1), when more than 292,000 cases (nine deaths) of hepatitis A (associated with the consumption of uncooked, contaminated cockles) were reported in the urban areas around Shanghai. This outbreak clearly demonstrated the need for effective sanitation programs to prevent the introduction of contaminated shellfish into the marketplace and what can happen when the system breaks down or when there are no effective programs in place.

Seasonality of Illness Reports

Gastroenteritis of an unknown or viral etiology seems to occur more frequently at certain times of the year. When grouped by month, both the outbreak (Fig. 3) and case (Fig. 4) data reveal two periods of increased illness: late spring and late fall. These incidents roughly coincide with times when bioaccumulation rates in shellfish are high. During certain times in spring (4) and fall (5) in temperate waters, hard clams (*Mercenaria mercenaria*) accumulate viruses (and other microbial indicators) at a significantly higher rate than at other times of the year and thus can be periodically contaminated with high levels of sewage-associated microorganisms, including microbial pathogens. This phenomenon is subsequently reflected in the human health effects data. The increased consumption of raw shellfish (particularly hard clams) during these periods may also be coincident with higher illness rates. Although data are lacking on the seasonal incidence of Norwalk and Norwalk-like viruses, they probably show a seasonal occurrence much like that of other viral groups that are epidemic only at certain times of the year. Their input into the marine and estuarine environment would then be intermittent and unpredictable as they pass through the infected individual into the wastewater stream.

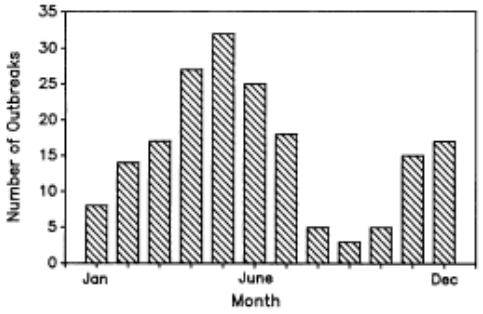

FIG. 3. Number of outbreaks by month of illness ascribed to viral pathogens or to illnesses of undetermined etiology (1894 to 1989).

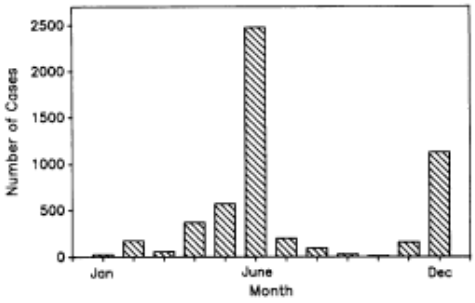

FIG. 4. Shellfish-associated illness cases ascribed to viral pathogens or to illnesses of undetermined etiology (1894 to 1989).

Relative Incidence of Allocthonous Microbial Agents Involved in Disease Outbreaks

The bacterial agents of shellfish-associated disease (Table 4) represent a small proportion of the outbreak (4.0%) and case (3.8%) reports. This may be because the indicator organisms used to assess and classify the sanitary quality of shellfish-growing areas (as open, restricted, or prohibited) effectively protect the health of the shellfish-consuming public against diseases of an allocthonous bacterial origin or because the etiological agents of gastroenteritis associated with shellfish outbreaks are infrequently isolated and identified.

Compared with bacteria, viral agents of shellfish-vectored disease represent a significantly greater proportion (Table 4) of the totals reported. However, those cases in which no agent was isolated represent the bulk of illness reports (more than 75% of the cases and 79% of the outbreaks). If the presumption is correct that most of these "unknowns" can be ascribed

TABLE 4. Shellfish-vectored disease outbreaks and cases by class of agent for sewage- and wastewater-associated pathogens (1905–1990)[a]

Class of agent	Cases		Outbreaks	
	No.	% of total	No.	% of total
Unknown	7,978	75.7	256	79.0
Viral	2,272	21.5	55	17.0
Bacterial	304	3.8	13	4.0

[a] Typhoid fever is not included in this table. The last reported shellfish-vectored case was in 1954.

TABLE 5. Incidents, outbreaks, and cases of shellfish-associated illnesses associated with members of the *Vibrio* genus (1967–1990)

Vibrio species	No. of cases	No. of incidents	No. of outbreaks
V. parahaemolyticus	159	60	14
V. vulnificus	160	133	8
V. cholerae non-O1	143	57	14
V. cholerae[a]	5	3	2
V. cholerae O1	14	14	
V. fluvialis	8	6	1
V. hollisae	15	15	
V. mimicus	14	14	
V. alginolyticus	1	1	
Vibrio spp.	6	4	1

[a] No serotype specified.

to a viral agent (symptomatically), enteric viral pathogens present the principal concern to the public health.

AUTOCHTHONOUS MARINE BACTERIA AS AGENTS OF SHELLFISH-VECTORED ILLNESSES

Several *Vibrio* spp., native to both marine and estuarine environments, have been identified as the causative agents of shellfish-vectored illnesses (Table 5). These halophilic, nonsporeforming bacteria occur in saline aquatic environments in densities that are related, at least in part, to water temperatures and salinity. Other factors that influence their occurrence and distribution in the aquatic environment are not well understood. The severity of human disease caused by the different species (Table 5) varies considerably. *Vibrio vulnificus* infections can result in septicemia with a high mortality rate; cholera has been reported among consumers of raw shellfish for the past two decades. All of the *Vibrio* spp. listed in Table 5, except *V. vulnificus*, are associated with gastroenteritis of varying severity. Among this group, the O1 serogroup of *Vibrio cholerae* is the most serious and debilitating. In general, all of these agents produce a much more severe gastroenteritis than that caused by enteric viral pathogens.

V. vulnificus

Oyster-associated *V. vulnificus* septicemia and death were first reported in 1975 (3). Since then, about ten cases (five deaths) of oyster-borne infections attributable to this species have been reported annually in the United States. The population at risk of developing this illness is well defined because they have certain health problems, such as liver cirrhosis, diabetes, hemochromatosis, and immunosuppressive disorders (17), which predispose them to infectious disease. The case fatality rate averages about 50% among this group. Given the numbers of individuals at risk and the frequency of raw shellfish consumption in certain areas of the United States (13), it is surprising that the number of cases and deaths is not higher. Clearly, the mechanisms of pathogenesis of this organism need further investigation. In addition, temperature abuse (i.e., the holding of shellfish at temperatures in excess of 45°C for prolonged periods of time in transit or in the marketplace) may contribute to illness associated with *Vibrio* spp. (or other bacteria) by providing a condition that would allow these pathogens to multiply in the molluscan shell stock. The significance of the role of temperature abuse in human morbidity or mortality is unknown.

V. vulnificus case reports show a seasonal pattern, with the highest frequencies occurring from midsummer through late

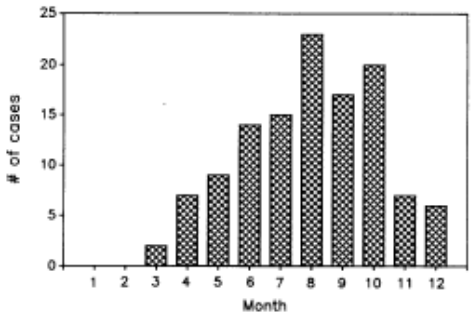

FIG. 5. Cases of shellfish-vectored *V. vulnificus* infections reported by month (1975 to 1989).

fall (Fig. 5). No shellfish-associated cases have been reported in the United States in January or February. Because of its temperature sensitivity, *V. vulnificus* is found in highest densities when water temperatures exceed 15°C (23); below this temperature, environmental densities decline rapidly. *V. vulnificus* is commonly found in all U.S. coastal waters and presumably in all species of near-coastal shellfish in densities that fluctuate with the season. However, case reports associate illness from this organism only with consumption of raw oysters and with shellstock harvested from waters of the U.S. Gulf Coast. The reason for this remains unexplained.

V. cholerae

Cholera was first identified in the United States in 1832, and the illness, involving several large food- and waterborne epidemics (20), was reported periodically until 1911. After that, it was believed to have been eradicated from this country. However, in 1973, a Texas fisherman was diagnosed with the illness (24), although the source of the organism could not be determined. Since that time, *V. cholerae* cases (and deaths), although rare, have been reported sporadically among shellfish consumers (Fig. 6). Both the O1 and other serotypes have been isolated from individuals with relatively severe gastroenteritis. Non-O1 serotypes are reported most frequently, and although the illness caused by them is generally less severe than that caused by O1 serotypes, these organisms have been associated with several oyster-vectored deaths. Non-O1 biotypes are indigenous to marine environments; however, there is no conclusive evidence for an autochthonous marine O1 popula-

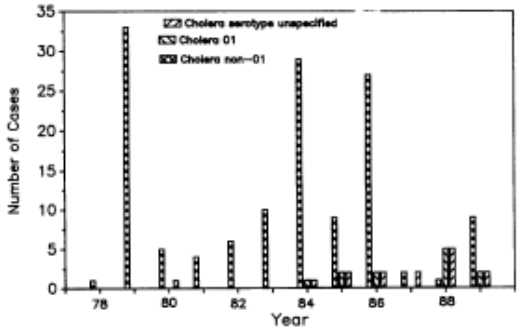

FIG. 6. Shellfish-associated cases of cholera (*V. cholerae* O1, non-O1 cholera, and unspecified serotypes) reported from 1978 to 1989.

TABLE 6. Shellfish-associated outbreaks and cases of infectious illness by type of shellfish (1898–1990)

Species	Agents other than *Vibrio* spp.[a]		*Vibrio* spp.[b]	
	No. of incidents	No. of cases	No. of incidents	No. of cases
Hard clams	232	6,201	4	4
Oysters	155	4,959	279	362
Soft clams	10	43	0	0
Mussels	14	174	1	1
Scallops	1	2	0	0

[a] Includes all illnesses from agents other than those in the *Vibrio* genus and of unknown etiology.

[b] Includes *V. parahaemolyticus*, *V. cholerae* O1 and non-O1, *V. vulnificus*, *V. fluvialis*, *V. mimicus*, and *V. hollisae*.

tion, although this possibility has been advanced (21). No cases of shellfish-vectored, domestically acquired cholera associated with the serotype responsible for the current South American epidemics (6) have been reported in the United States.

Other Vibrios

A number of other *Vibrio* spp. have been associated with shellfish-vectored illness outbreaks (Table 5). *Vibrio parahaemolyticus* cases are reported as frequently as *V. vulnificus* cases. However, illness caused by this bacterium is generally confined to gastroenteritis, although it can be severe and of relatively long duration. A problem in clearly establishing *Vibrio* spp. as etiological agents is that they are all native to marine waters (and presumably shellfish) and may be simply passing through the human gut after the ingestion of uncooked or lightly cooked shellfish. Classical epidemiological methods (e.g., isolation of the organism from the human host followed by reinfection) cannot be used to demonstrate the causality of a suspect organism in a foodborne outbreak. Therefore, the relationship between the isolation of a *Vibrio* sp. from a fecal sample and its role as the causative agent in a case report may be generally assumed but not conclusively established.

SPECIES AND SOURCES OF SHELLFISH FROM ILLNESS REPORTS

Most illness reports in which no causative agent was identified, or the agent was not identified as a *Vibrio* spp., have historically been associated with consumption of hard clams (Table 6). More than 56% of the outbreaks and 54% of the case reports identify hard clams (*M. mercenaria*) as the vector. Oysters (*Crassostrea virginica* and *Ostrea edulis*) are also significant vehicles for infectious illness, associated with 38% of outbreaks and 44% of cases. Soft clams, mussels, and scallops are of minimal public health concern with regard to infectious disease because they are usually cooked before consumption, or only the adductor muscle is usually consumed (scallops). The sources of shellfish (i.e., the original growing areas or last point of water immersion, such as a depuration facility or wet storage area) implicated in these illnesses are predominantly unknown. Of the 412 total outbreaks associated with species other than *Vibrio* spp. (Table 6), 317 (66%) were from shellfish of unknown or questionable origin. New York and Florida, the states most frequently reported as the sources of shellfish implicated in outbreaks, also reported the largest number of outbreaks and cases (Table 2). This association is probably not coincidental.

A completely different history is evident for infections

TABLE 7. Incidents of *Vibrio*-caused illnesses by source of shellfish (1898–1990)

Source of shellfish	No. of incidents[a]
Unknown	198
Louisiana	41
Florida	40
Texas	5
Alabama	5

[a] Incidents are defined as one or more cases from a common source.

associated with certain *Vibrio* spp., particularly *V. vulnificus*, the bacterial species of primary public health concern today. For illnesses caused by these autochthonous bacteria, oysters (principally *C. virginica*) are the predominant vector. More than 98% of the incident reports and 99% of the case reports (Table 6) are associated exclusively with this shellfish species. Most reports involve oysters whose original harvest (or wet storage) sites could not be reliably determined (Table 7); however, positively identified harvest areas were exclusively in Gulf Coast waters. This very interesting fact remains unexplained in light of the limited information available on the marine and estuarine ecology of these aquatic bacteria, particularly since their densities vary widely in the saline environments of all continental U.S. coasts (11, 23). In addition, there is little information on the mechanisms of pathogenesis of these organisms, although the predisposing factors that affect the susceptibility and morbidity of the human host are generally well described.

CONCLUSION

Infectious diseases attributable to the consumption of raw and lightly cooked molluscan shellfish are caused by bacterial agents that are native to the marine environment and by viral and bacterial agents from sewage effluents and other sources that contaminate environmental waters. As filter-feeding organisms, shellfish magnify public health problems associated with environmental contamination because they accumulate microbial pathogens, including viruses, manyfold over the densities found in overlying waters.

The current public health problems of greatest concern to consumers of molluscan shellfish are associated with viral, and suspected viral, pathogens. The numbers of cases and outbreaks caused by these pathogens far exceed those of all other infectious diseases. In terms of the severity of human illness and death, the *Vibrio* genus (specifically *V. vulnificus*) presents a serious problem. Although the number of cases reported yearly is quite low, the high mortality rates involved are of significant public health concern.

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Rate of Occurrence of False-Positive Results from Total Coliform Most-Probable-Number Analysis of Shellfish and Estuaries

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The incidence of confirmed test, false-positive coliform most-probable-number results was compared with environmental parameters and was found to be inversely related to water temperature. It is concluded that the completed coliform test must be done when water temperatures drop below 15°C.

Shellfish harvested from estuarine waters are examined for total numbers of coliforms, along with water and sediment samples from the harvesting areas. The most-probable-number (MPN) analysis (1, 3, 13) is routinely employed and is carried through the presumptive, confirmed, or completed sequence of tests. The completed tests are not always done when the sanitary quality of water is being assessed, notably in the cases of bathing and potable waters (2). To establish a balance between efficiency and accuracy, the incidence of false-positive and false-negative results at each stage of the analysis should be known.

It has been well documented that the presumptive test alone may be of limited reliability (11), historically because of those noncoliforms which may be present and capable of fermenting lactose aerogenically (4, 5, 6, 8, 9, 10, 12). In the study reported here, Chesapeake Bay oysters and oyster beds were examined over a 2-year period. Two sites in Chesapeake Bay, Tolly Point and Eastern Bay, were sampled on a routine basis. These sites were selected because they are commercially important oyster harvest areas and, in addition, the water column of both areas is subject to very little fecal contamination (mean total coliform completed test MPN, 8/100 ml). At approximately 1-month intervals during 1977 and 1978, bottom water samples were collected at one meter above the sediment by means of the Niskin sampler (General Oceanics, Inc.). Sediment samples were collected by using a Petite Ponar grab (Wildlife Supply Co.), and oysters were harvested using a drag-type dredge. All samples were processed within 30 min of collection.

Six oysters, each of which weighed ca. 16 to 20 g, including meat and liquor, after shucking, were scrubbed, rinsed, and aseptically shucked. The oyster tissue was pooled and homogenized in a solution consisting of sterile 0.5% (wt/vol) peptone (Difco Laboratories) in a 1:2 dilution of oyster tissue. Sediment samples were suspended in an estuarine three salts solution (3). Salinity and temperature were measured at the time of collection of the bottom water samples.

A five-tube, total coliform MPN analysis of each of the water, sediment, and oyster samples was performed in duplicate, and the results were normalized for 100 ml (or 100 g) of sample, following procedures recommended by the American Public Health Association (1). Samples (10, 1.0, 0.1, and, for sediment suspension, 0.01 ml) were transferred to appropriate tubes. Lactose broth (Difco), brilliant green bile (2%) broth (Difco), and eosin methylene blue agar (Difco) were employed. Total viable counts (TVC) of aerobic heterotrophic bacteria were enumerated on 30% strength 2216E Marine agar (Difco) (7) plates prepared in triplicate. The TVC plates were incubated at 17 ± 2°C for ca. 15 days before counts were made.

Within each MPN test series, the number of positive results at each successive step (Table 1) was compared, and the proportion of positive presumptive tests which failed to be confirmed as total coliform-positive was defined as the false-positive percent (FP%). The calculation was done using the formula: $FP\% = [(P - C)/P] \times 100$, where P is positive results and C is confirmed (or completed) positive results. This comparison was made for the presumptive-confirmed and also the confirmed-completed test steps. For each sample, the false-positive percentages were, in turn, compared with data for total coliform MPN, TVC, salinity, and temperature. Correlation coefficients were obtained using the Biomedical Computer Programs (BMDP) statistical package on the University of Maryland UNIVAC 1108 computer, and compared with values for critical r (15).

Bottom water salinities ranged from 7.4 to 15.0 ‰. TVC and temperature values are re-

TABLE 1. *Numbers of positive reactions obtained for presumptive, confirmed, and completed coliform MPN tests*

Date	Tolly Point			Eastern Bay			
	Temp (°C)[a]	No. of positive reactions[b]		Temp (°C)[a]	No. of positive reactions		
		Oyster	Bottom water		Oyster	Bottom water	Sediment
24 October 1977	15	21, 20, 20 (—)	12, 8, 8 (1.2E5)	13	10, 1, 1 (1.1E5)	10, 0, 0 (—)	21, 1, 1 (4.4E5)
18 November 1977	11	18, 15, 15 (—)	8, 5, 5 (—)	13	10, 7, 6 (—)	4, 3, 3 (—)	—, —, — (—)
20 December 1977	5	7, 4, 2 (2.0E3)	15, 9, 8 (—)	5	6, 3, 3 (—)	8, 4, 3 (—)	7, 4, 1 (—)
18 January 1978	1	2, 0, 0 (1.7E4)	20, 19, 10 (2.8E4)	1	7, 1, 0 (3.9E4)	9, 4, 1 (9.0E3)	22, 5, 1 (1.3E5)
28 March 1978	6	10, 9, — (5.0E3)	18, 17, — (—)	8	1, 0, — (—)	14, 4, — (—)	23, 0, — (—)
18 April 1978	10	13, 13, 13 (4.0E3)	7, 0, 0 (—)	9	1, 1, 1 (4.0E3)	7, 4, 4 (5.0E3)	23, 10, 3 (4.3E6)
19 May 1978	9	14, 14, 13 (4.7E4)	18, 10, 10 (7.4E4)	9	11, 4, 3 (3.6E5)	12, 3, 0 (6.0E4)	23, 13, 0 (—)
21 July 1978	26	5, 3, 3 (—)	3, 2, 2 (—)	24	5, 1, 1 (—)	1, 0, 0 (—)	4, 0, 0 (—)
6 September 1978	26	9, 7, 7 (9.3E4)	6, 5, 5 (2.0E3)	27	1, 1, 1 (2.7E4)	1, 1, 1 (5.0E3)	20, 1, — (2.3E7)
31 October 1978	14	11, 6, 6 (6.0E3)	9, 5, 5 (2.0E3)	15	17, 14, 14 (3.2E4)	0, 0, 0 (2.0E3)	16, 0, 0 (2.1E5)

[a] Temperature of water 1 to 2 m below surface.

[b] Positive results at each test level: first column is presumptive, second is confirmed, third is completed. Initial observations were recorded for 15 MPN tube series done in duplicate. Number within parentheses is the TVC for the corresponding sample. See text for procedures. —, No data.

ported in Table 1. Oyster total coliform MPN values were consistently low, averaging 81/100 g at Tolly Point and 34/100 g at Eastern Bay. Bottom water total coliform MPN values averaged ca. 12/100 ml and 3.2/100 ml, respectively. Sediment counts at Eastern Bay averaged 13/100 g.

The percent occurrence of false-positive presumptive and confirmed results are presented in Table 2 and Fig. 1, respectively. Overall, some parameters were not found to be correlated (probability, $P < 80\%$). For example, the percent occurrence of false-positive confirmed results did not correlate with: (i) percent occurrence of false-positive presumptive tests ($r = 0.068$); (ii) TVC ($r = 0.193$); or (iii) salinity ($r = -0.125$). Some equivocal correlations ($90\% < P < 95\%$) were noted, and these included total coliform MPN with false-positive percentages, both presumptive ($r = -0.269$) and confirmed ($r = -0.272$), and with salinity ($r = -0.113$).

The most important and definitive relationship detected was that of false-positive and confirmed results and sample temperature (Fig. 1). Although some variations were recorded between the stations as well as for each sample type, it was clear, particularly with regard to sediments (Table 1), that the number of false-positives detected in the confirmation tests increased significantly when the water temperature fell below 10°C. In fact, this relationship was statistically validated for each sample type and station. To cite composite data, water temperatures were found to have a strong negative correlation with percentage of false-positive confirmed tests (−0.593 correlation, significant at

TABLE 2. *Percentage of false-positive presumptive MPN results*

Date	% of false-positive results				
	Tolly Point		Eastern Bay		
	Oyster	Bottom water	Oyster	Bottom water	Sediment
24 October 1977	5	33	90	100	95
18 November 1977	17	38	30	25	—[a]
20 December 1977	43	40	50	50	43
18 January 1978	100	5	86	56	77
28 March 1978	10	6	100	71	100
18 April 1978	0	100	0	43	57
19 May 1978	0	44	64	75	43
21 July 1978	40	33	80	100	100
6 September 1978	22	17	0	0	95
31 October 1978	45	44	17	—	100

[a] —, No data.

the ≥99.9% confidence level; critical $r = 0.372$; $n - 3 = 45$) (Table 3). The temperature at which significant false-positive results begin to be observed may be related to changes in the composition of the bacterial population (14).

Based on the results of this study, it is concluded that, in the past, total coliform MPN (confirmed test) results for cold, estuarine water samples (i.e., <15°C) were subject to error, and reported values may have been higher than was, in fact, the case. It is recommended that the total coliform MPN evaluation of estuarine water, shellfish, and sediment samples include the completed test whenever the temperature of the water falls below 15°C.

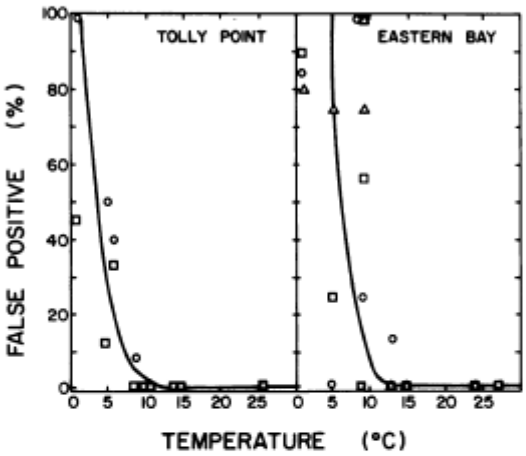

FIG. 1. *Effect of temperature on occurrence of false-positive confirmed MPN tests. Oysters (○), bottom water (□), and sediment (△) were sampled and processed as described in the text. Sample dates are listed in Table 1.*

TABLE 3. *Composite correlation matrix of salinity, temperature, and microbiological parameters*[a]

Parameter	Salinity (‰)	Temp (°C)	Log TVC	Presumptive FP%[b]	Confirmed FP%
Salinity	1.000				
Temperature	0.224	1.000			
Log TVC	0.376[c]	0.206	1.000		
Presumptive FP%	−0.014	−0.104	0.106	1.000	
Confirmed FP%	−0.125	−0.593[d]	0.193	0.068	1.000

[a] Results recorded for each sample, station, and date were combined, and the combined results were correlated.

[b] FP%, False-positive result, percentage of occurrence.

[c] Significant at the 0.05 level, critical $r = 0.367$.

[d] Significant at the 0.01 level, critical $r = 0.372$.

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Closing the Door on the Fecal Coliform Assay

The fecal coliform assay, the results of which have led to numerous misinterpretations over the years, may have outlived its usefulness

Michael P. Doyle and Marilyn C. Erickson

Since its inception in 1904, the fecal coliform assay has been used to assess the presence of fecal contamination in water and foods. Assays to detect *Escherichia coli*, a more specific indicator of fecal contamination, were previously not as popular due to the longer time period for detection required (five days) and their complexity. Recent advances in the detection of *E. coli*, however, have eliminated these impediments and detection occurs within 24 hours or less. Many limitations and complications have been associated with the fecal coliform assay, thereby raising questions about its continued appropriateness and usefulness in food and water testing. The microbiology literature is replete with reports of studies that correlate results of fecal coliform levels with the presence of *E. coli* including several recent examples that advocate the fecal coliform test as an acceptable indicator in manure composts and foods. However, the value of the fecal coliform assay as an indicator of fecal contamination is negated when bacteria of nonfecal origin are the principal microbes detected by the assay.

Historically, the definition of fecal coliforms has been based on methods used for their detection. Specifically, fecal coliforms are gram-negative bacilli, not sporulated, oxidase-negative, optional aerobic or anaerobic, able to multiply in the presence of bile salts or other surface agents that have equivalent properties, and are able to ferment lactose with acid and gas production in 48 h at the temperature of 44 ± 0.5°C. Several genera of bacteria that are common contaminants of nonfecal sources (e.g., plant materials and pulp or paper mill effluents) meet this definition. Examples include *Klebsiella*, *Enterobacter*, and *Citrobacter* species. Moreover, these bacteria which are false-positive indicators of fecal contamination can grow under appropriate conditions in nonfecal niches such as water, food, and waste. The International Commission on Microbiological Specifications for Foods in its evaluation of this issue reported the term fecal coliforms has arisen from attempts to find rapid, dependable methods for establishing the presence of *E. coli* and closely related variants without the need to purify cultures. Species of *Enterobacteriaceae* other than *E. coli* are associated with plants and do not indicate fecal contamination, yet they are identified as fecal coliforms by the fecal coliform assay. Hence, *E. coli* is the only valid index organism for the monitoring of foods containing fresh vegetables.

To reduce the possibility of false-positive results, a confirmatory test for *E. coli* is recommended. In spite of this precaution, there have been several instances where fecal coliform results have been incorrectly interpreted. One of the most sensational situations occurred in 1995 when the U.S. news media reported that high populations of fecal coliforms in restaurant-brewed tea indicated the presence of feces in tea. The dominant fecal coliforms identified were *Klebsiella pneumoniae* and some *Enterobacter* spp., but no *E. coli*. Although there was ample evidence of fecal coliform contamination of iced tea served in restaurants (e.g., 64% of samples at fecal coliform of >1,100 MPN/ml), there had been no history of outbreaks of illnesses resulting from consumption of iced tea.

Another instance where fecal coliform data have been inappropriately interpreted involved two Canadian recalls of sprouts where high levels of fecal coliforms were later identified to be *K. pneumoniae*. In the health hazard alert accompanying these recalls, a warning was issued that this organism could cause gastrointestinal illness in humans. While this bacterial strain is an opportunistic pathogen outside the intestinal tract causing respiratory and urinary tract infections, gastrointestinal illness rarely occurs. Hence, the overly cautious warning was likely due to the association of this bacterium with the fecal organism group.

A quick perusal of the Internet including both

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governmental and academic sites revealed information is being provided that fails to address the possibility that bacteria testing positive in the fecal coliform assay may originate from nonfecal sources. For example, a U.S. Environmental Protection Agency (EPA) page listing drinking water contaminants and their maximum contaminant levels stipulates that "fecal coliforms and *E. coli* only come from human and animal fecal waste." To the contrary, as noted above, there is a preponderance of data indicating that fecal coliforms do not only originate from fecal waste. Similarly, the Kentucky Division of Water site indicates that fecal coliform bacteria "are associated only with the fecal material of warm-blooded animals" and the Food Safety Authority of Ireland site reports that "faecal coliforms found in water are a direct indication that the water has been contaminated with animal or human effluent." Collegiate and K-12 academic sites also provide similar misleading information. Unfortunately, these generalizations can lead to misinterpretation of results by those who do not have a complete understanding of the fecal coliform assay and the subtleties associated with interpreting the results of such assays.

Concerns regarding the inappropriate interpretation of results of the fecal coliform assay and its limited usefulness as an indicator of fecal contamination are not new. They have surfaced several times over the past decade. When the issue of fecal coliforms in tea made media headlines, it was suggested that the fecal coliform assay be reevaluated for its usefulness in food testing. The following year, two commentaries published in *ASM News* opined that the fecal coliform term should be excluded from microbiology. This was further supported by investigators of a study comparing *E. coli*, total coliform, and fecal coliform populations as indicators of wastewater treatment efficiency, who concluded that *E. coli*-based effluent and stream standards (not fecal coliform standards) should be developed to protect public health. A subsequent review of the suitability of the coliform group as an indicator of microbial water safety led other investigators to recommend elimination of the fecal coliform assay. This proposal was further corroborated by studies revealing that only 50% of fecal coliform colonies enumerated as fecal coliforms in foods were identified as *E. coli*.

In the past few years, several changes in monitoring protocols have already been initiated by national and international regulatory agencies. In the European community as well as in Australia and New Zealand, the "fecal coliforms" term has been replaced by what is considered a more appropriate descriptor of this class of microorganisms, "thermotolerant coliforms". Both WHO's Guidelines for Drinking Water Quality and the Australian Drinking Water Guidelines, however, continue to advocate that thermotolerant coliform measurements are an acceptable alternative to *E. coli* measurements. While this change in terminology reduces the likelihood that positive results may be interpreted as meaning the presence of fecal contamination, it does not eliminate the possibility that nonfecal coliforms may be present and give positive results.

In 1986, the U.S. EPA published a document that encouraged states to use *E. coli* or enterococci as the basis of their water quality criteria to protect fresh recreational waters and to use enterococci as the basis for water quality criteria in marine waters. While these guidelines have been criticized, a systematic review and meta-analysis of data reaffirmed these recommendations. More specifically, this analysis revealed that *E. coli* was a more consistent predictor of gastrointestinal illness than other bacterial indicators in fresh water. Despite these recommendations, state and local authorities have been slow to respond in adopting these guidelines. To address some of the advantages and impediments to implementation of these guidelines, costs for the three bacterial indicators were surveyed in the Tacoma/Seattle region and were found to be fairly comparable and thus not a limiting factor. In contrast, an inherent weakness cited by the Washington State Department of Ecology was that using enterococci as an indicator organism in marine waters would complicate efforts to model data obtained from freshwater sources in which *E. coli* was monitored. Another weakness is the continuing requirement by the Food and Drug Administration to use fecal coliforms as an indicator microorganism in shellfish marketed across state borders. Despite this requirement, no significant relationship has been observed between levels of *E. coli* and enterococci and non-*E. coli* fecal coliforms in oysters. Consequently, the continued use of fecal coliforms as an indicator in shellfish would likely hinder widespread acceptance of more appropriate indicators. Moreover, in a National Academies of Science (NAS) report to evaluate candidate indicator organisms and/or indicator approaches, the committee was adverse to abandoning the current indicator microbes until new and better methods are developed and validated. While the NAS Committee foresaw the advent of increasingly sophisticated and powerful molecular biology techniques that would provide new opportunities for the development of improved assays for indicator microbes, we contend that immediate replacement of the fecal coliform assay with an *E. coli* assay would apply the best science available to providing public health protection.

In conclusion, physicians and public health officials have repeatedly misinterpreted results of the fecal coliform assay when applied to food, beverage, or water samples. To prevent future occurrences, the fecal coliform assay should at a minimum be redefined to specifically qualify that it is not a reliable indicator of either *E. coli* or the presence of fecal contamination. An even better alternative would be to eliminate the fecal coliform assay as an indicator of fecal contamination of foods, beverages, and water. The *E. coli* assay is a more reliable indicator of fecal contamination, although not absolute, and could serve as a replacement for the fecal coliform assay.

Literature citations and relevant references which provide the basis for this commentary can be found in the online version of this article.

Proposal Subject:	Total Coliform Method for Shellfish Dealer Process Water using the Membrane Filtration Techniques with mEndo LES agar
Specific NSSP Guide Reference:	2009 NSSP Section IV Guidance Documents Chapter II Growing Areas .10 Approved NSSP Laboratory Tests - Type I and Type II Microbiological Methods, UV treated Seawater
Text of Proposal/ Requested Action	Accept Total Coliform Method for Shellfish Dealer Process Water using the membrane filtration techniques with mEndo LES agar as an alternative method to the APHA MPN method for the presence/absence of total coliforms in UV treated seawater. Single Laboratory Validation Study Results and Method approval application attached.
Public Health Significance:	This method produces results in 24 hours and is a less labor intensive method for laboratories. This more rapid test method would allow operators of facilities who provide disinfected process water for shellfish in wet storage and depuration operations the ability to know they have a problem and take the required remediation action on a more timely basis. It would reduce the workload for the laboratory performing the testing.
Cost Information (if available):	This alternative test should be less costly to the laboratories.
Action by 2011 Laboratory Methods Review Committee	Recommended adoption of Proposal 11-111 as submitted.
Action by 2011 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 11-111.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 11-111.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 11-111.

ISSC Method Application and Single Lab Validation Checklist For Acceptance of a Method for Use in the NSSP

The purpose of single laboratory validation in the National Shellfish Sanitation Program (NSSP) is to ensure that the analytical method under consideration for adoption by the NSSP is fit for its intended use in the Program. A Checklist has been developed which explores and articulates the need for the method in the NSSP; provides an itemized list of method documentation requirements; and, sets forth the performance characteristics to be tested as part of the overall process of single laboratory validation. For ease in application, the performance characteristics listed under validation criteria on the Checklist have been defined and accompany the Checklist as part of the process of single laboratory validation. Further a generic protocol has been developed that provides the basic framework for integrating the requirements for the single laboratory validation of all analytical methods intended for adoption by the NSSP. Methods submitted to the Interstate Shellfish Sanitation Conference (ISSC) Laboratory Methods Review (LMR) Committee for acceptance will require, at a minimum, six (6) months for review from the date of submission.

Name of the New Method	Total Coliform Method for Shellfish Dealer Process Water using the membrane filtration techniques with mEndo LES agar
Name of the Method Developer	Mercuria Cumbo and Cathy L. Vining
Developer Contact Information	Maine Department of Marine Resources Lamoine Water Quality Laboratory 22 Coaling Station Rd. Lamoine, ME 04605 207-667-5654 Mercuria.cumbo@maine.gov Cathy.l.vining@maine.gov

Checklist	Y/N	Submitter Comments
A. Need for the New Method		
1. Clearly define the need for which the method has been developed.	Y	Alternative method which is more rapid than current NSSP approved method. Less labor intensive for laboratory
2. What is the intended purpose of the method?	Y	Shellfish dealer disinfected process water
3. Is there an acknowledged need for this method in the NSSP?	Y	NSSP requires testing of disinfected process water
4. What type of method? i.e. chemical, molecular, culture, etc.	Y	Microbiological culture
B. Method Documentation		
1. Method documentation includes the following information:		
Method Title	Y	Total Coliform Method for Shellfish Dealer Process Water using the membrane filtration technique with mEndo LES agar
Method Scope	Y	Presence/absence of total coliform in disinfected shellfish process water
References	Y	See attached document
Principle	Y	See attached document
Any Proprietary Aspects	Y	none
Equipment Required	Y	Membrane filtration apparatus
Reagents Required	Y	
Sample Collection, Preservation and Storage Requirements	Y	
Safety Requirements	Y	
Clear and Easy to Follow Step-by-Step Procedure	Y	

Quality Control Steps Specific for this Method	Y	
C. Validation Criteria		
1 Accuracy / Trueness	NA	Performance criteria previously established for this method.
2 Measurement Uncertainty	NA	Performance criteria previously established for this method.
3 Precision Characteristics (repeatability and reproducibility)	NA	Performance criteria previously established for this method.
4 Recovery	NA	Performance criteria previously established for this method.
5. Specificity	NA	Performance criteria previously established for this method.
6. Working and Linear Ranges	NA	Performance criteria previously established for this method.
7 Limit of Detection	NA	Performance criteria previously established for this method.
8 Limit of Quantitation / Sensitivity	NA	Performance criteria previously established for this method.
9. Ruggedness	NA	Performance criteria previously established for this method.
10. Matrix Effects	NA	Performance criteria previously established for this method.
11. Comparability (if intended as a substitute for an established method accepted by the NSSP)	Y	See attached document. Method is comparable for presence/absence with the NSSP approved APHA MPN Lactose Broth/Brilliant green Bile Broth Total Coliform method.
D. Other Information		
1. Cost of the Method		Comparable to approved method
2. Special Technical Skills Required to Perform the Method		Comparable to approved method
3. Special Equipment Required and Associated Cost		Membrane filtration apparatus
4. Abbreviations and Acronyms Defined		
5. Details of Turn Around Times (time involved to complete the method)		22 - 24 hours
6. Provide Brief Overview of the Quality Systems Used in the Lab		Study performed in two Maine State Shellfish Sanitation program laboratories which have quality assurance plans, have been evaluated and found to conform with the requirements of the NSSP for microbiological laboratories.
Submitters Signature **Mercuria Cumbo**	Date: **June 3, 2011**	
Submission of Validation Data and Draft Method to Committee	Date:	
Reviewing Members	Date:	
Accepted	Date:	

Recommendations for Further Work	Date:
Comments:	

DEFINITIONS

1. Accuracy/Trueness - Closeness of agreement between a test result and the accepted reference value.
2. Analyte/measurand - The specific organism or chemical substance sought or determined in a sample.
3. Blank - Sample material containing no detectable level of the analyte or measurand of interest that is subjected to the analytical process and monitors contamination during analysis.
4. Comparability - The acceptability of a new or modified method as a substitute for an established method in the NSSP. Comparability must be demonstrated for each substrate or tissue type by season and geographic area if applicable.
5. Fit for purpose - The analytical method is appropriate to the purpose for which the results are likely to be used.
6. HORRAT value - HORRAT values give a measure of the acceptability of the precision characteristics of a method.[4]
7. Limit of Detection - the minimum concentration at which the analyte or measurand can be identified. Limit of detection is matrix and analyte/measurand dependent.[4]
8. Limit of Quantitation/Sensitivity - the minimum concentration of the analyte or measurand that can be quantified with an acceptable level of precision and accuracy under the conditions of the test.
9. Linear Range - the range within the working range where the results are proportional to the concentration of the analyte or measurand present in the sample.
10. Measurement Uncertainty - A single parameter (usually a standard deviation or confidence interval) expressing the possible range of values around the measured result within which the true value is expected to be with a stated degree of probability. It takes into account all recognized effects operating on the result including: overall precision of the complete method, the method and laboratory bias and matrix effects.
11. Matrix - The component or substrate of a test sample.
12. Method Validation - The process of verifying that a method is fit for purpose.[1]
13. Precision - the closeness of agreement between independent test results obtained under stipulated conditions.[1, 2] There are two components of precision:
    a. Repeatability - the measure of agreement of replicate tests carried out on the same sample in the same laboratory by the same analyst within short intervals of time.
    b. Reproducibility - the measure of agreement between tests carried out in different laboratories. In single laboratory validation studies reproducibility is the closeness of agreement between results obtained with the same method on replicate analytical portions with different analysts or with the same analyst on different days.
14. Quality System - The laboratory's quality system is the process by which the laboratory conducts its activities so as to provide data of known and documented quality with which to demonstrate regulatory compliance and for other decision-making purposes. This system includes a process by which appropriate analytical methods are selected, their capability is evaluated, and their performance is documented. The quality system shall be documented in the laboratory's quality manual.
15. Recovery - The fraction or percentage of an analyte or measurand recovered following sample analysis.

16. Ruggedness - the ability of a particular method to withstand relatively minor changes in analytical technique, reagents, or environmental factors likely to arise in different test environments.[4]
17. Specificity - the ability of a method to measure only what it is intended to measure.[1]
18. Working Range - the range of analyte or measurand concentration over which the method is applied.

REFERENCES:

1. Eurachem Guide, 1998. The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics. LGC Ltd. Teddington, Middlesex, United Kingdom.
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4. MAF Food Assurance Authority, 2002. A Guide for the Validation and Approval of New Marine Biotoxin Test Methods. Wellington, New Zealand.
5. National Environmental Laboratory Accreditation. , 2003. Standards. June 5.
6. EPA. 2004. EPA Microbiological Alternate Procedure Test Procedure (ATP) Protocol for Drinking Water, Ambient Water, and Wastewater Monitoring Methods: Guidance. U.S. Environmental Protection Agency (EPA), Office of Water Engineering and Analysis Division, 1200 Pennsylvania Avenue, NW, (4303T), Washington, DC 20460. April.

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Application from Maine State Department of Marine Resources Single Laboratory Validation Study in support of acceptance of an alternative method for determining the presence/absence of total coliforms in disinfected shellfish process water.

Section A. Justification for New Method

Name of the New Method:	Total Coliform Method for Shellfish Dealer Process Water using the membrane filtration technique with mEndo LES agar
Specify the Type of Method:	microbiological, membrane filtration
Name of Method Developer:	Mercuria Cumbo and Cathy L. Vining
Developer Contact Information	Maine Department of Marine Resources Lamoine Water Quality Lab 22 Coaling Station Rd. Lamoine, ME 04605 Mercuria.Cumbo@maine.gov Cathy.L.Vining@maine.gov
Date of Submission:	June 3, 2011

Introduction:

This single laboratory validation study was conducted at both of the Maine Department of Marine Resources (MEDMR) Water Quality Laboratories, the laboratories that support the MEDMR growing area classification program. The Laboratories are referred to as Lamoine and Boothbay in this report. The study was carried out in each laboratory separately using disinfected recirculating wet storage process water from five facilities who submit samples on a weekly basis to the MEDMR laboratories for testing. The Lamoine study analyzed three of the facilities and the Boothbay study analyzed two of the facilities. The study was conducted over a one year period and represents all seasons.

The results of the study indicate that the MF method using mEndo LES agar is a viable alternative procedure for the APHA MPN as a presence/absence test for total coliforms in disinfected shellfish process water.

Purpose and Intended Use of the Method:

Where disinfection is applied to process waters in wet storage and depuration facilities, the NSSP Program Guide for the Control of Molluscan Shellfish requires that the disinfection system produces process water with no detectable coliforms using an NSSP approved method. We are proposing the use of the Total coliform membrane filtration method using mEndo LES agar for the presence or absence of total coliforms in disinfected shellfish process water as an alternative to the APHA multiple tube fermentation MPN total coliform method.

Need for the New Method in the NSSP, Noting Any Relationships to Existing Methods:

Currently there is one NSSP approved method; APHA multiple tube fermentation MPN method for total coliforms. This method requires two media and up to five days to complete. On the contrary the membrane filtration (MF) method is read in 22 to 24 hours. This method is comparable to the MPN method but has the advantage of providing sample results more quickly. When there are problems with the disinfection or process water system causing the presence of coliforms, the quicker analysis turnaround would allow the operator to take action on a timelier basis. The MF method requires less media and generally is less labor intensive for the laboratory.

Method Limitations and Potential Indications of Cases Where the Method May Not Be Applicable to Specific Matrix Types:

There are two limitations with membrane filtration methods. Turbidity in the samples can hinder filtration and the presence of high levels of non coliform bacteria can suppress the growth of coliforms. Neither of these limitations should be applicable to disinfected process water. Successful disinfection of process water requires that turbidity be eliminated. Shellfish process water is generally filtered before it goes to the disinfection process. The disinfection process should reduce the levels of all bacteria in the process water. Neither turbid process water samples nor non-coliform bacteria were encountered in any of the process waters analyzed for this validation study.

Other Comments:

This method has been in use for more than 40 years and is published in Standard Methods for the Examination of Water and Wastewater. It has been approved by EPA for use with potable water (for compliance with the US Drinking water program) and environmental waters including marine waters. The purpose of this validation study was to determine its comparability with the NSSP approved method for determining the presence/absence of total coliforms in disinfected shellfish process water. The membrane filtration technique as a microbiological tool for bacterial identification has a precedent in the NSSP with the MF method for fecal coliforms.

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Section B. Method Documentation

Total Coliform Method in Shellfish Dealer Process Water using the Membrane Filtration Techniques with mEndo LES Agar

Method Scope.

This method is a standard widely used method with a long history. It first appeared in the Twelfth Edition of Standard Methods for the Examination of Water and Wastewater in 1965 for fresh water applications. Since this time its uses have widened. It is an EPA approved method for the Safe Drinking Water Program and EPA approved for analyzing environmental waters including marine waters for Total Coliforms. The FDA has adopted this method for testing bottled water as an indication of insanitation or possible contamination. Within the US Safe Drinking Water Program the total coliform standard is no detectable coliforms and generally uses a presence/absence reporting format. Disinfected shellfish process waters must meet this standard. The purpose of this validation study was to determine the method's applicability to determine the presence/absence of total coliforms in disinfected shellfish process water. Specifically, the study was conducted on five different recirculating wet storage facilities in Maine. Each facility has a different process water system providing filtration followed by UV disinfection.

References:

American Public Health Association (APHA). 1992. *Standard Methods for the Examination of Water and Wastewater*, 20th Edition. APHA/AWWA/WEF, Washington, D.C. 9222B.

U.S. Environmental Protection Agency. 1978. *Microbiological Methods for Monitoring the Environment, Water and Wastes*. EPA/600/8/78/017. EPA, Washington, D.C. Part III Section B.

U.S. Food and Drug Administration (FDA).1995.*Bacteriological Analytical Manual*. U.S. FDA, 8th Edition, AOAC, Arlington,VA. Chapter 4 Section III.

Principle:

The membrane filtration method using mEndo LES agar provides a direct count of bacteria in processed water based on the development of colonies on the surface of the membrane filter. A quantity of water is filtered using a vacuum pump through the membrane which retains the bacteria. After filtration the membrane containing the bacterial cells is placed on mEndo agar, a selective and differential medium, incubated at 35°C for 24 hours. Following incubation, red colonies with or without metallic sheen are counted with the aid of a fluorescent lamp and stereo dissecting microscope. One volume of 100ml sample is used. Counting of colonies is not necessary; any presence of total coliforms is unacceptable.

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Analytes/Measurands:

Total coliform

Proprietary Aspects:

None

Method:

Sample Collection and Preservation:

1. Process water shall be collected in a labeled sterile bottle or sample bag large enough to contain 110 ml of sample. Sample container must be filled to allow an air space for allow shaking of the sample.
2. Paperwork must accompany the sample which identifies the sample collector, sample location, date and time of collection.
3. Sample shall be placed in a cooler with ice or ice packs to maintain the cooler temperature between 0 and 10°C during transport.
4. Samples are placed in the refrigerator when received at the laboratory.
5. Samples are analyzed as soon as possible and not longer than 30 hours from time of collection.

Equipment:

Reagent grade water
Sterile 1 liter media bottle w/ magnetic stir bar
Top loading balance
Petri dishes, sterile, plastic, 15 x 60 mm w/ loose lids
Membrane filtration units (filter base and funnel), sterilized
Ultraviolet unit for sanitization of filter funnel between filtrations
Filter manifold or filtering funnel
Carboy (vacuum capable) or Erlenmeyer vacuum flask to collect filtered waste water
Vacuum pump
Nalgene Autoclavable Low Boy Carboy, 8 liter and dispensing tubing, or autoclavable squirt bottle
Incubator maintained at 35 ± 5°C
Refrigerator maintained at 0-4°C
Timer for timing UV sterilization
Membrane filters, sterile, white, grid marked, 47 mm diameter with 0.45 ± 0.02 µm pore size
Forceps, straight or curved, with smooth tips to handle filters without damage
Alcohol burner
95% ethanol, methanol or isopropanol in small wide-mouth container for flaming forceps
Hand tally or electronic counting device
Stereo dissecting microscope with a cool white fluorescent lamp
Inoculation loops, 10 ul, sterile, disposable

Micropipetter (20-200ul)
Sterile Micropipetter tips
Quality control bacteria
Escherichia coli culture 10^{-7} dilution for QC (EC) or *Enterobacter aerogenes* culture 10^{-7} dilution for QC (EA)
Staphylococcus aureus culture 10^{-2} dilution for QC (SA)

Media and Reagents:
mEndo Agar LES
95%ethanol (not denatured)
Phosphate Buffered Saline (PBS)

1. Determine how many plates will be needed for testing. Each sample requires 1 plate plus start, end and 3 bacteria QC plates.
2. Use commercially available mEndo LES agar. Agar is used at the rate of 51 grams of mEndo LES agar in 1 liter of reagent grade water containing 20 ml of 95% ethanol (not denatured). Weigh appropriate amount of agar for volume of agar needed.
3. To prepare the media, heat slowly while stirring; boil for 1 minute in a large flask or sterile 1 liter bottle with cap which will help ensure complete boil time without boiling over. Do not autoclave.
4. Pour 5 to 7 grams mEndo agar into 60 mm Petri dishes. Weigh plates and record. If any plates fall below 5 grams they must be discarded.
5. Maximum storage time for plates is two weeks - preferably plates should be prepared shortly before use. Store prepared plates in the refrigerator in the dark.
6. Prepare phosphate buffered saline either in carboy or squeeze bottle. Autoclave appropriate amount of time for the volume.

Procedure:

1. Check bulbs in the UV sterilizer to insure that lights are working. Use appropriate eye protection to view UV bulbs.
2. Place sterile filter base unit(s) on filtering flask or filter manifold.
3. Use sterile forceps to aseptically place a sterile membrane filter on the filter base, grid side up. Filter forceps are sterilized by dipping into the 95% alcohol and flaming in the alcohol burner.
4. Begin series with a blank QC plates first by adding 20-40 ml of sterile PBS to the funnel with filter. Start filtration. Rinse sides of funnel at least twice with 20-30 ml of sterile PBS.
5. Use sterile forceps to aseptically remove the membrane filter from the filter base and roll it onto the mEndo Agar to avoid the formation of bubbles between the membrane and the agar surface. Place the filter grid side up on the media. Reseat the membrane if bubbles occur. Close the dish, invert.
6. In between filtering place the funnel(s) and base(s) in the UV sterilization unit for 2 minutes.
7. Repeat the above process for samples.

8. Process water samples must be shaken 25 times in a 12" arc in 7 seconds and 100 ml quantity is filtered.
9. Start filtration and rinse funnel sides with 20 to 30 ml of PBS twice. When sample is completely through the filter, turn off the vacuum pump and remove filter with sterile forceps (alcohol dip and flamed), then placing onto the appropriately labeled mEndo plate.
10. Remember to sterilize forceps between use by dipping in alcohol and flaming.
11. Finish with bacteria QC controls by adding 20-40 ml of PBS to a funnel for each of the controls. Aseptically add 100 µl of 10^{-7} EC or EA solution to one funnel. This should give a count of 10-20 bacteria per plate. Inoculate a second funnel with 10 µl loopful of SA containing 20-40 ml PBS for a negative control. Rinse, filter, then place filters on labeled mEndo plates.
12. Incubate all plates at 35 ± 0.5°C for 22 - 24 hours.
13. Read plates under a stereo dissecting microscope. All red colonies with or without a metallic sheen are counted.
14. Any number of colonies shall be reported out as Positive for total coliforms in 100 ml of sample.

Quality Control:

Quality control measures are all those required in the NSSP microbiology laboratory checklist for the lab in general and specifically for the membrane filtration method. A membrane filtration procedure currently is approved and quality control requirements are established in the checklist.

Validation Data:

This method, membrane filtration using mEndo LES agar, is a standard method that has been employed for testing coliforms for more than 40 years. Performance criteria have previously been established for use for drinking water and environmental fresh and marine waters. The purpose of this study was to determine whether it would be acceptable as an alternative to the current NSSP approved method for determining the presence or absence of total coliforms in disinfected shellfish process water.

Two studies were conducted, one in each of the MEDMR Water Quality Laboratories. In this report the Laboratories are referred to as Lamoine and Boothbay. The data was analyzed for each laboratory separately.

Disinfected recirculated shellfish process water from five Maine facilities was used for the two studies; three facilities submit samples on a weekly basis for total coliform testing to Lamoine and two facilities submit samples to Boothbay. The process waters are normally absent of total coliforms, so it was necessary to spike the samples. For each round of testing an unspiked sample was tested by both methods. All unspiked samples were negative for total coliforms. The study was conducted over a period of a year, so all seasons are represented. To determine comparability testing was performed by both methods. For each round of testing, process water from one facility was spiked and analyzed. The samples were either spiked with *Escherichia*

coli, ATTC 11775 or *Enterobacter aerogenes,* ATTC 13048. Five to six aliquots of each process water sample were spiked at levels to provide determinate numbers of bacteria. Any dilutions that produced indeterminate results for either method was deleted from the computations. The spiked samples were analyzed in triplicate by both the APHA MPN method and MF method using mEndo LES agar. Ten rounds of testing were performed in Lamoine with 156 data points for each method. Boothbay completed 11 rounds with 150 data points for each method. One hundred (100) ml of sample was analyzed for each replicate. MPN were divided between 20 tubes with 5 ml per tube. The range for the MPN is <1 to >60 MPN/100ml. The range for the MF test is <1 to >80 CFU/100 ml.

Comparability :

For each laboratory the replicate data for the individual dilutions was averaged. The two methods were plotted against each other and a linear regression computed. All computations were performed on the log 10 transformation. The result is provided in the Figure 1 and 2. All of the data is presented in Table 1 and 2.

The linear regression line for the Lamoine method comparison is $y = 0.9687x - 0.0195$. Since we are looking at a presence/absence condition, the area of concern when comparing the method is 0. The linear regression was computed with the MPN as the x value and MF as the y value. The y intercept of the equation for the Lamoine data is -0.0195, statistically less than 0. The linear regression for Boothbay is $y = 0.97x + 0.027$. The y intercept of the Boothbay data is 0.027, essentially 0.

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Figure 1 Lamoine Method Comparison

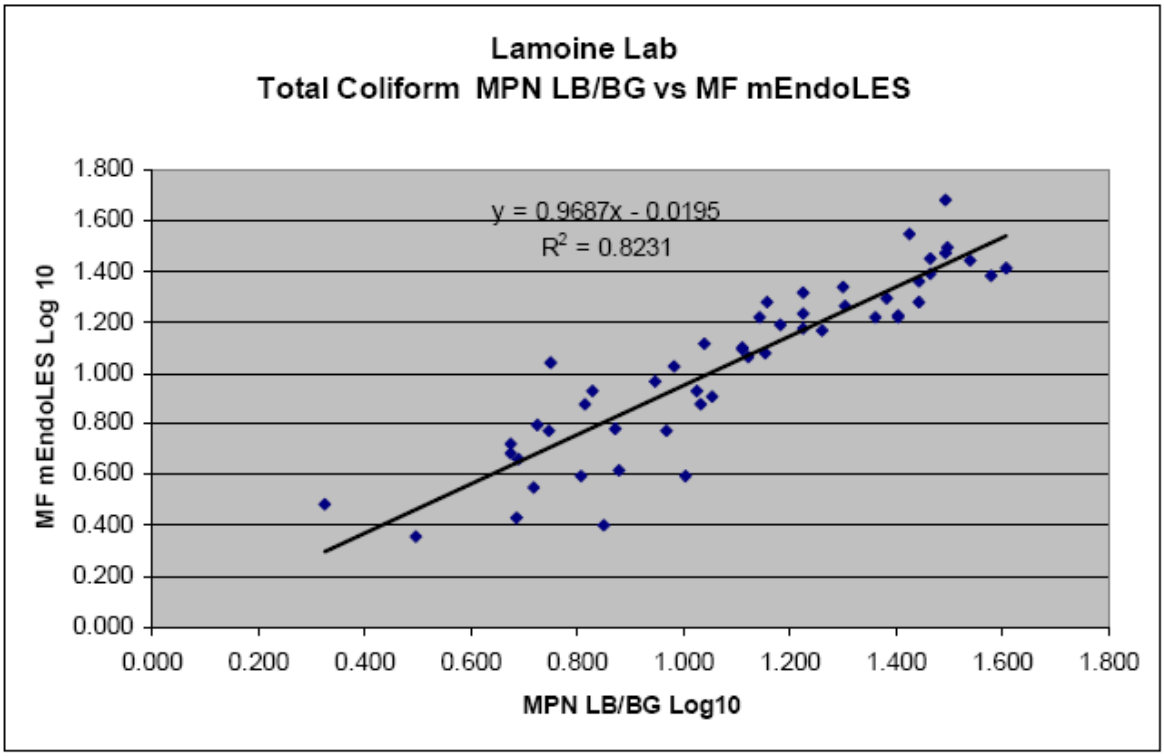

Figure 2. Boothbay Method Comparison

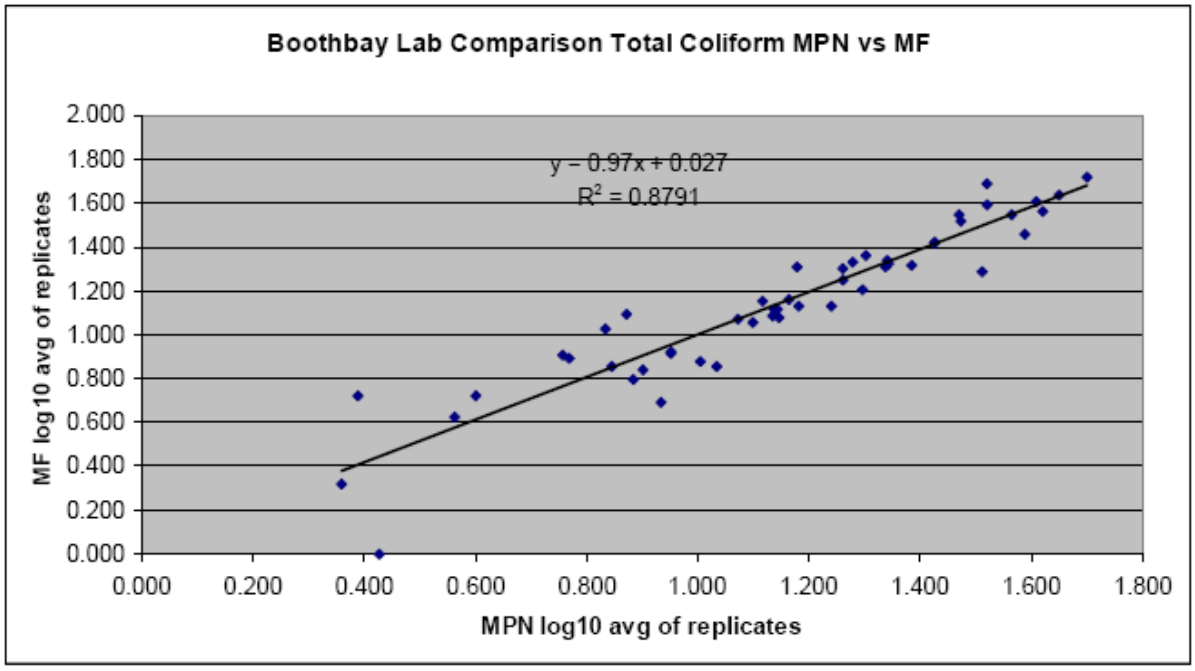

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Table 1 Lamoine method comparison data

DMR Water Quality Laboratory											
Lamoine, ME											
MF mENDO vs. MPN LB/BG Process Water Comparison											
				MPN LB/BG				MF mEndo LES			
Spiking Bacteria	Dealer	Date	Sample	Rep1	Rep2	Rep3	Geomean	Rep1	Rep2	Rep3	Geomean
Enterobacter aerogenes	TBR	9/21/2010	1	21	38	38	31	52	47	46	48
	TBR		2	21	38	38	31	24	28	39	30
	TBR		3	10	21	14	14	17	21	19	19
	TBR		4	12	16	12	13	12	13	10	12
	TBR		5	4.5	12	3.3	6	15	11	8	11
	TBR		6	8.6	8.6	5.8	8	9	4	2	4
	RDR	10/26/2010	1	28	32	24	28	25	25	19	23
	RDR		2	28	21	14	20	18	22	16	19
	RDR		3	21	8.6	12	13	13	11	13	12
	RDR		4	8.6	8.6	14	10	6	2	5	4
	RDR		5	2.1	5.8	8.6	5	3	7	7	5
	RDR		6	4.5	2.1	1	2	2	7	2	3
	MER	11/2/2010	1	28	28	18	24	24	19	17	20
	MER		2	21	24	12	18	16	13	15	15
	MER		3	12	10	10	11	10	12	5	8
	MER		4	5.8	7.1	10	7	7	4	8	6
	MER		5	10	4.5	5.8	6	4	5	3	4
	RDR	11/30/2010	2	60	32	28	38	31	23	20	24
	RDR		3	24	28	18	23	14	17	19	17
	RDR		4	14	21	12	15	18	17	12	15
	RDR		5	14	8.6	12	11	8	6	11	8
	RDR		6	5.8	4.5	4.5	5	4	3	8	5
	MER	12/7/2010	1	46	28	24	31	33	32	29	31
	MER		2	24	32	32	29	21	25	28	24
	MER		3	12	16	14	14	12	22	17	16
	MER		4	8.6	12	8.6	10	12	9	11	11
	MER		5	5.8	5.8	4.5	5	8	5	6	6
	MER		6	8.6	7.1	5.8	7	2	4	2	3
Escherichia coli	RDR	1/18/2011	4	38	38	46	40	23	24	32	26
	RDR		5	32	28	18	25	18	17	15	17
	RDR		6	14	24	14	17	14	21	17	17
	RDR	2/2/2011	2	21	28	32	27	34	34	38	35
	RDR		3	14	14	24	17	24	14	27	21
	RDR		4	12	10	18	13	14	18	8	13
	RDR		5	8.6	5.8	14	9	11	9	8	9
	RDR		6	7.1	8.6	4.5	7	6	8	9	8
	MER	2/8/2011	1	28	32	46	35	33	25	26	28
	MER		2	21	18	21	20	24	26	17	22

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DMR Water Quality Laboratory

Lamoine, ME

MF mENDO vs. MPN LB/BG Process Water Comparison

				MPN LB/BG				MF mEndo LES			
Spiking Bacteria	Dealer	Date	Sample	Rep1	Rep2	Rep3	Geomean	Rep1	Rep2	Rep3	Geomean
	MER		3	21	14	16	17	14	17	14	15
	MER		4	16	7.1	7.1	9	7	5	6	6
	MER		5	3.3	4.5	7.1	5	4	7	4	5
	MER		6	4.5	2.1	12	5	5	1	4	3
	RDR		2	32	24	32	29	21	34	32	28
	RDR		3	28	18	32	25	16	14	22	17
	RDR	3/8/2011	4	16	5.8	14	11	20	8	14	13
	RDR		5	12.0	12.0	8.6	11	8	6	9	8
	RDR		6	4.5	4.5	9	6	7	6	5	6
	MER		2	28	32	24	28	18	19	20	19
	MER		3	14	24	8.6	14	14	14	9	12
	MER	4/12/2011	4	12	12.0	2.1	7	10	5	12	8
	MER		5	4.5	4.5	7.1	5	3	5	3	4
	MER		6	3.3	2.1	4.5	3	3	2	2	2

Table 2 Boothbay method comparison data

DMR Boothbay Water Quality Lab

MF mEndo LES vs. MPN LB/BG Process Water Comparison

				MPN LB/BG				MF mEndo LES counts			
Spiking Bacteria	Dealer	Date	Sample	Rep1	Rep2	Rep3	Geomean	Rep 1	Rep 2	Rep 3	Geomean
Escherichia coli	CHS		1	60	46	46	50.3	52	54	51	52.3
	CHS		2	24	14	32	22.1	20	24	19	20.9
	CHS	7/6/2010	3	14.01	14	13.99	14.0	14	12	10	11.9
	CHS		4	7.1	5.8	4.5	5.7	11	6	8	8.1
	CHS		1	38	38	46	40.5	36	44	42	40.5
	CHS		2	28	32	38	32.4	14	22	23	19.2
	CHS	7/19/2010	3	10	4.5	7.1	6.8	12	11	9	10.6
	CHS		4	3.3	3.3	5.8	4.0	6	2	12	5.2
	CHS		5	1	5.8	3.3	2.7	1.01	1	1	1.0
	SMF		1	38	46	21	33.2	54	49	44	48.8
	SMF	8/30/2010	2	24	24	18	21.8	26	20	16	20.3
	SMF		3	9	10	14	10.8	9	10	4	7.1
	SMF		4	2.1	3.3	7.1	3.7	5	3	5	4.2
	CHS	9/13/2010	1	28	38	46	36.6	32	46	30	35.3
	CHS		2	18	16	24	19.0	23	24	18	21.5

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DMR Boothbay Water Quality Lab

MF mEndo LES vs. MPN LB/BG Process Water Comparison

				MPN LB/BG				MF mEndo LES counts			
Spiking Bacteria	Dealer	Date	Sample	Rep1	Rep2	Rep3	Geomean	Rep 1	Rep 2	Rep 3	Geomean
	CHS		3	16	12	16	14.5	20	11	14	14.5
	CHS		4	10	14	14	12.5	10	12	12	11.3
	CHS		5	4.5	10	10	7.7	6	10	4	6.2
	SMF		1	24	24	46	29.8	31	36	32	32.9
	SMF		2	21	32	21	24.2	22	14	29	20.7
	SMF	9/27/2010	3	10	12	21	13.6	16	14	8	12.1
	SMF		4	5.8	10	7.1	7.4	12	12	13	12.3
	SMF		5	10	4.5	4.5	5.9	12	5	8	7.8
	CHS		1	28	46	28	33.0	43	33	41	38.7
	CHS		2	18	28	21	22.0	29	15	24	21.9
	CHS	1/10/2011	3	18	14	14	15.2	14	12	15	13.6
	CHS		4	16	14	10	13.1	16	13	14	14.3
	CHS		5	7.1	7.1	10	8.0	12	4	7	7.0
	CHS		1	60	32	46	44.5	43	50	38	43.4
	CHS		2	24	28	28	26.6	30	27	23	26.5
	CHS	1/31/2011	3	24	18	18	19.8	21	11	18	16.1
	CHS		4	14	12	16	13.9	13	21	8	13.0
	CHS		5	12	7.1	12	10.1	5	12	7	7.5
Enterobacter Aerogenes	SMF		1	38	24	28	29.4	35	39	32	35.2
	SMF		2	16	21	18	18.2	25	19	17	20.1
	SMF	8/2/10	3	10	7.1	10	8.9	12	7	7	8.4
	SMF		4	2.1	3.3	2.1	2.4	6	8	3	5.2
	SMF		5	3	4	1	2.3	3	1	3	2.1
	CHS		2	32	18	14	20.1	21	21	28	23.1
	CHS	8/16/2010	3	10	10	7.1	8.9	6	9	10	8.1
	CHS		4	12	3.3	8.6	7.0	4	9	10	7.1
	SMF		2	38	60	32	41.8	38	34	38	36.6
	SMF	10/26/2010	3	28	24	28	26.6	29	29	22	26.4
	SMF		4	16	18	12	15.1	20	19	22	20.3
	SMF		5	12	12	18	13.7	13	14	12	13.0
	CHS	11/29/2010	1	60	21	46	38.7	18	33	39	28.5
	CHS		2	18	21	16	18.2	15	17	22	17.8
	CHS		3	21	18	14	17.4	12	19	11	13.6
	CHS		4	8.6	16	12	11.8	17	7	14	11.9

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DMR Boothbay Water Quality Lab											
MF mEndo LES vs. MPN LB/BG Process Water Comparison											
				MPN LB/BG				MF mEndo LES counts			
Spiking Bacteria	Dealer	Date	Sample	Rep1	Rep2	Rep3	Geomean	Rep 1	Rep 2	Rep 3	Geomean
	CHS		5	8.6	8.59	8.61	8.6	8	3	5	4.9

Summary of Results:

The membrane filtration method using mEndo LES agar for total coliform is a published standard method in use for over forty years. It is approved for use with drinking water (potable) and environmental water, fresh and marine. The performance criteria for this test has been previously established, therefore there was no need to complete performance criteria for this single laboratory validation study. The purpose of the study was to determine its comparability with the NSSP approved method.

As a presence/absence test the area of concern is zero (0). From the linear regression when the MPN is 0, at Lamoine the MF method is less than 0 (statistically) and at Boothbay it is 0.027, essentially 0.

This study indicates that the MF method is a viable alternative procedure for the APHA MPN as a presence/absence test for total coliforms in disinfected shellfish process water.

Proposal Subject:	Addition to the Requirements for the Authority During a Suspected Shellfish Related Outbreak
Specific NSSP Guide Reference:	2009 NSSP Section II Model Ordinance Chapter II @.01 Outbreaks of Shellfish-Related Illness Paragraph E, Section (1).
Text of Proposal/ Requested Action	E. When the investigation outlined in §.02B. cannot be completed within 24 hours, the Authority shall: (1) Follow the closure procedures. ~~outlined in § .01C; and if the investigation does not indicate a growing area problem, the area shall be immediately reopened and product recall terminated.~~
Public Health Significance:	N/A
Cost Information (if available):	Not available.
Research Needs:	There is a need of one type of post harvest processing technology that could be used as a determining factor that when applied as a process to recondition a batch of recalled oysters whether shucked, shellstock and post harvest processed oysters, all the rest of the oyster related pathogens causing foodborne illnesses are deemed clean. It is patterned after the analysis of water using fecal coliform as an identifier of the presence of pathogens in the water. In any oyster recall, dealers and processors often experience financially devastating product recalls and experience the loss of their investments on the product. The number of oyster dealers had decreased over the years for various reasons. Those remaining are finding it difficult to cope without alternatives to destruction of product.
Estimated Cost:	Not available at this time.
Proposed Source of Funding/Support:	Not available at this time.
Time Frame Anticipated:	Not available at this time.
Action by 2011 Task Force I	Recommended no action on Proposal 11-112. Rationale: Adequately addressed in Proposal 11-114.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 11-112.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 11-112.

Proposal Subject: Addition to the Requirements for the Authority During a Suspected Shellfish Related Outbreak

Specific NSSP Guide Reference: 2009 NSSP Section II Model Ordinance Chapter II @.01 Outbreaks of Shellfish-Related Illness Paragraph C.

Text of Proposal/ Requested Action C. When the investigation outlined in §.02B. ~~does not~~ indicates a growing area problem: ~~post harvest contamination problem, or illegal harvesting from a closed area~~, the Authority shall:

(1) Immediately place the implicated portion(s) of the harvest area(s) in the closed status;

(2) Notify receiving states and the FDA Regional Shellfish Specialist that a potential health risk is associated with shellfish harvested from the implicated growing area;

(3) As soon as determined by the Authority, transmit to the FDA and receiving states information identifying the dealers shipping the implicated shellfish; and

(4) Promptly initiate recall procedures consistent with the Recall Enforcement Policy, Title 21 Code of Federal Regulations Part 7. The recall shall include all implicated products.

Public Health Significance: The basis for this addition is to allow the Authority time to determine if suspected shellfish related outbreak is due to growing area problems or problems associated with the location where the shellfish were served. It would be expected that if the suspected outbreak were growing area related, illnesses would be seen at more than one location. It is difficult to determine the actual cause within 24 hours when faced with illness reported from a single location.

Cost Information (if available): None

Research Needs: There is a need of one type of post harvest processing technology that could be used as a determining factor that when applied as a process to recondition a batch of recalled oysters whether shucked, shellstock and post harvest processed oysters, all the rest of the oyster related pathogens causing foodborne illnesses are deemed clean. It is patterned after the analysis of water using fecal coliform as an identifier of the presence of pathogens in the water.

In any oyster recall, dealers and processors often experience financially devastating product recalls and experience the loss of their investments on the product. The number of oyster dealers had decreased over the years for various reasons. Those remaining are finding it difficult to cope without alternatives to destruction of product.

Estimated Cost: Not available at this time.

Proposed Source of Funding/ Support: Not available at this time.

Time Frame Anticipated:	Not available at this time.
Action by 2011 Task Force I	Recommended no action on Proposal 11-113. Rationale: Adequately addressed in Proposal 11-114.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 11-113.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 11-113.

Proposal Subject: Addition of the Requirements for the Authority During a Suspected Oyster Related Outbreak of *Norovirus*

Specific NSSP Guide Reference: Section II Model Ordinance Chapter II. Risk Assessment and Risk Management @.01 Outbreaks of Shellfish Related Illness

Key Words: *Norovirus*

Text of Proposal/ Requested Action: @.01 Outbreaks of Shellfish-Related Illness.

A. When shellfish are implicated in an illness outbreak involving two (2) or more persons not from the same household (or one or more persons in the case of paralytic shellfish poisoning [PSP]), and in the case of *Norovirus* being reported for more than one retail outlet or location of consumption), the Authority shall determine whether an epidemiological association exists between the illness and the shellfish consumption by reviewing:

(1) Each consumer's food history;
(2) Shellfish handling practices by the consumer and/or retailer;
(3) Whether the disease has the potential or is known to be transmitted by shellfish; and
(4) Whether the symptoms and incubation period of the illnesses are consistent with the suspected etiologic agent.

Public Health Significance: The basis for this addition is to allow the authority time to determine if the suspected oyster-related *Norovirus* outbreak is due to growing area problems or problems associated with the location where the oysters were served. Due to the nature of *Norovirus*, it would be expected that if the suspected outbreak were growing area related, illnesses would be seen at more than one location. With the known prevalence of *Norovirus* and the ease with which it can be spread by human to human and human to food contact, it is difficult to determine the actual cause within 24 hours when faced with illness reported from a single location.

The Centers for Disease Control and Prevention (CDC) estimates that *Norovirus* cause 23 million cases of acute gastroenteritis annually, making *Norovirus* the leading cause of gastroenteritis in the United States (CDC, 2006; Fankhauser, et al., 2002, Mead, et al., 1999).

Of viruses, only the common cold is reported more often than viral gastroenteritis (N*orovirus)* (Benson & Merano, 1998).
According to the CDC:

Food and drinks can very easily become contaminated with *Norovirus* because the virus is so small and because it probably takes fewer than 100 *Norovirus* particles to make a person sick. Food can be contaminated either by direct contact with contaminated hands or work surfaces that are contaminated with stool or vomit, or by tiny droplets from nearby vomit that can travel through air to land on food. Although the virus cannot multiply outside of human bodies, once on food or in water, it can cause illness.

People working with food who are sick with *Norovirus* gastroenteritis are a particular risk to others, because they handle the food and drink many other people will consume.

Since the virus is so small, a sick food handler can easily – without meaning to – contaminate the food he or she is handling. Many of those eating the contaminated food may become ill, causing an outbreak.

Outbreaks of *Norovirus* gastroenteritis have taken place in restaurants, cruise ships, nursing homes, hospitals, schools, banquet halls, summer camps, and family dinners – in other words, places where often people have consumed water and/or food prepared or handled by others. It is estimated that as many as half of all food-related outbreaks of illness may be caused by *Norovirus*. In many of these cases, sick food handlers were thought to be implicated.

Cost Information (if available):

Action by 2011 Growing Area Classification Committee

Recommended adoption of the suggested language to Chapter II @ .01 Outbreaks of Shellfish – Related Illness, B., C., D. and @ .02 Presence of Human Pathogens in Shellfish Meats. B. Growing Area Investigation (3), (4) & (5) as submitted by the Executive Office.

Modify Model Ordinance Chapter II. Risk Assessment and Risk Management:

@.01 Outbreaks of Shellfish-Related Illness.

A.When shellfish are implicated in an illness outbreak involving two (2) or more persons not from the same household (or one or more persons in the case of paralytic shellfish poisoning [PSP]), the Authority shall determine whether an epidemiological association exists between the illness
and the shellfish consumption by reviewing:

(1) Each consumer's food history;
(2) Shellfish handling practices by the consumer and/or retailer;
(3) Whether the disease has the potential or is known to be transmitted by shellfish; and
(4) Whether the symptoms and incubation period of the illnesses are consistent with the suspected etiologic agent.

NOTE: For additional guidance refer to the International Association of Milk, Food, and Environmental Sanitarians' *Procedures to Investigate Food Borne Illness*.

B. When the Authority has determined an epidemiological association between an illness outbreak and shellfish consumption, the Authority shall:

(1)Conduct an investigation of the illness outbreak within 24 hours to determine whether the illness is growing area related or is the result of post-harvest contamination or mishandling.
(2) Determine if the Authority should request voluntary recall by firms. If a firm or firms is requested by the Authority to recall, the firm will use procedures consistent with the Recall Enforcement Policy, Title 21 CFR Part 7. The recall shall include all implicated products.

C. When the investigation outlined in §.02B. does not indicate a post-harvest contamination problem, or illegal harvesting from a closed area, the Authority shall:

(1) Immediately place the implicated portion(s) of the harvest area(s) in the closed status;
(2) Notify receiving states, the ISSC and the FDA Regional Shellfish

Specialist that a potential health risk is associated with shellfish harvested from the implicated growing area;
(3) As soon as determined by the Authority, transmit to the FDA and receiving states information identifying the dealers shipping the implicated shellfish; and
(4)Promptly initiate recall procedures consistent with the Recall Enforcement Policy, Title 21 Code of Federal Regulations Part 7. The recall shall include all implicated products.

D. When the investigation outlined in §.02B demonstrates that the illnesses are related to post harvesting contamination or mishandling, growing area closure is not required. However, the Authority shall:
(1) Notify receiving states, the ISSC and the FDA Regional Shellfish Specialist of the problem; and
~~(2)Promptly initiate recall procedures consistent with the Recall Enforcement Policy Title 21Code of Federal Regulations Part 7. The recall shall include all implicated products.~~
(2) Determine if the Authority should request voluntary recall by firms. If a firm or firms is requested by the Authority to recall, the firm will use procedures consistent with the Recall Enforcement Policy, Title 21 CFR Part 7. The recall shall include all implicated products.

@. 02 Presence of Human Pathogens in Shellfish Meats.

B. Growing Area Investigation.
(1) The Authority shall review the following factors:
(a) The documentation to trace the shellfish to its source;
(b) (The classification assigned to the growing area and whether the sanitary survey data supporting that classification is current; and
(c) The probability of illegal harvesting from areas classified as restricted or prohibited, or in the closed status.
(2) The Authority shall take no further action when the Authority determines that:
(a) The growing area is properly classified;
(b) No illegal harvesting is taking place; and
(c) There is no reason to believe that the growing area is the source of the pathogens.
(3) When the Authority determines that the growing area is not properly classified, the Authority shall take immediate action to:
(a) Change the existing classification to the correct classification; or
(b) Close the growing area until the correct classification can be determined; and
~~(4)~~ (c) ~~Promptly initiate recall procedures consistent with the Recall Enforcement Policy Title 21 of Code of Federal Regulations Part 7.~~ Determine if the Authority should request voluntary recall by firms. If a firm or firms is requested by the Authority to recall, the firm will use procedures consistent with the Recall Enforcement Policy, Title 21 CFR Part 7. The recall shall include all implicated products.
(4)When the Authority determines that the growing area may be the source of pathogens and~~the Authority shall promptly initiate recall~~

~~procedures consistent with the Recall Enforcement Policy Title 21 of Code of Federal Regulations Part 7 if~~ the pathogens exceed tolerance levels, the Authority shall request a voluntary recall by firms. The firms will use procedures consistent with the Recall Enforcement Policy, Title 21 CFR Part 7. The recall shall include all implicated products.

(5) When the Authority determines that illegal harvesting is taking place, the Authority shall determine if the Authority should request a voluntary recall by firms The firms will use procedures consistent with the Recall Enforcement Policy, Title 21 CFR Part 7. The recall shall include all implicated products. ~~promptly initiate recall procedures consistent with the Recall Enforcement Policy Title 21 Code of Federal Regulations Part 7 for all shellfish that may be falsely represented.~~

C. Distribution and Processing Investigation.

(1) The Authority shall evaluate the distribution and processing of the shellfish. This investigation may include collection of additional meat samples.

(2) The Authority shall take no further action when the Authority determines that there is no reason to believe a problem exists in the distribution or processing of the shellfish.

(3) When the Authority determines that a problem exists in the distribution or processing of the shellfish, the Authority shall take immediate steps to correct the problem and determine if the Authority should request voluntary recall by firms. If a firm or firms is requested by the Authority to recall, the firm will use procedures consistent with the Recall Enforcement Policy, Title 21 CFR Part 7. The recall shall include all implicated products.

~~(3)promptly initiate recall procedures consistent with the Recall Enforcement Policy Title 21of Code of Federal Regulations Part 7.~~

Action by 2011 Task Force I

Recommended adoption of Substitute Proposal 11-114 as amended.

Modify Model Ordinance Chapter II. Risk Assessment and Risk Management:

@.01 Outbreaks of Shellfish-Related Illness.

A. When shellfish are implicated in an illness outbreak involving two (2) or more persons not from the same household (or one or more persons in the case of paralytic shellfish poisoning [PSP]), the Authority shall determine whether an epidemiological association exists between the illness and the shellfish consumption by reviewing:

(1) Each consumer's food history;

(2) Shellfish handling practices by the consumer and/or retailer;

(3) Whether the disease has the potential or is known to be transmitted by shellfish; and

(4) Whether the symptoms and incubation period of the illnesses are consistent with the suspected etiologic agent.

NOTE: For additional guidance refer to the International Association of Milk, Food, and Environmental Sanitarians' *Procedures to Investigate Food Borne Illness*.

B. When the Authority has determined an epidemiological association between an illness outbreak and shellfish consumption, the Authority shall:

(1) Conduct an investigation of the illness outbreak within 24 hours to determine whether the illness is growing area related or is the result of post-harvest contamination or mishandling.

(2) Determine whether to initiate a ~~if the Authority should request~~ voluntary recall by firms. If a firm or firms is requested by the Authority to recall, the firm will use procedures consistent with the Recall Enforcement Policy, Title 21 CFR Part 7. The recall shall include all implicated products.

C. When the investigation outlined in §.02B. does not indicate a post-harvest contamination problem, or illegal harvesting from a closed area, the Authority shall:

(1) Immediately place the implicated portion(s) of the harvest area(s) in the closed status;

(2) Notify receiving states, the ISSC and the FDA Regional Shellfish Specialist that a potential health risk is associated with shellfish harvested from the implicated growing area;

(3) As soon as determined by the Authority, transmit to the FDA and receiving states information identifying the dealers shipping the implicated shellfish; and

(4)Promptly initiate recall procedures consistent with the Recall Enforcement Policy, Title 21 Code of Federal Regulations Part 7. The recall shall include all implicated products.

D. When the investigation outlined in §.02B demonstrates that the illnesses are related to postharvesting contamination or mishandling, growing area closure is not required. However, the Authority shall:

(1) Notify receiving states, the ISSC and the FDA Regional Shellfish Specialist of the problem; and

(2) ~~Determine~~ Initiate a ~~if the Authority should request~~ voluntary recall by firms. If a firm or firms is requested by the Authority to recall, the firm will use procedures consistent with the Recall Enforcement Policy, Title 21 CFR Part 7. The recall shall include all implicated products.

@. 02 Presence of Human Pathogens in Shellfish Meats.

B. Growing Area Investigation.

(1) The Authority shall review the following factors:

(a) The documentation to trace the shellfish to its source;

(b) (The classification assigned to the growing area and whether the sanitary survey data supporting that classification is current; and

(c) The probability of illegal harvesting from areas classified as restricted or prohibited, or in the closed status.

(2) The Authority shall take no further action when the Authority determines that:

(a) The growing area is properly classified;

(b) No illegal harvesting is taking place; and

(c) There is no reason to believe that the growing area is the source of the pathogens.

(3) When the Authority determines that the growing area is not properly classified, the Authority shall take immediate action to:

(a) Change the existing classification to the correct classification; or

(b) Close the growing area until the correct classification can be determined; and

(c) Determine whether to initiate a ~~if the Authority should request~~ voluntary recall by firms. If a firm or firms is requested by the Authority to recall, the firm will use procedures consistent with the Recall Enforcement Policy, Title 21 CFR Part 7. The recall shall include all implicated products.

(4) When the Authority determines that the growing area may be the source of pathogens and the pathogens exceed tolerance levels, the Authority shall request a voluntary recall by firms. The firms will use procedures consistent with the Recall Enforcement Policy, Title 21 CFR Part 7. The recall shall include all implicated products.

(5) When the Authority determines that illegal harvesting is taking place, the Authority shall determine whether to initiate a~~if the Authority should request a~~ voluntary recall by firms The firms will use procedures consistent with the Recall Enforcement Policy, Title 21 CFR Part 7. The recall shall include all implicated products.

C. Distribution and Processing Investigation.

(1) The Authority shall evaluate the distribution and processing of the shellfish. This investigation may include collection of additional meat samples.

(2) The Authority shall take no further action when the Authority determines that there is no reason to believe a problem exists in the distribution or processing of the shellfish.

(3) When the Authority determines that a problem exists in the distribution or processing of the shellfish, the Authority shall take immediate steps to correct the problem and determine whether to initiate a~~if the Authority should request~~ voluntary recall by firms. If a firm or firms is requested by the Authority to recall, the firm will use procedures consistent with the Recall Enforcement Policy, Title 21 CFR Part 7. The recall shall include all implicated products.

Action by 2011 General Assembly Adopted recommendation of 2011 Task Force I on Proposal 11-114.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-114.

Proposal Subject: Addition to the Requirements for the Authority During a Suspected Shellfish Related Outbreak

Specific NSSP Guide Reference: 2009 NSSP Section II Model Ordinance
Chapter II @.01 Outbreaks of Shellfish-Related Illness J.

Text of Proposal/ Requested Action:

I. Whenever an Authority or dealer initiates a recall of shellfish products because of public health concerns, the Authority will monitor the progress and success of the recall. The Authority will immediately notify the FDA and the Authorities in other states involved in the recall. The Authority shall submit periodic recall status reports to the FDA Regional Shellfish Specialist consistent with the Recall Enforcement Policy Title 21 Code of Federal Regulations Part 7, Subpart C, §7.53 (b) (1-6) until such time that the Authority deems the recall to be completed. Each Authority involved in a recall will implement actions to ensure removal of recalled product from the market, issue public warnings if necessary to protect public health and provide periodic reports to the Authority in the state of product origin regarding recall efforts within their state until such time that the Authority in the state of product origin deems the recall to be completed. FDA will decide whether to audit or issue public warnings after consultation with the Authority/Authorities, and after taking into account the scope of the product distribution and other related factors. If the FDA determines that the Authority in any state involved in the recall fails to implement effective actions to protect public health, the FDA may classify, publish and audit the recall, including issuance of public warnings when appropriate.

J. Whenever the Molluscan shellfish products are deemed to be contaminated with a pathogen that would subject it to a recall, reconditioning of the product will be permitted as an alternative to control the hazard. Any such reconditioning process that is used must be validated to reduce the level of the pathogen in question to a level which is not reasonably likely to cause illness or alter the product to a form that is intended to be cooked.

~~J~~K. The Authority shall assess annually Vibrio parahaemolyticus illnesses associated with the consumption of molluscan shellfish. The assessment will include a record of all V. parahaemolyticus shellfish-associated illnesses reported within the state and from receiving states, the numbers of illnesses per event, and actions taken by the Authority in response to the illnesses.

Public Health Significance:

Cost Information (if available):

Action by 2011 Task Force I Recommended referral of Proposal 11-115 to the appropriate committee as determined by the Conference Chairman.

Action by 2011 General Assembly Adopted recommendation of 2011 Task Force I on Proposal 11-115.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-115.

Proposal Subject: Control of Marine Biotoxins

Specific NSSP Guide Reference: Section II Model Ordinance Chapter IV. Shellstock Growing Areas
@. 04. Marine Biotoxin Control
D. Controlled Harvest From Closed Federal Waters

Section IV Guidance Documents Chapter II. Growing Areas
.03 Example of Protocol for Onboard Screening and Dockside Testing for PSP in Closed Federal Waters

Text of Proposal/ Requested Action: Chapter IV Shellfish Growing Areas @.04 Marine Biotoxin Control. Insert new item A. (5)

(5) Prior to allowing the landing of shellfish harvested from waters closed due to periodic toxic algal blooms associated with PSP, and where routine monitoring of saxitoxin levels is not conducted, the State Authority in the landing State in cooperation with appropriate Federal agencies shall develop agreements or memorandums of understanding between the Authority and individual shellfish harvesters or individual shellfish dealers. The agreements or memorandums of understanding shall provide strict safety assurances. At a minimum agreements or memorandums of understanding shall include provisions for:

(a) harvest permit requirements.
(b) training for individuals conducting onboard toxicity screening using NSSP methods.
(c) vessel monitoring;
(d) identification of shellfish for each harvesting trip to include:
(i) Vessel name and owner
(ii) Captain's name
(iii) Person conducting onboard screening tests
(iv) Port of departure name and date
(v) Port of landing name and date
(vi) Latitude and longitude coordinates of designated harvest area
(vii) Onboard screening test results
(viii)Volume and species of shellfish harvested
(ix) Intended processing facility name, address and certification number
(x) Captain's signature and date
(e) Pre-harvested sampling that includes a minimum of five (5) samples from the intended harvest area be tested for saxitoxins. Harvesting shall not be permitted if any of the pre-harvested samples contain saxitoxin levels in excess of 44ug/l00g.
(f) Submittal of onboard screening homogenates and test results to the authority in the state of landing.
(g) The collection and saxitoxin level testing of a minimum of seven (7) dockside samples. The SSCA may require more samples based on the size of the vessel and the volume of shellfish harvested.
(h) Holding and providing separation until dockside samples verifiy that saxitoxin levels are below 80ug/100g.
(i) Disposal of shellfish should dockside test results exceed 80ug /100g.
(j) Notification prior to unloading.
(k) Unloading schedule.
(l) Access for Dockside Sampling.
(m) Record Keeping.
(n) Early Warning/Alert System

NOTE: The plan may include other requirements, as deemed necessary by the authority in the state of landing, to ensure adequate public health protection under the NSSP.

Insert new Additional Guidance reference at Model Ordinance Chapter IV@ .04. A. (5) as follows:

Additional Guidance – Section IV Guidance Documents Chapter II. Growing Areas .03 Protocol for the Landing of Shellfish from Federally Closed Waters due to PSP

Add new guidance to Section IV. Guidance Documents, Chapter II. Growing Areas .03 and re-number Section IV. Guidance Documents, Chapter II. Growing Areas .03 through .15 as .04 through .16.

Protocol for the Landing of Shellfish from Federally Closed Waters due to PSP

When the harvest of molluscan shellfish is closed in Federal Waters due to Paralytic Shellfish Poison (PSP), exceptions to the prohibitions may be authorized provided the Authority in the State of landing in cooperation with appropriate Federal agencies shall develop agreements or memorandums of understanding between the Authority and individual shellfish harvesters or individual shellfish dealers. This guidance provides descriptions of the specific information to be included in the protocol.

A. Harvest Permit Requirements

The Authority in the landing state will only allow the landing of shellfish from federal waters closed due to PSP from vessels in possession of an appropriate Exempted Fishing Permit (EFP) issued by the National Marine Fisheries Service (NMFS). The NMFS shall receive concurrence from the SSCA in the State of landing.

B. Training

The Authority shall ensure that all shipboard persons conducting onboard sampling have been trained by a National Shellfish Sanitation Program (NSSP) Laboratory Evaluation Officer (LEO) or a US Food and Drug Administration (FDA) marine biotoxin expert to conduct onboard PSP screening using a NSSP recognized method(s).

C. Vessel Monitoring

The Authority shall ensure that the harvesting location(s) of each landing vessel has been appropriately monitored. This requirement may be met by the vessel participating in the Federal Vessel Monitoring System (VMS).

D. Identification of Shellfish

Prior to landing each vessel shall provide the Authority with a record identifying each lot of shellfish as follows: For each harvesting trip the Captain or Mate shall record the following information on a "Harvest Record." Electronic logging of this information may be permitted provided it is made available to the authorized individual at dockside.

1. Vessel name and Federal Fishing Permit number
2. Name and telephone number of the vessel Captain and vessel owner
3. Date(s) of harvest
4. Number of lots and volume of catch per lot or number of containers per lot
5. Location(s) of harvest (GPS coordinates or latitude/longitude coordinates in degrees:minutes:seconds)
6. Identification of each harvest lot, including cage tag numbers for surfclams and ocean quahogs, and container numbers or identification codes for other shellfish species.
7. Location (GPS coordinates or latitude/longitude coordinates in degrees:minutes:seconds) of each PSP screening sample
8. Results of each PSP screening test. Screening test kits for each sample shall be submitted to the authorized authority along with the "Harvest Record" as stated in Section D.
9. Destination(s) and purchaser(s) of each lot and amount of each lot to each destination

The Captain or Mate shall sign the "Harvest Record." The "Harvest Record" shall be checked by the individual authorized to sample the harvested shellfish. Failure to provide complete and accurate information will result in revocation or suspension of the NMFS EFP and rejection of the entire lot(s) of harvested shellfish. Four (4) copies of the "Harvest Record" shall be prepared. One (1) copy shall remain with the vessel, one (1) copy shall be provided to the SSCA in the state of landing, one (1) copy shall accompany the catch to the processing firm(s), and one (1) copy shall be retained by the laboratory authorized to conduct lot sample analyses.

CONTAINER LABELING:

Each container of shellfish shall be clearly labeled with the following NSSP required information at the time of harvest:

1. For surfclams and ocean quahogs existing NMFS tagging requirements
2. For all other molluscan shellfish (including Stimpson clams also known as Arctic surfclams) using Tyvek tags:
   a. Vessel name
   b. Type and quantity of shellfish
   c. Date of harvest
   d. Harvest lot area defined by GPS coordinates or latitude/longitude coordinates in degrees:minutes:seconds

E. Pre-Harvest Sampling

Prior to commercial harvesting of molluscan shellfish, a minimum of five (5) screening samples shall be collected within each area of intended harvest (lot area) and tested for PSP toxins in accordance with a NSSP recognized screening method. Each screening sample shall be collected during a separate and distinct gear tow. Screening sample tows shall be conducted in a manner that evenly distributes the five (5) samples throughout the intended harvest area for each area of intended harvest (see Section H.). Only shipboard officials trained in the use of the designated NSSP screening method may conduct these tests. Each of the five (5) samples must test negative for PSP toxins. A positive result from any one (1) sample shall render the "lot area" unacceptable for harvest. The harvest vessel

captain shall immediately report all positive screening test results, by telephone, to the SSCA within the intended state of landing and the NMFS. The Captain should also notify other permitted harvest vessels of the positive screening test and advise them to avoid the questionable area. For each screening test, positive and negative, the remaining sample material (homogenate) shall be maintained under refrigeration. Test kits, positive and negative, shall accompany the remaining sample homogenates to the certified laboratory. Confirmatory testing shall be performed on homogenate from each positive screening test using a NSSP recognized test method. Upon request by the SSCA in the state of landing, confirmatory testing of homogenate from negative screening tests shall be conducted using a NSSP recognized test method.

Each screening sample shall be comprised of at least twelve (12) whole animals with the exception of mussels and "whole" or "roe-on" scallops. For mussels each sample shall be comprised of thirty (30) animals. For "whole" scallops each sample shall be comprised of twenty (20) scallop viscera and gonads. For "roe-on" scallops each sample shall be comprised of twenty (20) scallop gonads.

F. Submittal of Onboard Screening Homogenates and Test Results

All screening results shall be recorded on the "Harvest Record" as stipulated in Section D. of this Protocol. Test kits used to screen each lot shall accompany the "Harvest Record". Upon landing of the harvest vessel, the "Harvest Record" and accompanying test kits shall be provided to the individual (state shellfish official, FDA official, NMFS official) authorized to sample the harvested shellfish as described in Section G. of this Protocol.

G. Dockside Sampling

After dockside samples are collected, molluscan shellfish may be processed while awaiting PSP analytical results. Each lot must be identified and segregated during storage while awaiting dockside sample test results. Under no circumstances will product be released from the processor prior to receiving satisfactory paralytic shellfish toxin test results.

The dockside sampling protocol for molluscan shellfish shall be as follows:

1. For each lot of molluscan shellfish, a minimum of seven (7) composite samples, each comprised of at least twelve (12) whole animals, shall be taken at random by the individual authorized to sample, with the following exceptions:
   a. For each lot of mussels, a minimum of seven (7) composite samples, each comprised of at least thirty (30) whole animals, shall be taken at random by the individual authorized to sample.
   b. For each lot of "whole" scallops, a minimum of seven (7) composite samples, each comprised of twenty (20) scallop viscera and gonads, shall be taken at random by the individual authorized to sample.
   c. For each lot of "roe-on" scallops, a minimum of seven (7) composite samples, each comprised of twenty (20) scallop gonads, shall be taken at random by the individual authorized to sample.
2. Shellfish samples collected in accordance with G.1 shall be tested for the presence of paralytic shellfish toxins using NSSP recognized methods.
3. Laboratory test results for each lot of shellfish shall be forwarded to the

SSCA in the state in which the shellfish is being held prior to the product being released by the SSCA.

H. Holding and Lot Separation

A harvest lot is defined as all molluscan shellfish harvested during a single period of uninterrupted harvest activity within a geographic area not to exceed three (3) square miles. Once harvesting has ceased and the harvest vessel moves to another location, regardless of the distance, a new harvest lot will be established. Any harvest vessel containing more than one lot shall clearly mark and segregate each lot while at sea, during off loading, and during transportation to a processing facility. Prior to harvesting in Federal waters, each harvest vessel shall submit to the NMFS a written onboard lot segregation plan. The SSCA in the intended state of landing and the FDA Regional Shellfish Specialist must approve the proposed lot segregation plan.

I. Disposal of Shellfish

If test results of any one (1) of the seven (7) samples collected in accordance with G.1 equal or exceed 80ug of paralytic shellfish toxins/100g of shellfish tissue (n=7, c=0), the entire lot must be discarded or destroyed at the cost of the harvester under the supervision of the SSCA in accordance with state laws and regulations except when:

A lot of "whole" or "roe-on" scallops equals or exceeds 80ug paralytic shellfish toxins/100g of tissue, the adductor muscle may be shucked from the viscera and/or gonad and marketed. The remaining materials (viscera and/or gonad) must be discarded or destroyed under supervision of the SSCA in accordance with state laws and regulations.

Confirmatory PSP analyses shall be according to NSSP recognized methods and shall be conducted by laboratories certified in accordance with NSSP guidelines. Private laboratories may be used if certified by a Federal or state shellfish Laboratory Evaluation Officer (LEO) in accordance with NSSP guidelines.

J. Notification Prior to Unloading

Prior to the issuance of an EFP, the harvester shall be responsible for notifying the SSCA in the state of landing and in a manner approved by the SSCA that molluscan shellfish is being harvested for delivery to the intended receiving processor.

Each vessel shall give at least twelve (12) hours notice to the individual authorized to sample prior to unloading shellfish. Notice of less than twelve (12) hours may be approved by the authorized individual at his/her discretion. SSCAs may approve industry sampling and sample transport to the NSSP certified testing laboratory in accordance with the practices and procedures used by the SSCA under the NSSP. Such procedures may be approved by the SSCA only when sample collection and sample transport training is provided by the SSCA.

Shellfish from a federally closed harvest area must be kept separate and not sold until so authorized by the SSCA.

Failure to comply with the provisions of this Protocol will result in the suspension or revocation of the vessel's EFP.

K. Unloading Schedule

Unloading shall take place between 7:00 A.M. and 5:00 P.M. Monday through Friday, unless otherwise mutually agreed upon by the individual authorized to sample, the processing plant manager, the harvest vessel captain, and the SSCA in the state of landing, sample testing, and processing.

L. Access for Dockside Sampling

Individuals authorized to sample shall be provided access to the catch of shellfish.

M. Record Keeping

Record keeping requirements shall be as follows:

1. The vessel shall maintain Harvest Records for at least one (1) year.
2. The processor(s) shall maintain Harvest Records for at least one (1) year or two (2) years if the product is frozen.
3. The SSCA in the State of landing shall retain Harvest Records for at least two (2) years.

N. Early Warning/Alert System

PSP sample data acquired as a result of onboard screening and dockside testing shall be transmitted to a central data register to be maintained by the FDA. These data, both screening and confirmatory, shall be transmitted to the FDA by the NSSP certified laboratory conducting PSP analyses of the sampled lot(s) within one week of the completion of the PSP analyses. The data provided shall include the following:

1. shellfish species
2. harvest location name and coordinates (GPS or latitude/longitude)
3. harvest date
4. onboard screening test method, date, and results
5. laboratory test date and test results

Results of all samples having acceptable levels of paralytic shellfish toxins (<80ug/100g) shall immediately be reported to the SSCA in the state of landing. If the results of any one (1) sample equal or exceed 80ug/100g the testing laboratory shall immediately notify the FDA Regional Shellfish Specialist, the SSCA, and the processor by telephone. The FDA shall notify the NMFS. The NMFS shall notify permitted harvesters to advise them to cease fishing in the affected area(s).

NOTE: Due to the resources necessary to meet the requirements of this Protocol, State Shellfish Control Authorities (SSCAs) may find it necessary to require industry to fund associated costs. These costs may include sample collection, screening, transportation, analysis, inspection, enforcement, and other related expenses.

Public Health Significance:

The surf clam and ocean quahog fishery is one of the largest shellfish fisheries in the U.S. producing up to 130 million pounds of meats per year, generating about $75 million ex-vessel per year.

Atlantic surf clams and ocean quahogs are found in the North Atlantic from North Carolina to the Gulf of St. Lawrence. The surf clam and ocean quahog fisheries in the U.S. are managed by the National Marine Fisheries Services (NMFS) in accordance with a management plan prepared by the Mid Atlantic Fishery Management Council under an individual transferable quota system implemented in 1990.

The management plan includes requirements for trip announcements, landings time andport, and each vessel is equipped with a Vessel Monitoring System (VMS). The VMS allows the regulators to identified, tracked locations of harvest within 100 feet and steaming speed, for every clam vessel authorized to operate in federal waters.

Allocations are issued to quota holders each year in the form specifically identified tags that must be attached to containers of surf clams or ocean quahogs. Ownership of the tags and harvest activities are closely monitored by NOAA Fisheries.

Surf clams and ocean quahogs are processed for use in strips, soups, chowders, and sauces.Although surf clams and ocean quahogs are not consumed raw they are shipped alive in interstate commerce and are subject to NSSP regulation. Thirteen processing facilities are located in six states: MA, RI, NJ, DE, MD, and VA. A fleet of approximately 40 vessels land their catch in five states; MA, RI, NJ, MD, and NY.

Because the U.S. FDA does not have the resources necessary to routinely monitor the Northwest Atlantic Ocean where Alexandrium blooms responsible for PSP have historically occurred, waters of the Northwestern Atlantic west of 69.00° W Longitude have been closed since 1990. In 2005 federal waters east of 69.00° W Longitude and north of 40.00° N Latitude were also closed in response to an unprecedented toxic algal bloom (PSP) that occurred throughout the Northewest Atlantic Ocean, affecting state and federal waters. Much of this area remains closed today to the harvest of all molluscan shellfish, all of the area remains closed to the harvest of whole and roe-on scallops. These areas combined represent approximately 50% of the total surf and ocean quahog resource along the Atlantic coast. The result has been increased pressure on the remaining resource and economic loss to the fishery and its affiliated land based components.

Beginning in 2008, a pilot program was initiated to evaluate the Onboard Screening and Dockside Testing Protocol (Protocol), outlined in this ISSC Proposal and developed by FDA, NMFS, EPA, North and Mid Atlantic State shellfish authorities, and representatives of the Atlantic Fishery Management Council. The purpose of the pilot, which was given ISSC Executive Board concurrence, was to test the effectiveness of the Protocol for ensuring the safe harvest of shellfish harvested from Federal waters closed because of the historical occurrence of significant PSP episodes. Harvesting was conducted under an Experimental Fishing Permit issued to a single vessel by NMFS. Four States participated in the Pilot including NJ, DE, RI, and MA.

Under the Pilot, shellfish are tested at sea to ensure that harvest levels do not exceed 44ug PSP/100g meat. Once landed the shellfish is again tested using the traditional Mouse Bioassay (MBA) and only permitted to leave the processing facility for entry into the commercial market when all samples have demonstrated PSP levels compliant with NSSP requirements. To date there have been over 70 sucessful harvest trips to offshore Federal waters on Georges Bank, accounting for the safe landing of approximately 330,000

bushels of clams. The Pilot has demonstrated the efficacy of the Protocol in all regards.

Adoption of this Proposal by the ISSC will pave the way for additional vessels, operating under NMFS permit in accordance with Protocol requirements, to safely harvest from offshore Federal waters closed as a result of histoicall episodes of toxic PSP blooms.

Cost Information (if available):

Action by 2011 Task Force I

Recommended adoption of Proposal 11-116 as amended.

Chapter IV Shellfish Growing Areas @.04 Marine Biotoxin Control. Insert new item A. (5)

(5) Prior to allowing the landing of shellfish harvested from federal waters closed due to periodic toxic algal blooms associated with PSP, and where routine monitoring of saxitoxin levels is not conducted, the State Authority in the landing State in cooperation with appropriate Federal agencies shall develop agreements or memorandums of understanding between the Authority and individual shellfish harvesters or individual shellfish dealers. The agreements or memorandums of understanding shall provide strict safety assurances. At a minimum agreements or memorandums of understanding shall include provisions for:

(a) harvest permit requirements.
(b) training for individuals conducting onboard toxicity screening using NSSP methods.
(c) vessel monitoring;
(d) identification of shellfish for each harvesting trip to include:
(i) Vessel name and owner
(ii) Captain's name
(iii) Person conducting onboard screening tests
(iv) Port of departure name and date
(v) Port of landing name and date
(vi) Latitude and longitude coordinates of designated harvest area
(vii) Onboard screening test results
(viii)Volume and species of shellfish harvested
(ix) Intended processing facility name, address and certification number
(x) Captain's signature and date
(e) Pre-harvested (onboard) sampling that includes a minimum of five (5) samples from the intended harvest area be tested for saxitoxins. Harvesting shall not be permitted if any of the pre-harvested samples contain saxitoxin levels in excess of 44ug/l00g -when using a quantitative test or a positive at a limit of detection of 40ug/100g for the qualitative screening test.
(f) Submittal of onboard screening homogenates and test results to the authority in the state of landing.
(g) The collection and saxitoxin level testing of a minimum of seven (7) dockside samples. The SSCA may require more samples based on the size of the vessel and the volume of shellfish harvested.
(h) Holding and providing separation until dockside samples verifiy that saxitoxin levels are below 80ug/100g.
(i) Disposal of shellfish should dockside test results exceed 80ug /100g.
(j) Notification prior to unloading.
(k) Unloading schedule.
(l) Access for Dockside Sampling.

(m) Record Keeping.
(n) Early Warning/Alert System

NOTE: The plan may include other requirements, as deemed necessary by the authority in the state of landing, to ensure adequate public health protection under the NSSP.

Insert new Additional Guidance reference at Model Ordinance Chapter IV@ .04. A. (5) as follows:

Additional Guidance – Section IV Guidance Documents Chapter II. Growing Areas .03 Protocol for the Landing of Shellfish from Federally Closed Waters due to PSP

Add new guidance to Section IV. Guidance Documents, Chapter II. Growing Areas .03 and re-number Section IV. Guidance Documents, Chapter II. Growing Areas .03 through .15 as .04 through .16.

Protocol for the Landing of Shellfish from Federally Closed Waters due to PSP

When the harvest of molluscan shellfish is closed in Federal Waters due to Paralytic Shellfish Poison (PSP), exceptions to the prohibitions may be authorized provided the Authority in the State of landing in cooperation with appropriate Federal agencies shall develop agreements or memorandums of understanding between the Authority and individual shellfish harvesters or individual shellfish dealers. This guidance provides descriptions of the specific information to be included in the protocol.

A. Harvest Permit Requirements

The Authority in the landing state will only allow the landing of shellfish from federal waters closed due to PSP from vessels in possession of an appropriate Exempted Fishing Permit (EFP) issued by the National Marine Fisheries Service (NMFS). The NMFS shall receive concurrence from the SSCA in the State of landing.

B. Training

The Authority shall ensure that all shipboard persons conducting onboard sampling have been trained by a National Shellfish Sanitation Program (NSSP) Laboratory Evaluation Officer (LEO) or a US Food and Drug Administration (FDA) marine biotoxin expert to conduct onboard PSP screening using a NSSP recognized method(s).

C. Vessel Monitoring

The Authority shall ensure that the harvesting location(s) of each landing vessel has been appropriately monitored. This requirement may be met by the vessel participating in the Federal Vessel Monitoring System (VMS).

D. Identification of Shellfish

Prior to landing each vessel shall provide the Authority with a record identifying each lot of shellfish as follows: For each harvesting trip the Captain or Mate shall record the following information on a "Harvest Record." Electronic logging of this

information may be permitted provided it is made available to the authorized individual at dockside.

1. Vessel name and Federal Fishing Permit number
2. Name and telephone number of the vessel Captain and vessel owner
3. Date(s) of harvest
4. Number of lots and volume of catch per lot or number of containers per lot
5. Location(s) of harvest (GPS coordinates or latitude/longitude coordinates in degrees:minutes:seconds)
6. Identification of each harvest lot, including cage tag numbers for surfclams and ocean quahogs, and container numbers or identification codes for other shellfish species.
7. Location (GPS coordinates or latitude/longitude coordinates in degrees:minutes:seconds) of each PSP screening sample
8. Results of each PSP screening test. ~~Screening test kits for each sample shall be submitted to the authorized authority along with the "Harvest Record" as stated in Section D.~~
9. Destination(s) and purchaser(s) of each lot and amount of each lot to each destination

The Captain or Mate shall sign the "Harvest Record." The "Harvest Record" shall be checked by the individual authorized to sample the harvested shellfish. Failure to provide complete and accurate information will result in revocation or suspension of the NMFS EFP and rejection of the entire lot(s) of harvested shellfish. Four (4) copies of the "Harvest Record" shall be prepared. One (1) copy shall remain with the vessel, one (1) copy shall be provided to the SSCA in the state of landing, one (1) copy shall accompany the catch to the processing firm(s), and one (1) copy shall be retained by the laboratory authorized to conduct lot sample analyses.

CONTAINER LABELING:

Each container of shellfish shall be clearly labeled with the following NSSP required information at the time of harvest:

1. For surfclams and ocean quahogs existing NMFS tagging requirements
2. For all other molluscan shellfish (including Stimpson clams also known as Arctic surfclams) using Tyvek tags:
   a. Vessel name
   b. Type and quantity of shellfish
   c. Date of harvest
   d. Harvest lot area defined by GPS coordinates or latitude/longitude coordinates in degrees:minutes:seconds

E. Pre-Harvest Sampling

Prior to commercial harvesting of molluscan shellfish, a minimum of five (5) screening samples shall be collected within each area of intended harvest (lot area) and tested for PSP toxins in accordance with a NSSP recognized screening method. Each screening sample shall be collected during a separate and distinct gear tow. Screening sample tows shall be conducted in a manner that evenly distributes the five (5) samples throughout the intended harvest area for each area of intended harvest (see Section H.). Only shipboard officials trained in the use of the

designated NSSP screening method may conduct these tests. Each of the five (5) samples must test negative for PSP toxins. A positive result from any one (1) sample shall render the "lot area" unacceptable for harvest. The harvest vessel captain shall immediately report all positive screening test results, by telephone, to the SSCA within the intended state of landing and the NMFS. The Captain should also notify other permitted harvest vessels of the positive screening test and advise them to avoid the questionable area. For each screening test, positive and negative, the remaining sample material (homogenate) shall be maintained under refrigeration. Test kits, positive and negative, shall accompany the remaining sample homogenates to the certified laboratory. Confirmatory testing shall be performed on homogenate from each positive screening test using a NSSP recognized test method. Upon request by the SSCA in the state of landing, confirmatory testing of homogenate from negative screening tests shall be conducted using a NSSP recognized test method.

Each screening sample shall be comprised of at least twelve (12) whole animals with the exception of mussels and "whole" or "roe-on" scallops. For mussels each sample shall be comprised of thirty (30) animals. For "whole" scallops each sample shall be comprised of twenty (20) scallop viscera and gonads. For "roe-on" scallops each sample shall be comprised of twenty (20) scallop gonads.

F. Submittal of Onboard Screening Homogenates and Test Results

All screening results shall be recorded on the "Harvest Record" as stipulated in Section D. of this Protocol. Test kits used to screen each lot shall accompany the "Harvest Record". Upon landing of the harvest vessel, the "Harvest Record" and accompanying test kits shall be provided to the individual (state shellfish official, FDA official, NMFS official) authorized to sample the harvested shellfish as described in Section G. of this Protocol.

G. Dockside Sampling

After dockside samples are collected, molluscan shellfish may be processed while awaiting PSP analytical results. Each lot must be identified and segregated during storage while awaiting dockside sample test results. Under no circumstances will product be released from the processor prior to receiving satisfactory paralytic shellfish toxin test results.

The dockside sampling protocol for molluscan shellfish shall be as follows:

1. For each lot of molluscan shellfish, a minimum of seven (7) composite samples, each comprised of at least twelve (12) whole animals, shall be taken at random by the individual authorized to sample, with the following exceptions:
   a. For each lot of mussels, a minimum of seven (7) composite samples, each comprised of at least thirty (30) whole animals, shall be taken at random by the individual authorized to sample.
   b. For each lot of "whole" scallops, a minimum of seven (7) composite samples, each comprised of twenty (20) scallop viscera and gonads, shall be taken at random by the individual authorized to sample.
   c. For each lot of "roe-on" scallops, a minimum of seven (7) composite samples, each comprised of twenty (20) scallop gonads, shall be taken at random by the individual authorized to sample.

2. Shellfish samples collected in accordance with G.1 shall be tested for the presence of paralytic shellfish toxins using NSSP recognized methods.
3. Laboratory test results for each lot of shellfish shall be forwarded to the SSCA in the state in which the shellfish is being held prior to the product being released by the SSCA.

H. Holding and Lot Separation

A harvest lot is defined as all molluscan shellfish harvested during a single period of uninterrupted harvest activity within a geographic area not to exceed three (3) square miles. Once harvesting has ceased and the harvest vessel moves to another location, regardless of the distance, a new harvest lot will be established. Any harvest vessel containing more than one lot shall clearly mark and segregate each lot while at sea, during off loading, and during transportation to a processing facility. Prior to harvesting in Federal waters, each harvest vessel shall submit to the NMFS a written onboard lot segregation plan. The SSCA in the intended state of landing and the FDA Regional Shellfish Specialist must approve the proposed lot segregation plan.

I. Disposal of Shellfish

If test results of any one (1) of the seven (7) samples collected in accordance with G.1 equal or exceed 80ug of paralytic shellfish toxins/100g of shellfish tissue (n=7, c=0), the entire lot must be discarded or destroyed at the cost of the harvester under the supervision of the SSCA in accordance with state laws and regulations except when:

> A lot of "whole" or "roe-on" scallops equals or exceeds 80ug paralytic shellfish toxins/100g of tissue, the adductor muscle may be shucked from the viscera and/or gonad and marketed. The remaining materials (viscera and/or gonad) must be discarded or destroyed under supervision of the SSCA in accordance with state laws and regulations.

Confirmatory PSP analyses shall be according to NSSP recognized methods and shall be conducted by laboratories certified in accordance with NSSP guidelines. Private laboratories may be used if certified by a Federal or state shellfish Laboratory Evaluation Officer (LEO) in accordance with NSSP guidelines.

J. Notification Prior to Unloading

Prior to the issuance of an EFP, the harvester shall be responsible for notifying the SSCA in the state of landing and in a manner approved by the SSCA that molluscan shellfish is being harvested for delivery to the intended receiving processor.

Each vessel shall give at least twelve (12) hours notice to the individual authorized to sample prior to unloading shellfish. Notice of less than twelve (12) hours may be approved by the authorized individual at his/her discretion. SSCAs may approve industry sampling and sample transport to the NSSP certified testing laboratory in accordance with the practices and procedures used by the SSCA under the NSSP. Such procedures may be approved by the SSCA only when sample collection and sample transport training is provided by the SSCA.

Shellfish from a federally closed harvest area must be kept separate and not sold until so authorized by the SSCA.

Failure to comply with the provisions of this Protocol will result in the suspension or revocation of the vessel's EFP.

K. Unloading Schedule

Unloading shall take place between 7:00 A.M. and 5:00 P.M. Monday through Friday, unless otherwise mutually agreed upon by the individual authorized to sample, the processing plant manager, the harvest vessel captain, and the SSCA in the state of landing, sample testing, and processing.

L. Access for Dockside Sampling

Individuals authorized to sample shall be provided access to the catch of shellfish.

M. Record Keeping

Record keeping requirements shall be as follows:

1. The vessel shall maintain Harvest Records for at least one (1) year.
2. The processor(s) shall maintain Harvest Records for at least one (1) year or two (2) years if the product is frozen.
3. The SSCA in the State of landing shall retain Harvest Records for at least two (2) years.

N. Early Warning/Alert System

PSP sample data acquired as a result of onboard screening and dockside testing shall be transmitted to a central data register to be maintained by the FDA. These data, both screening and confirmatory, shall be transmitted to the FDA by the NSSP certified laboratory conducting PSP analyses of the sampled lot(s) within one week of the completion of the PSP analyses. The data provided shall include the following:

1. shellfish species
2. harvest location name and coordinates (GPS or latitude/longitude)
3. harvest date
4. onboard screening test method, date, and results
5. laboratory test date and test results

Results of all samples having acceptable levels of paralytic shellfish toxins (<80ug/100g) shall immediately be reported to the SSCA in the state of landing. If the results of any one (1) sample equal or exceed 80ug/100g the testing laboratory shall immediately notify the FDA Regional Shellfish Specialist, the SSCA, and the processor by telephone. The FDA shall notify the NMFS. The NMFS shall notify permitted harvesters to advise them to cease fishing in the affected area(s).

NOTE: Due to the resources necessary to meet the requirements of this Protocol, State Shellfish Control Authorities (SSCAs) may find it necessary to require industry to fund associated costs. These costs may include sample collection, screening, transportation, analysis, inspection, enforcement, and other related expenses.

Action by 2011 General Assembly

Adopted recommendation of 2011 Task Force I on Proposal 11-116.

Action by FDA February 26, 2012

Concurred with Conference action on Proposal 11-116 with the following recommendation.

FDA concurs with Conference action to adopt Proposal11-116 to approve use of the Onboard Screening and Dockside Testing Protocol for controlling PSP in closed Federal waters. In the adopted Model Ordinance language the Protocol requires delivery of all onboard screening homogenates and test results to the authority in the State of landing. However, there is a discrepancy between what the adopted Model Ordinance language requires and what the adopted Guidance Document language recommends. To be consistent with the Model Ordinance requirement for onboard screening homogenates and test results to be submitted to the authority in the State of landing, the Guidance Document should be amended as follows: (strikethrough = *deleted text,* underline = *added text)*

Change Guidance Document item "E" first paragraph to read, *"Prior to commercial harvesting of molluscan shellfish, a minimum of five (5) screening samples shall be collected…For each screening test, positive and negative, the remaining sample material (Homogenate) shall be maintained under refrigeration for later use should the SSCA in the State of landing request confirmatory testing using a NSSP recognized test method. ~~Test kits, positive and negative, shall accompany the remainig sample homogenates to the certified laboratory. Confirmatory testing shall be performed on homogenate from eachpositive screening test using a NSSP recognized test method. Upon request by the SSCA in the state of landing, confirmatory testing of homogenate from negative screening tests shall be conducted using a NSSP recognized test method."~~*

Change Guidance Document item "F" to read, *"All screening results shall be recorded on the "Harvest Record" as stipulated in Section D. of this Protocol. ~~Test kits used to screen each lot shall accompany the "Harvest Record".~~ Upon landing of the harvest vessel, the "harvest Record" ~~and accompanying test kits~~ and screening homogenates shall be provided to the ~~individual (state shellfish official, FDA official, NMFS official)~~ authority in the State of landing authorized to sample the harvested shellfish as described in Section G. of this Protocol.*

Proposal Subject:	Recall Notification
Specific NSSP Guide Reference:	2009 NSSP Section II Model Ordinance Chapter II Risk Assessment and Risk Management @ .01 Outbreaks of Shellfish Related Illness I.
Text of Proposal/ Requested Action	I. Whenever an Authority or dealer initiates a recall of shellfish products because of public health concerns, the Authority will monitor the progress and success of the recall. The Authority will immediately notify the FDA, ISSC and the Authorities in other states involved in the recall.
Public Health Significance:	Presently shellfish recalls are not listed on the USFDA website. In an effort to assure public notification of shellfish recalls, which would be consistent with other food programs, FDA is asking ISSC to include recalls on the ISSC website. FDA will provide a link in its website to the ISSC website for shellfish recalls. Should this option not be acceptable to ISSC, FDA will include notification on the FDA website.
Cost Information (if available):	
Action by 2011 Task Force I	Recommended adoption of Proposal 11-117 as submitted.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 11-117.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 11-117.

Proposal Subject: *Vibrio vulnificus* Risk Management Plan

Specific NSSP Guide Reference: 1999 NSSP Guide Model Ordinance
Chapter II. Risk Assessment and Risk Management

Text of Proposal/ Requested Action Modify 1999 Model Ordinance Chapter II. by adding new Section @. 04:

Chapter II. Risk Assessment and Risk Management.

@. 04 *Vibrio vulnificus* Risk Management

Risk Management Plan

(1) For states having 2 or more etiologically confirmed shellfish-borne *Vibrio vulnificus* illnesses traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state, the Authority shall develop and implement a *Vibrio vulnificus* risk management plan. Etiologically confirmed means those cases in which laboratory evidence of a specific agent is obtained and specified criteria are met.

(2) The plan may include the following elements and shall define the administrative procedures and resources necessary to accomplish (i.e., establish and maintain) them;

(a) Education/Consumer intervention;

(b) Pre-harvest controls to reduce *Vibrio vulnificus* levels in oyster shellstock; and

(c) Post-harvest controls to reduce *Vibrio vulnificus* levels in oyster shellstock.

(3) The plan shall include controls and interventions that are designed to reduce the rate of etiologically confirmed shellfish-borne *Vibrio vulnificus* septicemia illnesses reported in core states from the consumption of commercially harvested raw or undercooked oysters by 40 percent by the end of 2005 and by 60 percent by 2007. The rate of illness shall be calculated as the number of illnesses divided by the production of oysters from the states bordering the Gulf of Mexico, based on National Marine Fisheries Service landing data. Core states shall be Florida, Texas, California, Louisiana, Georgia, South Carolina, and Alabama. The baseline data for measuring illness reduction shall be the reported illnesses in the core states for the period 1996 to 1999, inclusive, as compiled by the Southeast Regional Office of the U.S. Food and Drug Administration. The data used for measuring goal attainment shall begin with 2001 data. See §B. (1) below.

(4) At a minimum, the plan shall include the following controls and interventions:

(a) Education/Consumer intervention - Implementing ~~of those~~ portions of the ISSC Education/Consumer Intervention Plan that are relevant to the state;

(b) Pre-harvest Controls - Based on the results of the annual FDA state shellfish program evaluation, assuring that all certified dealers comply with the time/temperature requirements contained in VIII.03, IX.05, XI.01A. (3), XII.01A. (3), XIII.01A. (3), and XIV.01A. (3). *[Ed. note: see proposed language for XI.01A. (3), XII.01A. (3), XIII.01A. (3), and XIV.01A. (3) in Issue 00-208.]*

(c) Post-harvest Controls

(i) Providing assistance, as necessary, for the further study of dockside icing to investigate its effects on shelf-life and variations in the effectiveness of the method as a result of seasonal and regional differences;

(ii) Implementing dockside icing requirements if the study results are favorable and illness reduction targets are not met as described in §(5) below;

(iii) Supporting, as necessary, the commercialization of existing post-harvest technologies and the development of new technologies;

(iv) Providing incentives to add refrigeration capacity to harvest vessels; and

(v) Selecting and preparing for the implementation of one or more of the controls contained in II. @. 04A. (6), in case such implementation becomes necessary, as described in that paragraph.

(5) If the illness reduction goal contained in II. @. 04A. (3) is less than 25 percent by the end of Year 4 (2004); the goal must be reassessed through a thorough review of the more intensive epidemiological investigations of illnesses for years 2001-2004.

[Submitter's note: The details of this more intensive epidemiological investigation are being discussed by the Vibrio Management Committee (VMC). Final recommendations will be made available following the VMC meeting on June 13 and 14.]

(6) Affected states must implement one or more of the following control strategies on January 1, 2008, if the illness reductions fail to meet the requirements of §(5) above.

[*Submitter's note: The Committee is discussing multiple options for appropriate control strategies. They include:*

(a) Labeling oysters when water temperatures reach a certain level (65° Fahrenheit is being discussed);

(b) Requiring post-harvest treatment when water temperatures exceed a certain level (65□ Fahrenheit is being discussed);

(c) Closing growing areas when water temperatures exceed a certain level (65° Fahrenheit is being discussed);

(d) Labeling shellfish, "For shucking and cooking only" based on Vibrio vulnificus levels in meats;

(e) Requiring post-harvest treatment based on levels of Vibrio vulnificus in meats at harvest;

(f) Closing growing areas based on Vibrio vulnificus levels in meats at harvest;

(g) Labeling oysters "For shucking and cooking only" during certain months;

(h) Requiring post-harvest treatment during certain months;

(i) Closing certain shellfish growing areas during certain months.

Submitter's note: Final recommendations will be made available following the VMC meeting on June 13 and 14.]

Epidemiological Plan

(1) Core states referenced in §A. above will administer a survey to determine the Vibrio vulnificus disease reporting practices in each state

for the period 1996-1999. The development and implementation plan for the survey will be initiated through the ISSC with participation of state public health officers, epidemiologists and others as determined. Continued surveillance will be necessary to indicate changes to reporting practices during 2000-2007. This is fundamental to establishing the illness baseline as described in §A. (3) above and in tracking future illness report data.

(2) Beginning in calendar year 2001, a new shellfish-borne Vibrio vulnificus disease investigation team will rapidly investigate any case of etiologically confirmed shellfish-borne Vibrio vulnificus septicemia illnesses in core states. This team will gather customary epidemiological information as well as the level of awareness of risk in those who have suffered etiologically confirmed shellfish-borne Vibrio vulnificus septicemia illnesses. The ISSC will assist in initiating this team.

Public Health Significance: This plan is aimed at reducing exposure to *Vibrio vulnificus*, especially in at-risk populations. These controls, by potentially decreasing exposure, can in turn potentially reduce oyster-borne *Vibrio vulnificus* septicemia illnesses.

Cost Information (if available): Unknown

Action by 2000 *Vibrio* Management Committee Recommended adoption of 00-201 as substituted by the Vibrio Management Committee (VMC).

Text of Proposal:

Modify Model Ordinance Chapter II. by adding Section @. 04:

@. 04 *Vibrio vulnificus* Risk Management

(A) For states having 2 or more etiologically confirmed shellfish-borne *Vibrio vulnificus* illnesses since 1995 traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state (Source State), the Authority shall develop and implement *a Vibrio vulnificus* risk management plan.

(B) The plan shall define the administrative procedures and resources necessary to accomplish (i.e. establish and maintain) involvement by the state in a collective illness reduction program. The goal of the program will be to reduce the rate of etiologically confirmed shellfish-borne *Vibrio vulnificus* septicemia illnesses reported in core states (Florida, Texas, California, Louisiana, Georgia, South Carolina, and Alabama) from the consumption of commercially harvested raw or undercooked oysters by 40 percent, collectively, by the end of 2005 and by 60 percent, collectively, by the end of 2007. The rate of illness shall be calculated as the number of illnesses adjusted for population and rate of reporting divided by the production of oysters from the states bordering the Gulf of Mexico, based on National Marine Fisheries Service landing data verified by Silver Spring, Maryland, headquarters. The goal may be reevaluated prior to the year 2005 and adjusted in the event that new science, data or information becomes available.

(C) The plan shall also include identification and preparation for implementation of one or more of the following controls, or equivalent controls, which shall be implemented should the 60 percent illness reduction goal not be achieved by 2007. This portion of the plan shall be completed no later than December 2006. The temperature and month-of the-year parameters identified in the following controls may be adjusted as needed to achieve the established illness reduction goal.

(1) Labeling all oysters, "For shucking by a certified dealer," when the Average Monthly Maximum Water Temperature exceeds 75°F;

(2) Subjecting all oysters to an Authority-approved post-harvest treatment that reduces the *Vibrio vulnificus* levels to 3MPN/g or less," when the Average Monthly Maximum Water Temperature exceeds 75°F;

(3) Closing shellfish growing areas when the Average Monthly Maximum Water Temperature exceeds 75°F;

(4) Labeling all oysters, "For shucking by a certified dealer," during the months of May through September, inclusive;

(5) Subjecting all oysters to a post-harvest treatment that is both approved by the Authority and reduces the *Vibrio vulnificus* levels to 3MPN/g or less during the months of May through September, inclusive;

(6) Closing shellfish growing areas during the months of May through September, inclusive.

Modify the NSSP Guide for Control of Molluscan Shellfish by adding the following Guidance Document (numbering to be determined at time of publication of the next revision).

***Vibrio vulnificus* Management Guidance Document**

***Vibrio vulnificus* Management**

The voting delegates at the 1999 Annual Meeting in New Orleans created the Vibrio Management Committee (VMC). At the 2000 annual meeting the voting delegates will be asked to adopt the VMC's recommendation of reducing the rate of etiologically confirmed shellfish-borne *Vibrio vulnificus* septicemia. The goal is to reduce those illnesses reported in core states (Florida, Texas, California, Louisiana, Georgia, South Carolina, and Alabama) from the consumption of commercially harvested raw or undercooked oysters by 40 percent by the end of 2005 and by 60 percent by the end of 2007. The Core States are the states that have consistently reported *Vv* cases since 1995. The rate of illness shall be calculated as the number of illnesses adjusted for population and rate of reporting divided by the production of oysters from the states bordering the Gulf of Mexico, based on National Marine Fisheries Service landing data verified by Silver Spring, Maryland, headquarters. This adjustment will be performed in consultation with statisticians and epidemiologists from core states and federal agencies. The baseline data and all future data for measuring illness reduction shall be the reported illnesses in the core states for the period 1996 to 1999, inclusive, as compiled by the Southeast Regional Office of the U.S. Food and Drug Administration. The data used for measuring goal attainment shall begin with 2001 data. The formula for calculating for the rate of illness is as follows:

(number of cases) x (CDC adjustment factor)
population

production

The VMC members will include, at a minimum, industry and state shellfish control authority representatives from *Vibrio vulnificus* Illness Source and Core States, FDA, NOAA, EPA, CDC, state epidemiologists; as well as industry and shellfish control representatives from other regions. *Vibrio vulnificus* Illness Source States are those states reporting 2 or more etiologically confirmed shellfish-borne *Vibrio vulnificus* illnesses since 1995 traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state. Core states are Florida, Texas, California, Louisiana, Georgia, South Carolina and Alabama. Etiologically confirmed means those cases in which laboratory evidence of a specific agent is obtained and specified criteria are met.

The VMC will meet at least annually to develop and approve work plans and review progress. The first plan will be in place for a one-year period, followed by three biennial plans. The first work plan and progress review period will be from January 2001 to December 31, 2001. The next work plan period will be from January 1, 2002 to December 31, 2003, January 1, 2004 to December 31, 2005; then January 1, 2006 to December 31, 2007.

Work plans will include goals, tasks, performance measures and assessment methods to track and achieve progress towards the illness reduction goals. The work plans will be developed by the VMC and approved by the VMC membership. The chair of the VMC will deliver a written annual progress report, including a summary of the previous year's progress made in the education program, to the ISSC March executive board meeting. The report shall be made available to the general membership. The biennial work plan structure, outlined below, provides adaptive management and assures consistent progress towards the illness reduction goals.

Work plans developed by the VMC shall include the following elements and shall define the administrative procedures and resources necessary for accomplishment (i.e. establishment and maintenance):

(a) An ISSC Consumer Education Program targeted toward individuals who consume raw oysters and whose health condition(s) increase their risk for *Vibrio vulnificus* infection. The Education Program's objectives will be 1) to increase the target audience's awareness that eating raw oysters can be life-threatening to them, and; 2) to change the at-risk group's oyster-eating behavior, i.e., to reduce or stop eating raw oysters. The ISSC Education Committee and the *Vibrio vulnificus* Education Subcommittee will assist in the development and oversight for this program.

(i) The Consumer Education Program will focus educational efforts in the Core States. The Education Program will make educational materials available to states upon request.
(ii) Educational approaches will emphasize partnerships with health and advocacy organizations, and include dissemination of printed materials, posting materials on the Internet, broadcast of television spots, press releases, and other measures

deemed effective such as the USDA Physician Notification Program.

(iii) Periodic administration of Behavior Risk Factor State Surveys (BRFSS) and other survey assessments at the state level shall be explored as a means of assessing the effectiveness of educational interventions.

(b) Administration of a survey to determine the current *Vibrio vulnificus* disease reporting and education in each state;

(c) Creation of a shellfish-borne *Vibrio vulnificus* disease investigation team that will be available to assist in collection of epidemiological information associated with confirmed shellfish-borne *Vibrio vulnificus* septicemia illness. This team will assist in gathering customary epidemiological information as well as the level of awareness of risk in those who have suffered etiologically confirmed shellfish-borne *Vibrio vulnificus* septicemia illnesses. A small ISSC team with recognized epidemiological officers will assist in rapid investigation of any case. This team will work cooperatively with existing local, state and federal disease investigation programs.

(d) Industry-implemented post-harvest controls to reduce *Vibrio vulnificus* levels in oyster shellstock which may include: time-temperature, post harvest treatment (i.e. hydrostatic pressure, cool pasteurization, IQF, and irradiation--pending approval), rapid chilling and other emerging technologies.

(e) To encourage implementation of post harvest controls the Conference will pursue options such as SBA low interest loans; revolving loans; cost sharing; demonstration projects; state-industry partnerships; FDA label incentives; PHT specific growing area classifications; targeted time/temperature assessment by FDA during annual shellfish program evaluations; assistance, as necessary, for the further study and possible implementation of dockside icing to investigate its effects on shelf life and variations in the effectiveness of the method as a result of seasonal and regional differences and incentives to add refrigeration capacity to harvest vessels. The goal will be to provide incentives necessary to post-harvest treat 20 percent of all oysters intended for the raw, half-shell market during the months of May through September harvested from a source state by the end of the third year (December 31, 2003). The assessment will include the capacity of all operational plants and the capacity of plants under construction. Should the 20 percent goal not be accomplished, the VMC will pursue additional incentives to achieve the goals.

(f) A VMC compilation and review of the data on rates of illness will be made available to the ISSC at the ISSC Biennial meeting following the year in which the data was gathered. In the event that the data is not available at the time of the meeting, the VMC shall meet and review the data when it becomes available and issue a compilation report, which will be made available to the entire ISSC

membership. In the event there is no Biennial meeting scheduled for a certain year, the VMC shall meet and review the data when it becomes available and issue a compilation report which will be made available to the entire conference.

(g) A VMC evaluation of the effectiveness of reduction efforts will be conducted at the end of the fifth year (December 31, 2005). The evaluation will determine whether the 40 percent, 5-year illness reduction goal or education/consumer intervention or post harvest controls performance measures set forth in prior work plans have been achieved. Should the VMC evaluation indicate the 40 percent, 5 year goal has not been accomplished; the committee will identify additional harvest controls in the 2006 - 2007 work plan to assure achievement of the 60 percent illness reduction goal by the close of the seventh year. In addition, the VMC will evaluate the requirements in Section 04.C. with the possibility of changing the controls to achieve remaining illness reduction goals.

PUBLIC HEALTH SIGNIFICANCE: The purpose of the National Shellfish Sanitation Program is to promote and improve the sanitation of shellfish (oysters, clams, mussels and scallops) moving in interstate commerce through federal/state cooperation and uniformity of State Shellfish Programs. This includes protection of the public health by reducing the prevalence of food borne hazards. Complete elimination of illness is difficult to attain but public health programs should be designed to provide the greatest level of public health protection possible. The vision of public health officials must focus on maximizing protection with the most practical public health measures available. This plan is designed to assure a significant reduction in *Vibrio vulnificus* septicemia illnesses through a combination of consumer education, processing incentives and, if necessary, mandatory harvesting or processing controls.

COST INFORMATION: Unknown.

In addition the Committee recommended:

(1) Issue 00-201 become effective October 1, 2000; and the requirement for the *Vibrio vulnificus* Management Plans specified in Section .04A. be developed by these states by April 1, 2001;
(2) Establish a new VMC technical subcommittee that would come up with a list of research and market-related questions and needs relative to the design of a PHT incentive program; and
(3) Ensure that the VMC establishes and performs all necessary evaluations of goals, tasks, performance measures, assessment measures and data collection elements contained in the new Model Ordinance Section @. 04 *Vibrio vulnificus* Risk Management, and in the *Vibrio vulnificus* Management Guidance Document.

Action by 2000 Task Force II

Recommended adoption of Issue 00-201 as substituted by the Vibrio Management Committee (VMC) and further amended as follows:

TEXT OF PROPOSAL:

Modify Model Ordinance Chapter II. By adding Section @. 04:

@. 04 *Vibrio vulnificus* Risk Management **for Oysters**.

(A) For states having 2 or more etiologically confirmed shellfish-borne *Vibrio vulnificus* illnesses since 1995 traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state (Source State), the Authority shall develop and implement *a Vibrio vulnificus* risk management plan.

(B) The plan shall define the administrative procedures and resources necessary to accomplish (i.e. establish and maintain) involvement by the state in a collective illness reduction program. **The Plan shall include, at a minimum, the ISSC Consumer Education Program targeted toward individuals who consume raw oysters and whose health condition(s) increase their risk for *Vibrio vulnificus* illnesses.** The goal of the Vibrio Risk Management Plan will be to reduce the rate of etiologically confirmed shellfish-borne *Vibrio vulnificus* septicemia illnesses, reported in core states, ~~which may include (Florida, Texas, California, Louisiana, Georgia, South Carolina, and Alabama) to be determined by the VMC after a thorough review of statistical and epidemiological information~~ from the consumption of commercially harvested raw or undercooked oysters by 40 percent, collectively, by the end of 2005 and by 60 percent, collectively, by the end of 2007. **The core states include Florida, Texas, California, Louisiana, Georgia, South Carolina, and Alabama.** T**he list of core states may be adjusted if after a thorough review, epidemiological and statistical data demonstrates that it would be appropriate.** The rate of illness shall be calculated as the number of illnesses adjusted for population and rate of reporting divided by the production of oysters from the states bordering the Gulf of Mexico, based on National Marine Fisheries Service landing data verified by Silver Spring, Maryland, headquarters. The goal may be reevaluated prior to the year 2005 and adjusted in the event that new science, data or information becomes available.

(C) The plan shall also include identification and preparation for implementation of one or more of the following controls, or equivalent controls, which shall be implemented should the 60 percent ~~illness~~ **rate of illness** reduction goal not be achieved by 2007. This portion of the plan shall be completed no later than December 2006. The temperature and month-of the-year parameters identified in the following controls may be adjusted as needed to achieve the established illness reduction goal.

(1) Labeling all oysters, "For shucking by a certified dealer," when the Average Monthly Maximum Water Temperature exceeds 75°F;

(2) Subjecting all oysters **intended for the raw, half-shell market** to an Authority-approved post-harvest treatment that reduces the *Vibrio vulnificus* levels to 3MPN/g or less," when the Average Monthly Maximum Water Temperature exceeds 75°F;

(3) Closing shellfish growing areas **for the purpose of harvest of oysters intended for the raw, half-shell market** when the Average Monthly Maximum Water Temperature exceeds 75°F;

(4) Labeling all oysters, "For shucking by a certified dealer," during the months of May through September, inclusive;

(5) Subjecting all oysters **intended for the raw, half-shell market** to a post-harvest treatment that is both approved by the Authority and reduces the *Vibrio vulnificus* levels to 3MPN/g or less during the months of May through September, inclusive;

(6) Closing shellfish growing areas **for the purpose of harvesting oysters intended for the raw, half-shell market** during the months of May through September, inclusive.

Modify the NSSP Guide for the Control of Molluscan Shellfish by adding the following Guidance Document (numbering to be determined at time of publication of the next revision.)

***Vibrio vulnificus* Management Guidance Document**

***Vibrio vulnificus* Management**

The voting delegates at the 1999 Annual Meeting in New Orleans created the Vibrio Management Committee (VMC). At the 2000 annual meeting the voting delegates will be asked to adopt the VMC's recommendation of reducing the rate of etiologically confirmed shellfish-borne *Vibrio vulnificus* septicemia. The goal is to reduce ~~those~~ **the rate of** illness reported in core states ~~from~~ **due to** the consumption of commercially harvested raw or undercooked oysters by 40 percent by the end of 2005 and by 60 percent by the end of 2007. The Core States are the states that have consistently reported *Vibrio vulnificus* cases since 1995. **The list of core states may be adjusted if after a thorough review, epidemiological and statistical data demonstrates that it would be appropriate.** The rate of illness shall be calculated as the number of illnesses adjusted for population and rate of reporting divided by the production of oysters from the states bordering the Gulf of Mexico, based on National Marine Fisheries Service landing data verified by Silver Spring, Maryland, headquarters. This adjustment will be performed in consultation with statisticians and epidemiologists from core states and federal agencies. The baseline data and all future data for measuring illness reduction shall be the reported illnesses in the core states for the period 1996 to 1999, inclusive, as compiled by the Southeast Regional Office of the U.S. Food and Drug Administration. The data used for measuring goal attainment shall begin with 2001 data. The formula for calculating the rate of illness is as follows:

$$\frac{\text{(number of cases) x (}\sout{\text{CDC}}\text{ illness reporting adjustment factor)}}{\text{population}}$$

$$\frac{}{\text{production}}$$

The VMC members will include, at a minimum, **balanced representation from** industry and state shellfish control **authorities** from *Vibrio vulnificus* Illness Source and Core States, FDA, NOAA, EPA, CDC, state epidemiologists; as well as industry and shellfish control representatives from other regions. *Vibrio vulnificus* Illness Source States are those states reporting 2 or more etiologically confirmed shellfish-borne *Vibrio vulnificus* illnesses since 1995 traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state. Core states are Florida, Texas, California, Louisiana, Georgia, South Carolina and Alabama **or those states determined to be appropriate after a thorough review of epidemiological and statistical data.** Etiologically confirmed means those cases in which laboratory evidence of a specific agent is obtained and specified criteria are met.

Recognizing the increasing importance and roles for the VMC, the Committee leadership will be expanded and structured in a similar manner as stated in the ISSC By-Laws for Task Forces (reference: ISSC By-Law, Article I Task Forces). The VMC Chair shall alternately be selected from a state shellfish control authority and from industry. The Board Chairman, with approval of the Board, shall appoint a VMC

Chair and Vice-Chair. If the VMC Chair represents a state shellfish control authority, the Vice-Chair shall be an industry representative. At the end of the VMC Chair's term of office, the Vice Chair will become Chairman and a new Vice Chair will be appointed who represents the same segment of the Conference as the outgoing VMC Chair. A VMC Chair and Vice Chair should be appointed before October 1, 2000 in order to be consistent with plans for annual VMC meetings and with the effective date of *Vibrio vulnificus* Risk Management Plans. Likewise, the term of office should be for (2) years.

The VMC will meet at least annually to develop and approve work plans and review progress. The first plan will be in place for a one-year period, followed by three biennial plans. The first work plan and progress review period will be from January 2001 to December 31, 2001. The next work plan period will be from January 1, 2002 to December 31, 2003, January 1, 2004 to December 31, 2005**;** then January 1, 2006 to December 31, 2007.

Work plans will include goals, tasks, performance measures and assessment methods to track and achieve progress towards the illness reduction goals. The work plans will be developed by the VMC and approved by the VMC membership. The chair of the VMC will deliver **a written annual progress report, including a summary of the previous year's progress made in the education program, to the ISSC March executive board meeting. The report shall be made available to the general membership. The biennial work plan structure, outlined below,** provides adaptive management and assures consistent progress towards the illness reduction goals.

Work plans developed by the VMC shall include the following elements and shall define the administrative procedures and resources necessary for accomplishment (i.e. establishment and maintenance):

(a) An ISSC Consumer Education Program targeted toward individuals who consume raw oysters and whose health condition(s) increase their risk for *Vibrio vulnificus* infection. The Education Program's objectives will be 1) to increase the target audience's awareness that eating raw, untreated oysters can be life-threatening to them, and; 2) to change the at-risk group's oyster-eating behavior, i.e., to reduce or stop eating raw, untreated oysters. The ISSC Education Committee and the *Vibrio vulnificus* Education Subcommittee will assist in the development and oversight for this program.

(i) The Consumer Education Program will focus educational efforts in the Core States. The Education Program will make educational materials available to states upon request.
(ii) Educational approaches will emphasize partnerships with health and advocacy organizations, and include dissemination of printed materials, posting materials on the Internet, broadcast of television spots, press releases, and other measures deemed effective such as the USDA Physician Notification Program.
(iii) Periodic administration of Behavior Risk Factor State Surveys (BRFSS) and other survey assessments at the state level shall be explored as a means of assessing the effectiveness of educational interventions.

(b) Administration of a survey to determine the current *Vibrio vulnificus* disease reporting and education in each state.

(c) Creation of a shellfish-borne *Vibrio vulnificus* disease investigation team that will be available to assist in collection of epidemiological information associated with confirmed shellfish-borne *Vibrio vulnificus* septicemia illness. This team will assist in gathering customary epidemiological information as well as the level of awareness of risk in those who have suffered etiologically confirmed shellfish-borne *Vibrio vulnificus* septicemia illnesses. A small ISSC team with recognized epidemiological officers will assist in rapid investigation of any case. This team will work cooperatively with existing local, state and federal disease investigation programs.

(d) Industry-implemented post-harvest controls to reduce *Vibrio vulnificus* levels in oyster shellstock which may include: time-temperature, post harvest treatment (i.e. hydrostatic pressure, cool pasteurization, IQF, and irradiation--pending approval), rapid chilling and other emerging technologies.

(e) To encourage implementation of post harvest controls the Conference will pursue options such as ~~SBA low interest loans; revolving loans; cost sharing; demonstration projects; state-industry partnerships;~~ **market development;** FDA label incentives; PHT specific growing area classifications; targeted time/temperature assessment by FDA during annual shellfish program evaluations; assistance, as necessary, for the further study and possible implementation of dockside icing to investigate its effects on shelf life and variations in the effectiveness of the method as a result of seasonal and regional differences and incentives to add refrigeration capacity to harvest vessels. The goal will be to provide incentives necessary to post-harvest treat 20 percent of all oysters intended for the raw, half-shell market during the months of May through September harvested from a source state by the end of the third year (December 31, 2003). The assessment will include the capacity of all operational plants and the capacity of plants under construction. Should the 20 percent goal not be accomplished, ~~the VMC will pursue additional incentives to achieve the goals.~~ **the VMC will investigate and report their findings as to why the goal was not reached.**

(f) The VMC will develop a list of issues relating to public health, various technologies; including Post-harvest treatments; marketability; shelf -life and similar matters that lend themselves to investigation. The VMC will work with FDA, NOAA, CDC, EPA, the shellfish industry and other entities as appropriate to obtain or facilitate the investigation of the issues listed and take the results into account as it develops plans or recommended Issues for the ISSC.

~~(f)~~**(g)**A VMC compilation and review of the data on rates of illness will be made available to the ISSC at the ISSC Biennial meeting following the year in which the data was gathered. In the event that the data is not available at the time of the meeting, the VMC shall meet and review the

data when it becomes available and issue a compilation report, which will be made available to the entire ISSC membership. In the event there is no Biennial meeting scheduled for a certain year, the VMC shall meet and review the data when it becomes available and issue a compilation report which will be made available to the entire conference.

~~(g)~~**(h)** A VMC evaluation of the effectiveness of reduction efforts will be conducted at the end of the fifth year (December 31, 2005). The evaluation will determine whether the 40 percent, 5-year ~~illness reduction~~ goal to **reduce the rate of illness** or education/consumer intervention or post harvest controls performance measures set forth in prior work plans have been achieved. Should the VMC evaluation indicate the 40 percent, 5 year goal has not been accomplished, the committee will identify additional harvest controls in the 2006 - 2007 work plan to assure achievement of the 60 percent ~~illness~~ reduction **in the rate of illness** goal by the close of the seventh year. In addition, the VMC will evaluate the requirements in Section 04.C. with the possibility of changing the controls to achieve remaining illness reduction goals.

PUBLIC HEALTH SIGNIFICANCE: The purpose of the NSSP is to promote and improve the sanitation of shellfish (oysters, clams, mussels and scallops) moving in interstate commerce through federal/state cooperation and uniformity of State Shellfish Programs. This includes protection of the public health by reducing the prevalence of food borne hazards. Complete elimination of illness is difficult to attain but public health programs should be designed to provide the greatest level of public health protection possible. The vision of public health officials must focus on maximizing protection with the most practical public health measures available. This plan is designed to assure a significant reduction in *Vibrio vulnificus* septicemia illnesses through a combination of consumer education, processing incentives and, if necessary, mandatory harvesting or processing controls.

COST INFORMATION: Unknown.
The Task Force further recommended adoption of the 2000 Vibrio Management Committee recommendations # 1, 2, and 3.

Action by 2000 General Assembly

The 2000 General Assembly referred Issue 00-201 to appropriate committee as determined by the Conference Chairman.

Action by USFDA

Concurred with Conference action.

Action by 2001 *Vibrio vulnificus* Subcommittee

Recommended adoption of Issue 00-201 as amended and presented in the 2001 Issue packet:

TEXT OF PROPOSAL:

Modify Model Ordinance Chapter II. By adding Section @. 04:

@. 04 *Vibrio vulnificus* Risk Management for Oysters.

(A) For states having 2 or more etiologically confirmed shellfish-borne *Vibrio vulnificus* illnesses since 1995 traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state (Source State), the Authority shall develop and implement *a Vibrio*

vulnificus risk management plan.

(B) The Source State's *Vibrio vulnificus* management plan shall define the administrative procedures and resources necessary to accomplish (i.e. establish and maintain) involvement by the state in a collective illness reduction program. The Plan shall include, at a minimum, the ISSC Consumer Education Program targeted toward individuals who consume raw oysters and whose health condition(s) increase their risk for *Vibrio vulnificus* illnesses. The goal of the *Vibrio vulnificus* Risk Management Plan will be to reduce the rate of etiologically confirmed shellfish-borne *Vibrio vulnificus* septicemia illnesses reported collectively by ~~core reporting states, collectively~~ California, Florida, Louisiana, Texas, from the consumption of commercially harvested raw or undercooked oysters by 40 percent, ~~collectively, by the end of~~ for years 2005 and 200~~5~~6 (average) and by 60 percent for years 2007 and ~~collectively, by the end of~~ 200~~7~~8 (average~~) from the current rate of 0.306/million~~ from the average illness rate for the years 1995 - 1999 of 0.306/million~~. The core reporting states include Florida, Texas, California, and Louisiana.~~ The list of ~~core reporting~~ states (California, Florida, Louisiana, Texas) used to calculate rate reduction may be adjusted if after a thorough review, epidemiological and statistical data demonstrates that it would be appropriate. The illness rate shall be calculated as the number of illnesses per unit of population. The goal may be reevaluated prior to the year 200~~5~~6 and adjusted in the event that new science, data or information becomes available.

(C) The Source States' *Vibrio vulnificus* management plan shall also include identification and preparation for implementation of one or more of the following controls, or equivalent controls, which shall be implemented should the 60 percent rate of illness reduction goal not be achieved collectively by 200~~7~~8. The control measures identified in the plan shall be appropriate to the state and reflect that state's contribution to the number of Vv illnesses and the controls that have been implemented by each state. This portion of the plan shall be completed no later than December 200~~6~~7. The temperature and month-of the-year parameters identified in the following controls may be adjusted by the ISSC Executive Board as recommended by the Vibrio Management Committee (VMC) on a state by state basis, as needed to achieve the established illness reduction goal. The adjustment to the State's plan can take into account the illness rate reduction that has occurred since the last review of the plan.

(1) Labeling all oysters, "For shucking by a certified dealer," when the Average Monthly Maximum Water Temperature exceeds 75°F;

(2) Subjecting all oysters intended for the raw, half-shell market to an Authority-approved post-harvest treatment that reduces the *Vibrio vulnificus* levels to 3MPN/g or less," when the Average Monthly Maximum Water Temperature exceeds 75°F;

(3) Closing shellfish growing areas for the purpose of harvest of oysters intended for the raw, half-shell market when the Average Monthly Maximum Water Temperature exceeds 75°F;

(4) Labeling all oysters, "For shucking by a certified dealer," during the months of May through September, inclusive;

(5) Subjecting all oysters intended for the raw, half-shell market to a post-harvest treatment that is both approved by the Authority and reduces the *Vibrio vulnificus* levels to 3MPN/g or less during the months of May through September, inclusive;

(6) Closing shellfish growing areas for the purpose of harvesting oysters intended for the raw, half-shell market during the months of May through September, inclusive.

Modify the NSSP Guide for the Control of Molluscan Shellfish by adding the following Guidance Document (numbering to be determined at time of publication of the next revision.)

***Vibrio vulnificus* Management Guidance Document**

***Vibrio vulnificus* Management**

The voting delegates at the 1999 Annual Meeting in New Orleans created the Vibrio Management Committee (VMC). Subsequently, *Vibrio vulnificus* and *Vibrio parahaemolyticus* subcommittees have been charged to develop appropriate illness control measures for these two pathogens. The VMC provides guidance and oversight to the subcommittees. Subcommittee recommendations are reviewed by the VMC before submittal to Task Forces. At the 2001 annual meeting, Task Forces will review the VMC's recommendation of reducing the rate of etiologically confirmed shellfish-borne *Vibrio vulnificus* septicemia with the intention to submit the recommendation to the voting delegates. The goal is to reduce the rate of illness reported in ~~core reporting states~~ California, Florida, Louisiana and Texas due to the consumption of commercially harvested raw or undercooked oysters by 40 percent by the end of 200~~5~~6 and by 60 percent by the end of 200~~7~~8. ~~The Core Reporting States are Louisiana, California, Florida, and Texas. The list of core reporting.~~ The list of states may be adjusted if after a thorough review, epidemiological and statistical data demonstrates that it would be appropriate. The rate of illness shall be calculated as the number of illnesses adjusted for population. This adjustment will be performed in consultation with statisticians and epidemiologists from ~~core reporting states~~ California, Florida, Louisiana and Texas and Federal agencies. The baseline data and all future data for measuring illness reduction shall be the reported illnesses in the ~~core reporting states~~ California, Florida, Louisiana and Texas for the period 1995 to 1999, inclusive, as compiled by the Southeast Regional Office of the U.S. Food and Drug Administration. The data used for measuring goal attainment shall begin with 200~~1~~2 data. For the purpose of maintaining an accurate count of the number of illnesses report by each state (California, Florida, Louisiana and Texas) ~~Core Reporting State~~, the following will apply:

(a) Illness cases counted are those reported by ~~Core Reporting States~~ California, Florida, Louisiana and Texas;
(b) Each illness case is recorded under the state that reports it;
(c) Each case is not counted more than once; and
(d) In the event more than one report per case is filed, the case is recorded under the state of diagnosis.

The formula for calculating the rate of illness is as follows:

$$\frac{\text{(number of cases)}}{\text{population}}$$

The ~~VMC~~ Vv subcommittee members will include, at a minimum, balanced representation from industry and state shellfish control authorities from *Vibrio vulnificus* Illness Source States ~~and Core Reporting States~~ California, Florida, Louisiana and Texas, FDA, NOAA, EPA, CDC, state epidemiologists; as well as industry and shellfish control representatives from other regions. *Vibrio vulnificus* Illness Source States are those states reporting two (2)

or more etiologically confirmed shellfish-borne *Vibrio vulnificus* illnesses since 1995 traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state. ~~Core reporting states are Florida, Texas, California, and Louisiana, or those states determined to be appropriate after a thorough review of epidemiological and statistical data.~~ Etiologically confirmed means those cases in which laboratory evidence of a specific agent is obtained and specified criteria are met.

Recognizing the increasing importance and roles for the, the Committee leadership will be expanded and structured in a similar manner as stated in the ISSC By-Laws for Task Forces (reference: ISSC By-Law, Article I Task Forces). The VMC Chair shall alternately be selected from a state shellfish control authority and from industry. The Board Chairman, with approval of the Board, shall appoint a VMC Chair and Vice-Chair. If the VMC Chair represents a state shellfish control authority, the Vice-Chair shall be an industry representative. At the end of the VMC Chair's term of office, the Vice Chair will become Chairman and a new Vice Chair will be appointed who represents the same segment of the Conference as the outgoing VMC Chair. A VMC Chair and Vice Chair should be appointed before October 1, 200~~0~~1 in order to be consistent with plans for annual VMC meetings and with the effective date of *Vibrio vulnificus* Risk Management Plans. Likewise, the term of office ~~should~~ shall be for (2) years.

The VMC will meet at least annually to develop and approve annual VMC work plans for *Vibrio vulnificus* illness reduction and review progress. ~~The first plan will be in place for a one-year period, followed by three biennial plans.~~ A series of work plans, each covering a one-year period shall be adopted. The first work plan and progress review period will ~~be from January 2001 to December 31, 2001.~~ cover a seventeen-month period from August 1, 2001 to December 31, 2003 followed subsequently by annual work plans. ~~The next work plan period will be from January 1, 2002 to December 31, 2003, January 1, 2004 to December 31, 2005; then January 1, 2006 to December 31, 2007.~~

Work plans will include goals, tasks, performance measures and assessment methods to track and achieve progress towards the illness reduction goals. The work plans will be developed by the VMC and approved by the VMC membership. The chair of the VMC will deliver a written annual progress report, including a summary of the previous year's progress made in the education program, to the ISSC March executive board meeting. The report shall be made available to the general membership. The ~~biennial~~ annual work plan structure, outlined below, provides adaptive management and assures consistent progress towards the illness reduction goals. If annual assessment of progress towards achieving the illness rate reduction goals show inadequate progress the VMC shall incorporate actions into current and subsequent work plans to assure success in achieving those goals. In addition, if annual review shows inadequate progress the VMC will develop issues for deliberation at the 2005 biennial meeting to consider actions such as:

- increased educational efforts,
- limited harvest restriction,
- reduction in time from harvest to refrigeration,
- phased-in post-harvest treatment requirements, or
- other equivalent controls.

Work plans developed by the VMC shall include the following elements and shall define the administrative procedures and resources necessary for accomplishment (i.e. establishment and maintenance):

(a) An ISSC Consumer Education Program targeted toward individuals who consume raw

oysters and whose health condition(s) increase their risk for *Vibrio vulnificus* infection. The Education Program's objectives will be 1) to increase the target audience's awareness that eating raw, untreated oysters can be life-threatening to them, and; 2) to change the at-risk group's oyster-eating behavior, i.e., to reduce or stop eating raw, untreated oysters. The ISSC Vibrio Management Committee and the *Vibrio vulnificus* Education Subcommittee will ~~assist~~ evaluate Year 2001 survey results ~~will be~~ and compare~~d~~ ~~to~~ them with the Year 2003 or 2004 survey results to ~~demonstrate that determine~~ the effectiveness in meeting the two objectives of the *Vv* education effort: (1) Show 40% increase in awareness of risk from Vv; and (2) Show 15% increase in at-risk consumers no longer eating raw oysters while minimizing impacts to non-at-risk consumer raw oyster consumption. ~~in the development and oversight for this program~~.

(i) The Consumer Education Program will focus educational efforts ~~in the Core Reporting States~~ California, Florida, Louisiana and Texas. The Education Program will make educational materials available to additional states upon request.

(ii) Educational approaches will emphasize partnerships with health and advocacy organizations, and include dissemination of printed materials, posting materials on the Internet, broadcast of television spots, press releases, and other measures deemed effective such as the USDA Physician Notification Program.

(iii) Survey assessments at the state level shall be used as a means of assessing the baseline knowledge and effectiveness of educational interventions.

(b) Administration of a survey to determine the current *Vibrio vulnificus* disease reporting and education in each state;

(c) Creation of a ~~A committee~~ working group ~~will be created~~ to work cooperatively with local, state, and federal agencies and ~~program~~ programs to assist in the collection of environmental and epidemiological data to further expand on the current information available. A coordinator may be utilized to facilitate the activities of this ~~subcommittee~~ working group to develop standardized collection of environmental and epidemiological information from harvest to consumer.

(d) Industry-implemented post-harvest controls to reduce *Vibrio vulnificus* levels in oyster shellstock which may include: time-temperature, post harvest treatment (i.e. hydrostatic pressure, cool pasteurization, IQF, and irradiation--pending approval), rapid chilling and other emerging technologies.

(e) Pursuit of ISSC options ~~To encourage implementation of post harvest controls the Conference will pursue options~~ such as industry education and communication; FDA label incentives; PHT specific growing area classifications; targeted time/temperature assessment by FDA during annual shellfish program evaluations; assistance, as necessary, for the further study and possible implementation of dockside icing to investigate its effects on shelf life and variations in the effectiveness of the method as a result of seasonal and regional differences and incentives to add refrigeration capacity to harvest vessels. The goal will be to provide incentives necessary to post-harvest treat 20 percent of all oysters intended for the raw, half-shell market during the months of May through September harvested from a ~~source state~~ Source State by the end of the third year (December 31, 200~~3~~4. The assessment will include the capacity of all operational plants and the capacity of plants under construction. Should the 20 percent goal not be accomplished, the VMC will investigate and report their findings as to why the goal was not reached.

(f) Development by the VMC of ~~The VMC will develop~~ a list of issues relating to public health, various technologies~~;~~ including Post-harvest treatments; marketability; shelf -life and similar matters that lend themselves to investigation. The VMC will work with FDA, NOAA, CDC, EPA, the shellfish industry and other entities as appropriate to obtain or facilitate the investigation of the issues listed and take the results into account as it develops plans or recommended Issues for the ISSC.

(g) Provision for a ~~A~~ VMC compilation and review of the data on rates of illness which will be made available to the ISSC at the ISSC Biennial meeting following the year in which the data was gathered. In the event that the data is not available at the time of the meeting, the VMC shall meet and review the data when it becomes available and issue a compilation report, which will be made available to the entire ISSC membership. In the event there is no Biennial meeting scheduled for a certain year, the VMC shall meet and review the data when it becomes available and issue a compilation report which will be made available to the entire ~~conference~~ membership.

(h) Provision for a ~~A~~ VMC evaluation of the effectiveness of reduction efforts which will be conducted at the end of the fifth year (December 31, 200~~5~~6). The evaluation will determine whether the 40 percent, 5-year goal to reduce the rate of illness or education/consumer intervention or post harvest controls performance measures set forth in prior work plans have been achieved. Should the VMC evaluation indicate the 40 percent, 5 year goal has not been accomplished, the committee will identify additional harvest controls in the 200~~6~~7 - 200~~7~~8 work plan to assure achievement of the 60 percent reduction in the rate of illness goal by the close of the seventh year. In addition, the VMC will evaluate the requirements in Section 04.C. with the possibility of changing the controls to achieve remaining illness reduction goals.

(i) Should a disagreement arise between FDA and the Authority on the equivalency of a control as described in .04c, the Vv Subcommittee will be requested to provide guidance.

PUBLIC HEALTH SIGNIFICANCE: The purpose of the National Shellfish Sanitation Program is to promote and improve the sanitation of shellfish (oysters, clams, mussels and scallops) moving in interstate commerce through federal/state cooperation and uniformity of State Shellfish Programs. This includes protection of the public health by reducing the prevalence of food borne hazards. Complete elimination of illness is difficult to attain but public health programs should be designed to provide the greatest level of public health protection possible. The vision of public health officials must focus on maximizing protection with the most practical public health measures available. This plan is designed to assure a significant reduction in *Vibrio vulnificus* septicemia illnesses through a combination of consumer education, processing incentives and, if necessary, mandatory harvesting or processing controls.

COST INFORMATION: Unknown.

Action by 2001 *Vibrio vulnificus* Subcommittee

Recommended the following changes to Issue 00-201 at the July 22, 2001 subcommittee meeting:

TEXT OF PROPOSAL:

Modify Model Ordinance Chapter II. By adding Section @. 04:

@. 04 *Vibrio vulnificus* Risk Management for Oysters.

(A) For states having 2 or more etiologically confirmed shellfish-borne *Vibrio vulnificus* illnesses since 1995 traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state (Source State), the Authority shall develop and implement *a Vibrio vulnificus* management plan.

(B) The Source State's *Vibrio vulnificus* management plan shall define the administrative procedures and resources necessary to accomplish (i.e. establish and maintain) involvement by the state in a collective illness reduction program. ~~The Plan shall include, at a minimum, the ISSC Consumer Education Program targeted toward individuals who consume raw oysters and whose health condition(s) increase their risk for *Vibrio vulnificus* illnesses.~~ The goal of the *Vibrio* vulnificus Management Plan will be to reduce the rate of etiologically confirmed shellfish-borne *Vibrio vulnificus* septicemia illnesses reported collectively by California, Florida, Louisiana, Texas, from the consumption of commercially harvested raw or undercooked oysters by 40 percent, for years 2005 and 2006 (average) and by 60 percent for years 2007 and 2008 (average) from the average illness rate for the years 1995 - 1999 of 0.306/million. The list of states (California, Florida, Louisiana, Texas) used to calculate rate reduction may be adjusted if after a thorough review, epidemiological and statistical data demonstrates that it would be appropriate. The illness rate shall be calculated as the number of illnesses per unit of population. The goal may be reevaluated prior to the year 2006 and adjusted in the event that new science, data or information becomes available.

(C) The Source States' *Vibrio vulnificus* management plan **shall include, at a minimum:**

(1) The ISSC Consumer Education Program targeted toward individuals who consume raw oysters and whose health condition(s) increase their risk for *Vibrio vulnificus* illnesses;

(2) A process to collected standardized information for each *Vibrio vulnificus* illness: including underlying medical conditions; knowledge of disease status; prior counseling on avoidance of high risk foods, including raw oysters; existence of consumer advisories at point of purchase or consumption; and, if possible, whether consumer was aware and understood the advisories;

(3) A standardized process for tracking products implicated in *Vibrio vulnificus* illnesses;

(4) Identification and preparation for achieving a goal of post-harvest treatment capacity of 25 percent of all oysters intended for the raw, half-shell market during the months of May through September harvested from a Source State by the end of the third year (December 31, 2004). The percentage of post harvest treatment will include the capacity of all operational plants and the capacity of plants under construction;

(5) Identification and preparation for implementation of required post harvest treatment capacity of 50% of all oysters intended for the raw, half-shell market during the months of May through September, harvested from a Source State, which shall be implemented should the 40 percent illness reduction goal not be achieved by December 31, 2006. The percentage of post harvest treatment will include the capacity of all operational plants and the capacity of plants under construction. In the alternative, the state may utilize the control measures, or equivalent control measures, listed in .04, (C), (6) (a), (b), (c), and (d) below for such periods of time which, in combination with post harvest treatment, will provide

equivalent outcomes. This portion of the plan shall be completed no later than December 31, 2005; and

(6) Identification and preparation for implementation of one or more of the following controls, or equivalent controls, which shall be implemented should the 60 percent rate of illness reduction goal not be achieved collectively by 2008. The control measures identified in the plan shall be appropriate to the state and reflect that state's contribution to the number of Vv illnesses and the controls that have been implemented by each state. This portion of the plan shall be completed no later than December 2007. The temperature and month-of the-year parameters identified in the following controls may be adjusted by the ISSC Executive Board as recommended by the Vibrio Management Committee (VMC) on a state by state basis, as needed to achieve the established illness reduction goal. The adjustment to the State's plan can take into account the illness rate reduction that has occurred since the last review of the plan.

(a) Labeling all oysters, "For shucking by a certified dealer," when the Average Monthly Maximum Water Temperature exceeds 75°F;

(b) Subjecting all oysters intended for the raw, half-shell market to an Authority-approved post-harvest treatment that reduces the *Vibrio vulnificus* levels to 3MPN/g or less," when the Average Monthly Maximum Water Temperature exceeds 75°F;

(c) Closing shellfish growing areas for the purpose of harvest of oysters intended for the raw, half-shell market when the Average Monthly Maximum Water Temperature exceeds 75°F;

(d) Labeling all oysters, "For shucking by a certified dealer," during the months of May through September, inclusive;

(e) Subjecting all oysters intended for the raw, half-shell market to a post-harvest treatment that is both approved by the Authority and reduces the *Vibrio vulnificus* levels to 3MPN/g or less during the months of May through September, inclusive;

(f) Closing shellfish growing areas for the purpose of harvesting oysters intended for the raw, half-shell market during the months of May through September, inclusive.

Modify the NSSP Guide for the Control of Molluscan Shellfish by adding the following Guidance Document (numbering to be determined at time of publication of the next revision.)

***Vibrio vulnificus* Management Guidance Document**

***Vibrio vulnificus* Management**

The voting delegates at the 1999 Annual Meeting in New Orleans created the Vibrio Management Committee (VMC). Subsequently, *Vibrio vulnificus* and *Vibrio parahaemolyticus* subcommittees have been charged to develop appropriate illness control measures for these two pathogens. The VMC provides guidance and oversight to the subcommittees. Subcommittee recommendations are reviewed by the VMC before submittal to Task Forces. At the 2001 annual meeting, Task Forces will review the VMC's recommendation of reducing the rate of etiologically confirmed shellfish-borne *Vibrio vulnificus* septicemia with the intention to submit the recommendation to the voting delegates. The goal is to reduce the rate of illness reported in California, Florida, Louisiana and Texas due to the consumption of commercially harvested raw or undercooked oysters ~~by 40 percent by the end of 2006 and by 60 percent by the end of 2008.~~ **by 40 percent, for**

years 2005 and 2006 (average) and by 60 percent for years 2007 and 2008 (average) from the average illness rate for the years 1995 - 1999 of 0.306/million. The list of states may be adjusted if after a thorough review, epidemiological and statistical data demonstrates that it would be appropriate. The rate of illness shall be calculated as the number of illnesses adjusted for population. This adjustment will be performed in consultation with statisticians and epidemiologists from California, Florida, Louisiana and Texas and Federal agencies. The baseline data and all future data for measuring illness reduction shall be the reported illnesses in the California, Florida, Louisiana and Texas for the period 1995 to 1999, inclusive, as compiled by the Southeast Regional Office of the U.S. Food and Drug Administration. The data used for measuring goal attainment shall begin with 2002 data. For the purpose of maintaining an accurate count of the number of illnesses report by each state (California, Florida, Louisiana and Texas), the following will apply:

(a) Illness cases counted are those reported by California, Florida, Louisiana and Texas;
(b) Each illness case is recorded under the state that reports it;
(c) Each case is not counted more than once; and
(d) In the event more than one report per case is filed, the case is recorded under the state of diagnosis.

The formula for calculating the rate of illness is as follows:

$$\frac{\text{number of cases}}{\text{population}}$$

The V.v. subcommittee members will include, at a minimum, balanced representation from industry and state shellfish control authorities from *Vibrio vulnificus* Illness Source States California, Florida, Louisiana and Texas, FDA, NOAA, EPA, CDC, state epidemiologists; as well as industry and shellfish control representatives from other regions. *Vibrio vulnificus* Illness Source States are those states reporting two (2) or more etiologically confirmed shellfish-borne *Vibrio vulnificus* illnesses since 1995 traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state. Etiologically confirmed means those cases in which laboratory evidence of a specific agent is obtained and specified criteria are met.

Recognizing the increasing importance and roles for the, the Committee leadership will be expanded and structured in a similar manner as stated in the ISSC By-Laws for Task Forces (reference: ISSC By-Law, Article I Task Forces). The VMC Chair shall alternately be selected from a state shellfish control authority and from industry. The Board Chairman, with approval of the Board, shall appoint a VMC Chair and Vice-Chair. If the VMC Chair represents a state shellfish control authority, the Vice-Chair shall be an industry representative. At the end of the VMC Chair's term of office, the Vice Chair will become Chairman and a new Vice Chair will be appointed who represents the same segment of the Conference as the outgoing VMC Chair. A VMC Chair and Vice Chair should be appointed before October 1, 2001 in order to be consistent with plans for annual VMC meetings and with the effective date of *Vibrio vulnificus* Risk Management Plans. Likewise, the term of office shall be for (2) years.

The VMC will meet at least annually to develop and approve annual VMC work plans for *Vibrio vulnificus* illness reduction and review progress. A series of work plans, each covering a one-year period shall be adopted. The first work plan and progress review period will cover a seventeen-month period from August 1, 2001 to December 31, 2003 followed subsequently by annual work plans. Work plans will include goals, tasks, performance measures and assessment methods to track and achieve progress towards the illness

reduction goals. The work plans will be developed by the VMC and approved by the VMC membership. The chair of the VMC will deliver a written annual progress report, including a summary of the previous year's progress made in the education program, to the ISSC March executive board meeting. The report shall be made available to the general membership. The annual work plan structure, outlined below, provides adaptive management and assures consistent progress towards the illness reduction goals. If annual assessment of progress towards achieving the illness rate reduction goals show inadequate progress the VMC shall incorporate actions into current and subsequent work plans to assure success in achieving those goals. In addition, if annual review shows inadequate progress the VMC will develop issues for deliberation at the 2005 biennial meeting to consider actions such as:

- increased educational efforts,
- limited harvest restriction,
- reduction in time from harvest to refrigeration,
- phased-in post-harvest treatment requirements, or
- other equivalent controls.

Work plans developed by the VMC shall include the following elements and shall define the administrative procedures and resources necessary for accomplishment (i.e. establishment and maintenance):

(a) An ISSC Consumer Education Program targeted toward individuals who consume raw oysters and whose health condition(s) increase their risk for *Vibrio vulnificus* infection. The Education Program's objectives will be 1) to increase the target audience's awareness that eating raw, untreated oysters can be life-threatening to them, and; 2) to change the at-risk group's oyster-eating behavior, i.e., to reduce or stop eating raw, untreated oysters. The ISSC Vibrio Management Committee and the *Vibrio vulnificus* Education Subcommittee will evaluate Year 2001 survey results and compare them with the Year 2003 or 2004 survey results determine the effectiveness in meeting the two objectives of the *Vv* education effort: (1) Show 40% increase in awareness of risk from Vv; and (2) Show 15% increase in at-risk consumers no longer eating raw oysters while minimizing impacts to non-at-risk consumer raw oyster consumption.

(i) The Consumer Education Program will focus educational efforts **in** California, Florida, Louisiana and Texas. The Education Program will make educational materials available to additional states upon request.

(ii) Educational approaches will emphasize partnerships with health and advocacy organizations, and include dissemination of printed materials, posting materials on the Internet, broadcast of television spots, press releases, and other measures deemed effective such as the USDA Physician Notification Program.

(iii) Survey assessments at the state level shall be used as a means of assessing the baseline knowledge and effectiveness of educational interventions.

(b) Administration of a survey to determine the current *Vibrio vulnificus* disease reporting and education in each state.

(c) Creation of a working group to work cooperatively with local, state, and federal agencies and programs to assist in the collection of environmental and epidemiological data to further expand on the current information available. A coordinator may be utilized to facilitate the activities of this

working group to develop standardized collection of environmental and epidemiological information from harvest to consumer.

(d) Industry-implemented post-harvest controls to reduce *Vibrio vulnificus* levels in oyster shellstock which may include: time-temperature, post harvest treatment (i.e. hydrostatic pressure, cool pasteurization, IQF, and irradiation--pending approval), rapid chilling and other emerging technologies.

(e) Pursuit of ISSC options such as industry education and communication; FDA label incentives; PHT specific growing area classifications; targeted time/temperature assessment by FDA during annual shellfish program evaluations; assistance, as necessary, for the further study and possible implementation of dockside icing to investigate its effects on shelf life and variations in the effectiveness of the method as a result of seasonal and regional differences and incentives to add refrigeration capacity to harvest vessels. The goal will be to provide incentives necessary to post-harvest treat ~~20~~ **25** percent of all oysters intended for the raw, half-shell market during the months of May through September harvested from a Source State by the end of the third year (December 31, 2004). The assessment will include the capacity of all operational plants and the capacity of plants under construction. Should the ~~20~~ **25** percent goal not be accomplished, the VMC will investigate and report their findings as to why the goal was not reached.

(f) Development by the VMC of a list of issues relating to public health, various technologies~~,~~ including Post-harvest treatments; marketability; shelf -life and similar matters that lend themselves to investigation. The VMC will work with FDA, NOAA, CDC, EPA, the shellfish industry and other entities as appropriate to obtain or facilitate the investigation of the issues listed and take the results into account as it develops plans or recommended Issues for the ISSC.

(g) Provision for a VMC compilation and review of the data on rates of illness, which will be made available to the ISSC at the ISSC Biennial meeting following the year in which the data was gathered. In the event that the data is not available at the time of the meeting, the VMC shall meet and review the data when it becomes available and issue a compilation report, which will be made available to the entire ISSC membership. In the event there is no Biennial meeting scheduled for a certain year, the VMC shall meet and review the data when it becomes available and issue a compilation report which will be made available to the entire membership.

Provision for a VMC evaluation of the effectiveness of reduction efforts, which will be conducted at the end of the fifth year (December 31, 2006). The evaluation will determine whether the 40 percent, 5-year goal to reduce the rate of illness or education/consumer intervention or post harvest controls performance measures set forth in prior work plans have been achieved. Should the VMC evaluation indicate the 40 percent, 5 year goal has not been accomplished; the committee will identify additional harvest controls in the 2007 - 2008 work plan to assure achievement of the 60 percent reduction in the rate of illness goal by the close of the seventh year. In addition, the VMC will evaluate the requirements in Section 04.C. with the possibility of changing the controls to achieve remaining illness

reduction goals.

Should a disagreement arise between FDA and the Authority on the equivalency of a control as described in .04~~e~~**(C)**, the V.v. Subcommittee will be requested to provide guidance.

The *Vibrio vulnificus* Subcommittee further recommended the following:

1) **Request the Executive Board request FDA to meet with the Irradiation petition submitter to establish a timetable under which FDA will review the petition.**

2) **Request the Executive Board request FDA and the state of California seek additional funding to increase the education of at-risk consumers in California, particularly in southern California,**

3) **Recommended that the Chairman appoint a committee to develop further guidance language for implementation of .04 (C) (1)-(5).**

4) **Recommended adoption of an effective date of October 1, 2001, and further recommended an expedited review by FDA.**

Action by 2001 *Vibrio* Management Committee

Recommended adoption of the *V. vulnificus* Subcommittee Report recommendations.

Action by 2001 Task Force II

Recommended adoption of 2001 Vibrio Management Committee Report recommendations.

The Task Force further recommended the Executive Board Chairman appoint an appropriate committee which shall develop a threshold for adoption of *Vibrio vulnificus* management plans (.04)(A), and for development of an exit strategy for source states.

Action by 2001 General Assembly

Adopted recommendation of 2001 Task Force II.

Action by USFDA

Concurred with Conference action.

This issue was referred back to the ISSC V*ibrio vulnificus* Subcommittee following its marginal defeat at the 2000 ISSC. While FDA was disappointed that the 2000 Conference voted to refer Issue 00-201 back to committee, we believe the dedicated efforts of the *Vibrio vulnificus* Subcommittee over the ensuing year resulted in ISSC adoption of a stronger and more workable plan to reduce *Vibrio vulnificus* illnesses associated with raw shellfish consumption. Issue 00-201 was designed to reduce *Vibrio vulnificus* septicemia illnesses through post harvest treatment (PHT) processing, consumer education, and, if necessary, mandatory harvesting and/or processing controls. FDA looks forward to working with states as they develop and implement *Vibrio vulnificus* management plans. We also look forward to our continued participation on the ISSC *Vibrio* Management Committee (VMC*), Vibrio vulnificus* Subcommittee, and *Vibrio vulnificus* Education Subcommittee to implement measures (including data collection, data analysis, and development of annual work plans by the VMC) set forth in the "*Vibrio vulnificus* Management Guidance Document" which was adopted as part of Issue 00-201.

During review of Issue 00-201, FDA noted that adopted in the third sentence of Chapter II. @. 04(C)(5) did not include alternatives (e) and (f) of 04(C)(6) should the 40% illness reduction goal not be achieved. It is our understanding that alternatives (e) and (f), which appear to have been inadvertently omitted, will be considered at the January meeting of the ISSC Executive Board for inclusion as alternatives in 04(C)(5).

Action by 2003 *Vibrio vulnificus* Subcommittee Recommended that the baseline illness reduction rate of 1995 – 99 of 0.306 per million be modified in Chapter II @ 04 B to 0.303 per million to reflect the elimination of 1 case from the database.

Action by 2003 *Vibrio* Management Committee Recommended adoption of *Vv* Subcommittee recommendation on Proposal 00-201.

Action By 2003 Task Force II Recommended adoption of *Vibrio* Management Committee recommendation on Proposal 00-201.

Action By 2003 General Assembly Adopted recommendations of 2003 Task Force II.

Action By USFDA Concurred with Conference Action.

Action by 2005 *Vv* Subcommittee Recommended the *Vibrio* Management Committee communicate to the Executive Board that the Conference has made significant progress toward achieving the 40% illness reduction goal as reflected in the 2004 rates compared to the baseline in the core states. Additionally, FDA has found all states required to implement *Vv* Management Plan are in compliance with the Model Ordinance. It should be noted that this is not an indication for a reduction in current efforts.

Action by 2005 *Vibrio* Management Committee Recommended adoption of the *Vv* Subcommittee recommendation on Proposal 00-201. Additionally, the VMC adopted the following motion:

In the three (3) Gulf Core States the illness rate reduction was 32% from their baseline. In all four Core States the reduction was 47%. Likely factors that contributed to the illness reduction include increased voluntary post harvest processing, education of at-risk individuals and California's action to ban non-post harvest processed oysters. It is recommended that the Conference continue to pursue additional methods to measure success or failure of the Risk Management Plan in both the Core States and nationally.

Action by 2005 Task Force II Recommended adoption of the *Vibrio* Management Committee recommendations on Proposal 00-201.

Action by 2005 General Assembly Adopted recommendation of 2005 Task Force II.

Action by USFDA With reservation, FDA concurs with action taken on Proposal 00-201. Although FDA recognizes that a 47% reduction in *Vibrio vulnificus (Vv)* illnesses has been achieved in the Core reporting states, the Agency believes that this reduction is primarily the result of California's ban on non-post harvest processed Gulf oysters. At the 2005 Conference FDA proposed that California be removed from the list of Core states and that one or more additional states with consistent *Vv* illness reporting records be substituted. The *Vv*

Subcommittee did not concur with FDA's recommendation and retained California as a Core state for measuring the success of the *Vv* Action Plan. FDA maintains the position that California should be removed as a Core reporting state and that illness reduction rates that include California provide a false indication of success relative to the *Vv* Action Plan illness reduction goals. FDA requests that the ISSC Executive Board direct the *Vibrio* Management Committee (VMC), during its March meeting, to reconsider the decision of the *Vv* Subcommittee to retain California as a Core reporting state.

Action by 2007 *Vibrio* Mgmt Committee

Recommended that the *Vibrio* Management Committee continue to monitor the activities of Proposal 00-201.

Action by 2007 Task Force II

Recommended adoption of the *Vibrio* Management Committee recommendation on Proposal 00-201.

Action by 2007 General Assembly

Adopted recommendation of 2007 Task Force II.

Action by USFDA

December 20, 2007

Concurred with Conference action with the following comments and recommendations for ISSC consideration.

At the 2007 Biennial Meeting, Dr. Alvin Rainosek advised the Conference that current efforts under the *Vibrio vulnificus* Management Plan are not likely to achieve the ISSC's 60% illness reduction goal by the end of 2008. FDA strongly encourages source states and the shellfish industry to begin preparing for the implementation of controls outlined in NSSP Model Ordinance Chapter II @ .04 and intended to ensure a 60% illness reduction in years subsequent to 2008. FDA anticipates that source states will be prepared to implement these controls at the conclusion of 2008 should the 60% reduction goal not be met. FDA also anticipates that implementation of those controls, should they be needed, will achieve a 60% illness reduction by the end of 2009 as determined by the average number of illnesses for the years 2008 and 2009 combined.

Action by VMC October 2009

1. a. Recommended that FDA submit a proposal for deliberation by a Special ISSC conference to be held in 2010.

   b. In the interim, it is requested that FDA, in coordination with ISSC fund a robust economic impact and consumer acceptance analysis to inform the ISSC deliberations on the proposal. An impacts analysis guidance committee will be appointed to guide and make recommendations on the components of the impacts analysis study.

2. Recommended that a workgroup be established to develop criteria for an economic analysis. The workgroup will use the criteria for an economic impact analysis for rulemaking as a guide. The study should include a taste acceptance component. The workgroup should include, but not be limited to, at least one industry member and one regulatory member from the east, west and gulf coasts.

3. Recommended that May 1, 2011, be set as date for implementation of Model Ordinance Chapter II @ .04, *Vibrio* Management Plan for Oysters.

4. Recommended that the *Vibrio* Management Committee meet at the Spring 2010

meeting of the Executive Board.

5. Recommended that the findings of the *Vibrio* vulnificus Illness Review Subcommittee be accepted. The Subcommittee found that 17 cases in 2007 met the criteria and 13 cases in 2008 met the criteria. After adjusting for population changes, the illness rate reduction was calculated to be 35.2% from the baseline period.

Action by 2009 Task Force II

Recommended adoption of *Vibrio* Management Committee Recommendation No. 1.a. and b. on Proposal 00-201.

Recommended adoption of *Vibrio* Management Committee Recommendation No. 2. on Proposal 00-201 with instruction to add a consumer representative to the work group.

Recommended adoption of *Vibrio* Management Committee Recommendation No. 3 on Proposal 00-201.

Action by 2009 Task Force II (continued)

Recommended adoption of *Vibrio* Management Committee Recommendation No. 4 on Proposal 00-201.

Recommended adoption of *Vibrio* Management Committee Recommendation No. 5 on Proposal 00-201.

Action by 2009 General Assembly

Voted no action on Proposal 00-201 Recommendation 1.a.

Adopted recommendation of 2009 Task Force II on Proposal 00-201 Recommendation 1.b.

Adopted recommendation of 2009 Task Force II on Proposal 00-201 Recommendation 2.

Voted no action on Proposal 00-201 Recommendation 3. The previous implementation date of May 1, 2010 remains in effect.

Adopted recommendation of 2009 Task Force II on Proposal 00-201 Recommendation 4.

Adopted recommendation of 2009 Task Force II on Proposal 00-201 Recommendation 5.

Adopted a motion that the Vibrio Management Committee, at its fall 2010 meeting, evaluate the effects of the Vibrio Management Plans implemented May 1, 2010, and make recommendations to the Executive Board.

Adopted a motion that the Executive Board write a letter to FDA stating that the unilateral actions taken to regulate *Vv* under the Seafood HACCP Regulations are not consistent with the MOU between the ISSC and FDA.

Action by USFDA 02/16/2010

Concurred with Conference action on Proposal 00-201 with the following comments and recommendations for ISSC consideration.

FDA concurs with recommendations of the Conference as outlined in Proposal 00-201. Recognizing the difficult and sensitive nature of efforts to effectively control illnesses and deaths associated with *Vv*, FDA, in its January 26, 2010 letter to the ISSC, stated its desire to maintain an open dialog with the ISSC and its commitment to a process to ensure that the essential elements will be in place for the Executive Board to take action during its fall 2010 meeting to protect consumers from Vv illnesses and deaths. Toward that end, FDA is

contracting with Research Triangle Institute to conduct an assessment of Post Harvest Processing implementation by the Gulf industry. As you know, efforts to conduct a consumer acceptance component of that study will be conducted through a contract let by the ISSC. In that regard, FDA stands ready to offer assistance and guidance as appropriate.

Action by 2011 Task Force II

Recommended no action on Proposal 00-201.

Rationale: This proposal is addressed in Proposal 11-201-A.

Action by 2011 General Assembly

Adopted recommendation of 2011 Task Force II on Proposal 00-201.

Action by FDA February 26, 2012

Concurred with Conference action on Proposal 00-201.

Proposal Subject: Identification of Wet Stored Shellstock

Specific NSSP Guide Reference: NSSP Guide Section II Model Ordinance
Chapter X. General Requirements for Dealers
@ .05 Shellstock Identification B. Tags (2)

Text of Proposal/ Requested Action

.05 B. (2) The dealers tag…

(a) The dealer's name…
(b) The dealer's certification…
(c) The original shellstock …
(d) The date of harvest…
(e) If depurated …
(f) The most precise…
(g) **When the shellstock has been transported from the original area and wet stored in another approved growing area within the same state for at least two weeks, the dealer will:**
(i) use the date shellstock was harvested from the last growing area as the harvest date;
(ii) identify the last growing area as the harvest location.
~~(g)~~ **(h)** When the shellstock has been transported across state lines…
~~(h)~~ **(i)** The type and quantity …
~~(i)~~ **(j)** The following statement…
~~(j)~~ **(k)** All shellstock intended…

Public Health Significance: There is no guidance in the Model Ordinance on tagging shellstock that is moved from one growing area to another within the same state. After 2 weeks in a growing area, the shellstock would have the characteristics of the new growing area and the product should be tagged appropriately. This will facilitate product recall and trace backs in the event of human illnesses.

Cost Information (if available): None

Action by 2003 Task Force II Recommended referral of Proposal 03-204 to the appropriate committee as determined by the Conference Chairman.

Action by 2003 General Assembly Adopted recommendation of 2003 Task Force II.

Action by USFDA Concurred with Conference Action.

Action by 2005 Post Harvest Processing Committee Recommended adoption of Proposal 03-204 with the following change to (g):

(i) use the date shellstock was harvested from the ~~last~~ most recent growing area as the harvest date;
(ii) identify the ~~last~~ most recent growing area as the harvest location.

Action by 2005 Task Force II Recommended referral of Proposal 03-204 to appropriate committee as determined by the Conference Chairman.

Action by 2005 General Assembly	Adopted recommendation of 2005 Task Force II.
Action by USFDA	Concurred with Conference action.
Action by 2007 Traceability/PHP Committees	Recommended no action on Proposal 03-204. Rationale – No scientific information has been provided to support the suggestion that shellstock harvested and wet stored for a specified period of time in a site other than the original harvest site takes on the characteristics of the wet storage area.
Action by 2007 Task Force II	Recommended referral of Proposal 03-204 back to the Post Harvest Processing Committee with direction to address confusion over whether activity is wet storage, relay, or transplanting under aquaculture and to secure whatever science is available relative to length of time in growing area to take on new characteristics of that growing area.
Action by 2007 General Assembly	Adopted recommendation of 2007 Task Force II.
Action by USFDA	December 20, 2007 Concurred with Conference action.
2011 NOTE:	The only pending action associated with this proposal will be a report from FDA. The report will be shared with the membership when available.
Action by 2011 Task Force II	Recommended no action on Proposal 03-204. Rationale: No additional information has been provided on this proposal.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force II on Proposal 03-204.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 03-204.

Proposal Subject Post Harvest Processing

Specific NSSP Guide Reference: Model Ordinance Chapter I. Definitions
Model Ordinance Chapter IX. Transportation
Model Ordinance Chapter X. General Requirements for Dealers
Model Ordinance Chapter XVI. Post Harvest Treatment

Text of Proposal/ Requested Action

During its March 2004 meeting the ISSC Executive Board was made aware that changes were needed to address confusion associated with the handling and labeling of post harvest processed shellfish. A committee was appointed and recommendations developed for Board consideration at the August 2004 Executive Board meeting. The Board approved the following interim changes to the NSSP Model Ordinance. Included in the Model Ordinance changes adopted by the Executive Board is language in *Chapter X. General Requirements for Dealers .05 Shellstock Identification B. Tags*, which allows for inclusion of language, associated with USDA requirements for Country of Origin Labeling (COOL). The new Model Ordinance language does not require Country of Origin labeling but does allow dealers to include this information on tags and labels.

CHAPTER I. DEFINITIONS

Post Harvest Processing means processing of shellfish for the purpose of added safety or quality that involve hazards not addressed by controls in NSSP Model Ordinance Chapters XI. through XIV.

Raw means shellfish that have not been thermally processed:

(a) to an internal temperature of 145°F or greater for 15 seconds (or equivalent); or

(b) altering the organoleptic characteristics.

Shellfish means all species of:

(a) Oysters, clams or mussels, whether:
- (i) Shucked or in the shell;
- **(ii) Raw, including post harvest processed;**
- ~~(ii)~~**(iii)** Frozen or unfrozen;
- ~~(iii)~~**(iv)** Whole or in part; and

(b) Scallops in any form, except when the final product form is the adductor muscle only.

CHAPTER IX. TRANSPORTATION

Requirements for the Authority
@ .02 Shipment Acceptability

A. Shipments are properly identified with tags **and/or labels** and shipping documents;
B. Shellstock is alive…
C. Shucked **or post harvest processed** shellfish **are** ~~is~~ cooled to a temperature of 45° Fahrenheit (7.2° Centigrade) or less; and
D. The time-temperature…
E. All other conditions…

CHAPTER X. GENERAL REQUIREMENTS FOR DEALERS

.05 Shellstock Identification

B. Tags

(5) <u>The statement "Keep Refrigerated" or an equivalent statement must be included on the tag.</u>

(6) <u>Country of origin information (USDA 2004) may be included on the dealer tag.</u>

.06 Shucked Shellfish Labeling

A. Shellfish Labeling

(5) The dealer shall assure that:

(a) The shucker-packer's or repacker's certification number is on the label of each package of fresh or frozen shellfish;

(b) The statement "Keep Refrigerated" or an equivalent statement appears on the label;

(c) Packages containing less than 64 fluid ounces have:

(i) A "SELL BY DATE" which is a reasonable subsequent shelf-life or the words "BEST IF USED BY" followed by a date when the product would be expected to reach the end of its shelf-life; and

(ii) The date as a month and day of the month.

(d) Packages containing 64 fluid ounces or more have on the lid and sidewall or bottom the "DATE SHUCKED" indicated as the number of the day of the year or the month and day of the month.

.07 Post Harvest Process Labeling

A. If a dealer elects to post harvest process shellfish and the final product form is live, the dealer shall label in accordance with Chapter <u>X. . 05.</u>

B. If a dealer elects to post harvest process shellfish and the final product form is not live, the dealer shall label in accordance with Chapter X. .06 and include the following, or equivalent statement: These shellfish have been post harvest processed.

NOTE: The Consumer Advisory shall be required for both A and B.

.08 Shipping Documents and Records.

.09 Wet Storage in Artificial Bodies of Water.

CHAPTER XVI. POST HARVEST **<u>PROCESSING</u>** ~~TREATMENT~~

<u>All References in Chapter XVI. to post harvest treatment will be changed to post harvest processing.</u>

Public Health Significance: None submitted

Cost Information (if available): Although these changes have immediate effective dates, the Executive Board recognizes the financial impact associated with tagging and labeling changes. The

Executive Board requests states to establish reasonable implementation schedules to allow the shellfish industry to incorporate these changes into their tagging and labeling programs.

Action by 2005 PHP Committee

The PHP Committee reviewed Proposal 05-200 and acknowledged implementation concerns associated with Chapter X. .07 and directed a workgroup to propose language to address the concerns to Task Force II.

Action by 2005 Task Force II

Amended Proposal 05-200 by substituting the following language submitted by the PHP Workgroup to replace Chapter X. .07.

Chapter X. .07 Processed Shellstock Labeling

A. The dealer shall label all processed shellstock with tags meeting the requirements of § .05 B. (1).

B. Processed Shellstock Tags

(1) The dealer tag on processed shellstock shall contain the following indelible, legible information in the order specified below:

(a) The dealer's name and address;
(b) The dealer's certification number as assigned by the Authority;
(c) The original shellstock shipper's certification number. If depurated the original shellstock shipper's certification number is not required;
(d) A "SELL BY DATE" which is a reasonable subsequent shelf-life or the words "BEST IF USED BY" followed by a date when the product would be expected to reach the end of its shelf-life. The date shall include, month, day and year;
(e) If depurated, the depuration cycle number or lot number;
(f) The most precise identification of the harvest location as is practicable including the initials of the state of harvest, and the Authority's designation of the growing area by indexing, administrative or geographic designation. If the Authority has not indexed growing areas, then an appropriate geographical or administrative designation must be used (e.g. Long Bay, Decadent County, lease number, bed, or lot number).
(g) When the shellstock has been transported across state lines and placed in wet storage in a dealer's operation, the statement: "THIS PRODUCT IS A PRODUCT OF (NAME AND STATE) AND WAS WET STORED AT (FACILITY CERTIFICATION NUMBER) FROM (DATE) TO (DATE)";
(h) The type and quantity of processed shellstock; and
(i) The following statement in bold capitalized type on each tag: "THIS TAG IS REQUIRED TO BE ATTACHED UNTIL CONTAINER IS EMPTY OR IS RETAGGED AND THEREAFTER KEPT ON FILE FOR 90 DAYS."
(j) All processed shellstock intended for raw consumption shall include a consumer advisory. The following statement, from Section 3-602.11 of the 1999 Food Code, or an equivalent statement, shall be included on all shellstock: "RETAILERS, INFORM YOUR CUSTOMERS" "Consuming raw or undercooked meats, poultry, seafood, shellfish or eggs may increase your risk of foodborne illness, especially if you have certain medical conditions."

(k) The statement "Keep Refrigerated" or an equivalent statement must be included on the tag.

(2) If the processed shellstock is removed from the original container, the tag on the new container shall meet the requirements in §.07B.

(3) Country of origin information (USDA 2004) may be included on the shucker-packer or reshipper tag.

Additionally, the Task Force added the following definition to Proposal 05-200:

Chapter I - Definitions

(80) Processed shellstock means shellstock that has been Post Harvest Processed with a validated or non-validated process which results in a frozen or unfrozen end product which is no longer alive, and that is sold in the whole or half shell.

Task Force II recommended that Proposal 05-200, as amended by the PHP Workgroup, be referred to the appropriate committee as determined by the Conference Chairman for further deliberation and Proposal 05-200 as amended remain interim pending further Conference action.

Action by 2005 General Assembly

Adopted recommendation of 2005 Task Force II.

Action by 2005 USFDA

FDA concurs with action by the Conference to refer Proposal 05-200 to an appropriate committee for further deliberation. However, FDA does not concur with interim adoption of Proposal 05-200 language, as amended by Task Force II, pending further Conference action. FDA finds that the interim language needs clarification prior to inclusion in the NSSP Model Ordinance. Task Force II, in its decision to refer this Proposal back to committee, recognized the need for clarification relative to the "post harvest processing" and "processed shellstock" definitions and the potential confusion associated with labeling of such products. The concept of PHP has been expanded from its original intent, which focused on processing to reduce Vibrio levels to non-detect, to include other processes that do not necessarily achieve pathogen reduction of public health significance. As a result the ISSC is continuing to examine how the Model Ordinance can best address this broader approach to PHP and its associated labeling requirements. Until the ISSC has completed its deliberations on Proposal 05-200 it is in the best interest of industry and regulatory authorities not to include Proposal 05-200 interim language in the NSSP Model Ordinance at this time.

Action by 2007 Post Harvest Processing Committee

Recommended adoption of Proposal 05-200 as amended

1. Change the title of Chapter X. 07 from Processed Shellstock Labeling to In-shell Product or Post Harvest Processed In-Shell Product labeling. Replace all references to processed shellstock in the language adopted by the Conference in 2005 with "in-shell product".

2. Add a definition for "in shell product" to Chapter I (Definitions):

   "In Shell Product means non-living, processed shellfish with one or both shells present."

3. The Conference should appoint a work group to review Chapters VII. (Wet Storage in Approved and Conditionally Approved Growing Areas), XV. (Depuration), and XVI. (Post Harvest Processing) to determine if requirements are consistent for the risks involved with each process.
4. A transition period of up to 12 months should be allowed to allow dealers to utilize their current inventory of shellfish and supplies before the new labeling requirements must be met.

Action by 2007 Task Force II Recommended adoption of the Post Harvest Processing Committee recommendation on Proposal 05-200.

Action by 2007 General Assembly Adopted recommendation of 2007 Task Force II.

Action by USFDA December 20, 2007
Concurred with Conference action.

2009 Action No activity

Action by 2011 Post Harvest Processing Committee Recommended no action on Proposal 05-200.

Rationale: After reviewing the current NSSP controls and monitoring requirements for the processes listed above, the Committee determined that the controls and monitoring requirements for each process are not equivalent, but that public health risks are adequately addressed by NSSP controls. Therefore, no changes to the controls and monitoring in the Model Ordinance are warranted at this time.

Action by 2011 Task Force II Recommended adoption of Post Harvest Processing Committee recommendation of no action on Proposal 05-200.

Rationale: No changes to the controls and monitoring in the Model Ordinance are warranted at this time

Action by 2011 General Assembly Adopted recommendation of 2011 Task Force II on Proposal 05-200.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 05-200.

Proposal Subject:	Post Harvest Handling Definition
Specific NSSP Guide Reference:	NSSP Guide Section II. Model Ordinance B. Definitions of Terms
Text of Proposal/ Requested Action	Add a new definition for Post Harvest Handling as follows and renumber Definitions Section appropriately. Post Harvest Handling means any handling technique which has been established by a certified dealer and/or licensed harvester using the Hazard Analysis Critical Control Point guidelines that have been proven to result in a low historical risk of incidence of illnesses to consumers from naturally occurring bacteria as determined by the SSCA.
Public Health Significance:	The use of Post-Harvest Handling techniques by certified dealers and licensed harvesters are proven to provide consumers of raw molluscan shellfish with a low incidence of illnesses caused by naturally occurring bacteria using HACCP controls
Cost Information (if available):	Less than the cost of closing oyster harvest areas, requiring oysters be shucked when shucking oysters is not profitable or requiring post-harvest processing of oysters.
Action by 2009 Task Force II	Recommended referral of Proposal 09-201 to an appropriate committee as determined by the Conference Chairman.
Action by 2009 General Assembly	Adopted recommendation of 2009 Task Force II on Proposal 09-201.
Action by USFDA 02/16/2010	Concurred with Conference action on Proposal 09-201.
Action by 2011 Post Harvest Processing Committee	Recommended no action on Proposal 09-201. Rationale: Consider Proposal 09-231 as a substitute.
Action by 2011 Task Force II	Recommended adoption of Post Harvest Processing Committee recommendation of no action on Proposal 09-201. Rationale: This proposal was addressed by Committee action on Proposal 09-231.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force II on Proposal 09-201.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 09-201.

Proposal Subject: Continuing Education Requirement for Certified Shellfish Dealers

Specific NSSP Guide Reference: NSSP Guide Section II. Model Ordinance
Chapter I. Shellfish Sanitation Program
@.02 Dealer Certification A. General

Text of Proposal/ Requested Action

(2) Certification shall be given only to persons who meet the established requirements established for certification.

a. All persons prior to applying for plant certification shall complete 3 hours annually of continuing education hours to maintain certification by the Authority and listing the ICSSL. Continuing Education hours could include attendance at ISSC meetings attendance at regional shellfish sanitation conferences, attendance at regional shellfish association meetings, or any other conference or meeting approved by the Authority.

Public Health Significance: This requirement will better inform certified dealers of new guidelines set forth in the NSSP.

Cost Information (if available): The cost would include registration fee and certification certificate for dealer to attend continuing education course.

Action by 2009 Task Force II: Recommended referral of Proposal 09-203 to an appropriate committee as determined by the Conference Chairman.

Action by 2009 General Assembly Adopted recommendation of 2009 Task Force II on Proposal 09-203.

Action by USFDA 02/16/2010 Concurred with Conference action on Proposal 09-203.

Action by 2011 Education Committee Recommended no action on Proposal 09-203.

Rationale: Every state has certification requirements which include demonstration of knowledge through experience or education. No further education requirement is needed at this time.

Action by 2011 Task Force II Tabled consideration of Proposal 09-203 until Wednesday, October 5, 2011.

Additional Action by 2011 Task Force II Recommended adoption of Education Committee recommendation of no action on Proposal 09-203.

Rationale: This proposal is addressed in Proposal 09-212.

Action by 2011 General Assembly Adopted recommendation of 2011 Task Force II on Proposal 09-203.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 09-203.

Proposal Subject: Continuing Education Requirement for Licensed Shellfish Harvesters

Specific NSSP Guide Reference: NSSP Guide Section II. Model Ordinance Chapter VIII. Control of Shellfish Harvesting @.01 Control of Shellstock Growing Areas

Text of Proposal/ Requested Action

C. Licensing of Harvesting

(1) The Authority shall assure that a license is required to commercially harvest shellstock, including shellstock harvested from aquaculture.

(2) Each license shall:

(a) Not be valid for more than one year;

(b) Require the harvester to complete 3 hours annually of continuing education hours to attain a harvester license from the Authority Continuing Education hours could include attendance at ISSC meetings, attendance at regional shellfish sanitation conferences, attendance at regional shellfish association meetings, or any other conference or meeting approved by the Authority.

~~(b)~~ (c) Require the harvester to sell only to dealers listed on the Interstate Certified Shellfish Shippers List; and

~~(c)~~ (d) Allow the harvester, at his discretion, to place shellstock in containers for transport of shellstock from a growing area to land or to a dealer.

Public Health Significance: This requirement will better inform licensed shellfish harvesters of new guidelines set forth in the NSSP.

Cost Information (if available): The cost would include registration fee and certification certificate for the licensed harvester to attend a continuing education course.

Action by 2009 Task Force II Recommended referral of Proposal 09-211 to an appropriate committee as determined by the Conference Chairman.

Action by 2009 General Assembly Adopted recommendation of 2009 Task Force II on Proposal 09-211.

Action by USFDA 02/16/2010 Concurred with Conference action on Proposal 09-211.

Action by 2011 Education Committee Recommended no action on Proposal 09-211.

Rationale: Every state has certification requirements which include demonstration of knowledge through experience or education. No further education requirement is needed at this time.

Action by 2011 Task Force II Tabled consideration of Proposal 09-211 until Wednesday, October 5, 2011.

Additional Action by 2011 Task Force II Recommended adoption of Education Committee recommendation of no action on Proposal 09-211.

Rationale: This proposal is addressed in Proposal 09-212.

Action by 2011 General Assembly Adopted recommendation of 2011 Task Force II on Proposal 09-211.

Action by FDA
February 26, 2012

Concurred with Conference action on Proposal 09-211.

Proposal Subject: New Food Safety Training Requirements for Harvesters and Dealers

Specific NSSP Guide Reference: NSSP Guide Section II. Model Ordinance
Chapter VIII. Control of Shellfish Harvesting

Text of Proposal/ Requested Action

.02 Shellstock Harvesting and Handling.

A. Harvesters. Any harvester who engages in-shellfish packing as defined in this Ordinance shall:
(1) Be a dealer; or
(2) Pack shellstock for a dealer.

B. Harvester/Dealer Education

Requirement for States that have determined, through a *Vibrio* risk ~~assessment, that~~assessment that *Vibrio* illnesses are reasonably likely to occur.

(1) If a harvester or dealer elects to harvest oysters intended for raw consumption during months that are typically associated with *Vibrio* illnesses, the harvester or dealer shall obtain a minimum of two hours of training in harvest and post-harvest practices, held bi-annually; or an equivalent level of training, as determined by the State authority

(2) The training shall cover all phases of harvest and post harvest handling likely to result in temperature abuse or growth of *Vibrio* bacteria. The training shall include harvest and post harvest practices, transportation and handling and processing methods designed to minimize the growth of Vibrio and to reduce the risk of illness from *Vibrios*.

(3) Based upon harvest practices and environmental conditions, the State Authority may determine the exact requirements of the training program, including the length and frequency of the training session.

(4) Harvesters and dealers must receive a certificate for training that has been approved by the Authority prior to issuance of a new license, or before a license shall be renewed.

(5) At least one representative from each company with a harvester or dealer license shall obtain the training.

(6) The Authority may provide the required training course, or approve other training classes or courses provided by other government agencies, educational institutes, academic meetings, private institutions, non profit organizations or trade associations.

~~B~~C. Non-Vessel Harvesting
~~C~~D. Vessels
~~D~~E. Disposal of Human Sewage from Vessels
~~E~~F. Shellstock Washing
~~F~~G. Shellstock Identification

Public Health Significance: The risk of *Vibrio* illness can be greatly reduced through appropriate harvesting, post harvesting, transportation, handling, and processing of oysters intended for raw

consumption. Because harvesters are not required to obtain HACCP training, it has been recognized that critical information about temperature abuse and the growth of *Vibrio* bacteria is not being conveyed to a large number of growers that only have a harvester's license. Further, it is recognized that dealers will benefit from learning more about the advantages of utilizing certain harvest, post harvest, transportation, handling and processing techniques designed to prevent the growth of *Vibrio* bacteria.

Cost Information (if available): Undetermined cost implications. Recommend ISSC assistance in providing training materials or support.

Action by 2009 Task Force II: Recommended referral of Proposal 09-212 to an appropriate committee as determined by the Conference Chairman.

Action by 2009 General Assembly: Adopted recommendation of 2009 Task Force II on Proposal 09-212.

Action by USFDA 02/16/2010: Concurred with Conference action on Proposal 09-212.

Action by 2011 Education Committee Recommended no action on Proposal 09-212.

Rationale: Every State has certification requirements which include demonstration of knowledge through experience or education. In addition, source States that have implemented a *Vibrio* Management Plan have already implemented some type of training requirement into the Plan. No further education requirement is needed at this time.

Action by 2011 Task Force II Tabled consideration of Proposal 09-211 until Wednesday, October 5, 2011.

Action by 2011 Task Force II Recommended adoption of the following substitute proposal:

HARVESTER:

Model Ordinance Chapter VIII. Control of Shellfish Harvesting
Requirements for Harvesters

.01 General.

A. Each harvester shall have a valid license, and a special license if necessary, in his possession while engaged in shellstock harvesting activities.

B. Prior to licensing each harvester shall obtain Authority approved training every two years. The training shall include required harvest, handling, and transportation practices as determined by the Authority. A harvester shall be allowed ninety (90) days following initial licensing to obtain the required education.

(1) A harvester shall obtain proof of completion of the required training. Proof of training obtained by the harvester within the past two years shall be presented to the Authority prior to certification, recertification, or licensing.

(2) At a minimum, one individual involved in the shellfish operations shall obtain the required training.

(3) The harvester shall maintain record of the completed training.

~~B.~~C. Persons who are working in a boat crew under the supervision of a licensed harvester need not have a valid harvester's license.

~~C.~~D. In the case of riparian or leased land, unless the riparian owner or lessee employs a licensed harvester, the riparian owner or lessee shall be licensed as a harvester prior to harvesting his shellstock. A licensed riparian owner or lessee may employ unlicensed harvesters to work his property or lease.

DEALER:

Model Ordinance Chapter X General Requirements for Dealers
.04 Certification Requirements.

A. General.

(1) No person shall act as a dealer prior to obtaining certification.

(2) Any person who wants to be a dealer shall:

(a) Make application to the Authority for certification;

(b) Have and implement a HACCP Plan, and have a program of sanitation monitoring and record keeping in compliance with 21 CFR 123 as it appears in the *Federal Register* of December 18, 1995, except for the requirement for harvester identification on a dealer's tag.

(c) Obtain Authority approved training every two years. The training shall include required processing, handling, and transportation practices as determined by the Authority. A dealer shall be allowed ninety (90) days following initial licensing to obtain the required education.

(i) A dealer shall receive proof of completion of the required training. Proof of training obtained by the dealer within the past two years shall be presented to the Authority prior to certification, recertification, or licensing.

(ii) At a minimum, one individual involved in the shellfish operations shall obtain the required training.

(iii) The dealer shall maintain the record of the completed training.

(3) Each dealer shall have a business address at which inspections of facilities, activities, or equipment can be conducted.

NOTE: These provisions take effect January 1, 2014.

Action by 2011 General Assembly Adopted the recommendation of Task Force II on Proposal 09-212.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 09-212.

Proposal Subject: Research Need for Suitable Time-Temperature Monitoring Devices for Shipping Times Greater than Four Hours

Specific NSSP Guide Reference: Section II. Model Ordinance
Chapter IX. Transportation .05 Shipping Times

Text of Proposal/ Requested Action The Pacific Rim Shellfish Sanitation Conference requests that the ISSC create an educational committee with the purpose of establishing criteria, plus research and review of suitable time-temperature monitoring devices to adequately monitor the temperature of shellstock during shipping. The educational committee will also post and maintain a clearinghouse showing potential time-temperature monitoring devices on the ISSC organization website so as to support dealers who ship shellfish.

B. Shipping Time is Greater Than Four Hours.

(1) When the shipping …
  (a) Mechanically refrigerated conveyances …
  (b) Containers with an …

(2) Unless the dealer has an approved HACCP plan with an alternate means of monitoring time-temperature, the initial dealer shall assure that a suitable time temperature recording device accompanies each shipment of shellfish.
(3) The initial dealer shall note the date and time on the temperature-indicating device, if appropriate.
(4) Each receiving dealer shall write the date and time on the temperature-indicating device, if appropriate, when the shipment is received and the doors of the conveyance or the containers are opened.
(5) The final receiving dealer shall keep the time-temperature recording chart or other record of time and temperature in his files and shall make it available to the Authority upon request.
(6) An inoperative temperature-indicating device shall be considered as no recording device.

Public Health Significance: Shellfish dealers are required by the NSSP to ensure that shellfish is shipped under proper temperature control to prevent possible pathogen growth. Natural marine pathogens such as *Vibrio vulnificus* and *Vibrio parahaemolyticus* show substantial growth when temperature increases. Pathogen growth has a logarithmic relationship to temperature; therefore, maintaining proper temperature control during shipping can lessen or restrict the growth of these pathogens.

Dealers have requested guidance on what time-temperature devices and technologies are available and suitable for industry use. With ever-changing technologies, a central educational clearinghouse would best serve the conference and its members.

Cost Information (if available): None – research request

Proposed SpecificResearch Need/Problem to be Addressed:
Research into appropriate time-temperature monitoring devices in order to monitor the temperature of shellstock during shipping. The current problem to be addressed focuses on whether or not shellstock is being kept at proper and controlled temperatures during shipping in order to suppress or restrict the growth of pathogens such as *Vibrio vulnificus* and *Vibrio parahaemolyticus*. These time-temperature devices could serve to inform the

receiver if the product before them is safe for human consumption and the grower on whether or not their product is being shipped as agreed.

How will addressing this research support/improve the mission/role of the ISSC/NSSP/Industry? Support need with literature citations as appropriate.
This research support will improve the mission of the ISSC/NSSP/Industry by increasing the monitoring of shellstock once it leaves the growing area. Time to Temperature controls have been instituted and measured in the growing areas and people are still getting sick. The industry and regulators in the Pacific Rim are asking the questions: how can we measure whether or not the shellstock temperatures are being maintained during shipping? How can we collect this data to help narrow down where the pathogen growth may be occurring? By narrowing in on possible avenues for growth and collecting sound data to support the possibility, public health will be better served.

Relative Priority Rank in Terms of Resolving Research Need:

Immediate	☐
Valuable	☐
Important	☒
Required	☐
Other	☐

Estimated Cost:

Proposed Sources of Funding/Support:

Time Frame Anticipated:

Action by 2009 Task Force II Recommended adoption of Proposal 09-214 as submitted.

Action by 2009 General Assembly Adopted recommendation of 2009 Task Force II on Proposal 09-214.

Action by USFDA 02/16/2010 Concurred with Conference action on Proposal 09-214.

Action by 2011 Time Temperature Technology Committee Recommended that the Executive Board appoint a committee to design a survey, develop criteria for evaluating devices, continue discussions, and develop information to post on the ISSC website pertaining to available technologies.

Action by 2011 Task Force II Recommended adoption of the Time Temperature Technology Committee recommendation on Proposal 09-214.

Action by 2011 General Assembly Adopted recommendation of 2011 Task Force II on Proposal 09-214.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 09-214.

Proposal Subject: Post Harvest Handling

Specific NSSP Guide Reference: NSSP Guide Section II Model Ordinance Definitions and New Chapter XVII.

Text of Proposal/ Requested Action

Action #1

Add a new definition to B. Definition of Terms for Post Harvest Handling and renumber Definitions Section accordingly.

Post Harvest Handling means a control(s) employed by a dealer to further reduce, beyond controls currently in place under the NSSP, the post harvest growth of naturally occurring pathogens for the purposes of handling product outside of as an alternative to the Authority's existing NSSP management plans.

Action #2:

Add a new chapter to the NSSP Guide Section II. Model Ordinance as follows:

Chapter XVII. Post Harvest Handling

A. If a dealer elects to use a post harvest handling control(s) to reduce the levels of post harvest growth of a naturally occurring pathogen(s) of public health concern in shellfish, the dealer shall:

(1) Have a HACCP plan (approved by the Authority) for the control(s) that reduces post harvest growth of the target pathogen(s).

(a) The dealer must validate that the post harvest handling control(s) reduces the post harvest growth of naturally occurring pathogen(s). The validation study must be approved by the State Shellfish Control Authority with FDA concurrence.

(b) The ability of the post harvest handling control(s) to reliably achieve the appropriate reduction in post harvest growth of the target pathogen(s) shall be routinely verified at a frequency determined by the State Shellfish Control Authority.

(2) Package and label all shellfish in accordance with the requirements of this Ordinance.

(3) Keep records in accordance with Chapter X. 07.

Public Health Significance: The changes recommended by this proposal provide added opportunities for shellfish dealers to meet the required State Control Plans for naturally occurring pathogens.

Cost Information (if available):

Action by 2009 Task Force II: Recommended referral of Proposal 09-231 to an appropriate committee as determined by the Conference Chairman.

Action by 2009 General Assembly Adopted recommendation of 2009 Task Force II on Proposal 09-231.

Action by USFDA 02/16/2010 Concurred with Conference action on Proposal 09-231.

Action by 2011 Post Harvest Processing Committee

Recommended no action on Proposal 09-231.

Rationale: The proposed new definition and new chapter are not necessary because the State *Vibrio* Management Plans already allow handling practices to reduce levels of naturally occurring pathogens. The recommended changes are adequately addressed in the Model Ordinance.

Action by 2011 Task Force II

Recommended referral of Proposal 09-231 to an appropriate Committee as determined by the Conference Chairman with instructions that the Committee establish validation protocols for activities that reduce levels of naturally occurring pathogens so that a dealer can work outside the Authority's *Vibrio* Management Plan. Additionally, the Committee is charged with ensuring the Post Harvest Handling (PHH) definition and section in Chapter XVII is consistent so that they are directing a process that reduces levels not just growth.

The intent of Task Force II is that Post Harvest Handling activities are not intended to be used to support labeling claims.

Action by 2011 General Assembly

Adopted recommendation of 2011 Task Force II on Proposal 09-231.

Action by FDA February 26, 2012

Concurred with Conference action on Proposal 09-231.

Proposal Subject: Post Harvest Handling

Specific NSSP Guide Reference: Section II. Model Ordinance
Chapter XVII. Post Harvest Handling

Text of Proposal/ Requested Action:

Post Harvest Handling

A. If a dealer elects to use a post harvest handling process to reduce post harvest growth of some target pathogens of public health concern in shellfish, the dealer shall:

(1) Have a HACCP plan approved by the Authority for the process that reduces post harvest growth of the target pathogen(s).

(a) The dealer must demonstrate that the post harvest handling process reduces the post harvest growth of Vibrio vulnificus in the product to be determined by the State Shellfish Authority or other method approved for NSSP use.

(b) The dealer must demonstrate that post harvest handling process reduces the post harvest growth of Vibrio parahaemolyticus in the product to be determined by the State Shellfish Authority or other method approved for NSSP use.

(c) For handling procedure that target other pathogens the dealer must demonstrate that the level of those pathogens in the post harvest handled product has reduced post harvest growth to an adequate action level determined by the ISSC or SSCA.

(d) The ability of the post harvest handling to reliably achieve the appropriate reduction of growth in the target pathogen(s) shall require the certified dealer to conduct an annual validation study approved by the SSCA with the concurrence of FDA.

(e) The HACCP plan shall include:

(i) Post harvest handling controls to ensure that the end point criteria are met for every lot; and,

(ii) A sampling program to periodically verify that the end point criteria are met.

2. Package and label all shellfish in accordance with all requirements of this Ordinance.

3. Keep records in accordance with Model Ordinance Chapter X.07.

Public Health Significance: It is well documented that a HACCP based approach to handling oysters during and following harvest will reduce the growth of bacteria that may cause illnesses.

Cost Information (if available): The cost associated with this proposal is far less than those that currently exist to meet guidelines set in the *Vibrio vulnificus* and *parahaemolyticus* Management Plans for oysters.

Action by 2009 Task Force II: Recommended referral of Proposal 09-232 to an appropriate committee as determined by the Conference Chairman.

Action by 2009 General Assembly Adopted recommendation of 2009 Task Force II on Proposal 09-232.

Action by USFDA 02/16/2010 Concurred with Conference action on Proposal 09-232.

Action by 2011 Post Harvest Processing Committee	Recommended no action on Proposal 09-232. Rationale: The Committee opted to dispense with Proposal 09-232 and substitute the language found in Proposal 09-231 and consider 09-231 as a substitute.
Action by 2011 Task Force II	Recommended adoption of the Post Harvest Processing Committee recommendation of no action on Proposal 09-232. Rationale: The recommended action of Proposal 09-232 was addressed in Committee action on Proposal 09-231.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force II on Proposal 09-232.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 09-232.

Proposal Subject: Approval of the Use of End-Product Testing as an Alternative to Validation of Post Harvest Processes

Specific NSSP Guide Reference: Section IV. Guidance Documents
Chapter IV. Naturally Occurring Pathogens

Text of Proposal/ Requested Action

.04 Post Harvest Processing (PHP) Validation/Verification Guidance for *Vibrio vulnificus* and *Vibrio parahaemolyticus*

C. End Product Testing

Used as an alternative to validation of new shellfish processes to ensure that the end-product contains less than 30 MPN/g of Vv and/or Vp.

Prior to adding labeling claims to the product, the processor must analyze each lot of the finished product in accordance with the NSSP guidance document.

Only lots having less than 30 MPN/g will be allowed to be labeled as PHP. Processor must incorporate the sampling and testing into their HACCP plan and maintain records of HACCP controls as well as laboratory analytical results for all lots tested.

~~C~~D. Initial Load Testing

Initial level of *Vibrios* in shellfish for each lot of shellfish used in validation shall be 10,000 MPN per gram or greater based on the adjusted geometric mean (AGM) of the MPNs/g of four samples where the AGM is given by:

AGM = the geometric mean of the 4 MPNs/g multiplied by an adjustment factor of 1.3

Note: If 4 samples from a lot of shellfish with a true density of 100,000 cells per gram are examined by the MPN procedure, the probability of the geometric mean of the MPNs showing 100,000 or greater is about 50%. In an attempt to improve the probability of samples being accepted when the true density is 100,000/g an adjustment factor of 1.3 was selected based upon statistical analysis.

~~D~~E. Verification

Public Health Significance: None

Cost Information (if available): None

Action by 2009 Task Force II: Recommended referral of Proposal 09-235 to an appropriate committee as determined by the Conference Chairman.

Action by 2009 General Assembly Adopted recommendation of 2009 Task Force II on Proposal 09-235.

Action by USFDA	Concurred with Conference action on Proposal 09-235.
Action by 2011 Post Harvest Processing Committee	Recommended no action on Proposal 09-235. Rationale: Validation is a requirement for labeling claims.
Action by 2011 Task Force II	Recommended adoption of Post Harvest Processing Committee recommendation of no action on Proposal 09-235. Rationale: Validation is a requirement for processing shellfish with labeling claims.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force II on Proposal 09-235.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 09-235.

Proposal Subject: Restricted Use Shellstock Definition

Specific NSSP Guide Reference: Section II Model Ordinance
Definitions;
Chapter X. General Requirements for Dealers;
Chapter XI. Shucking and Packing;
Chapter XIII. Shellstock Shipping; and
Chapter XIV. Depuration

Section IV. Guidance Documents Chapter IV. Naturally Occurring Pathogens
.02 *Vibrio vulnificus* Management Plan
.03 *Vibrio parahaemolyticus* Control Plan Guidance

Section V. NSSP Approved Forms

Text of Proposal/ Requested Action: Approve interim controls adopted by the ISSC Executive Board effective November 15, 2010.

Definitions: Add new definition and renumber as appropriate:

Restricted Use Shellstock means shellstock that is harvested from growing areas classified as approved under conditions that do not allow the sale of the shellstock for direct marketing for raw consumption. Restricted use shellstock is identified with a tag indicating that the shellstock is intended for further processing prior to distribution to retail or food service.

Model Ordinance

Chapter X. General Requirements for Dealers

.01 General HACCP Requirements

C. Contents of the HACCP Plan

(2) List the critical control points…

(c) Critical control points shall be designed to ensure that shellstock received with restricted use tags is processed consistent with the stated purpose. For Shellstock tagged for restricted use, critical control points shall be included in the Certified Dealer's HACCP plan to ensure that the shellstock is shipped to another Certified Dealer with the restricted use tag or processed consistent with the stated purpose

.05 Shellstock Identification

B. Tags

(4) If the shellstock is removed from the original container, the tag on the new container shall meet the requirements in §.05 B. If the shellstock is received bearing a restricted use tag all specific use language shall be transferred to the new shipping tag.

E. All restricted use shellstock shall include a tag containing all information required in § .05 of Model Ordinance Chapter X. In addition the tag will include specific language detailing the intended use of the shellstock.

FE. Transaction Record. If shellstock are sold in bulk, the dealer shall provide a transaction record prior to shipment. This transaction record shall contain all the information required in §.05 B. with the addition of the name of the consignee.

Chapter XI. Shucking and Packing

.01 Critical Control Points

E. Shellstock Shipping Critical Control Point

(1) The dealer shall ensure that Shellstock that is received bearing a restricted use tag shall only be shipped to a certified dealer and shall include specific language detailing the intended use of the shellstock.

Chapter XIII. Shellstock Shipping

.01 Critical Control Points

E. Shellstock Shipping Critical Control Point

(1) Shellstock that is received bearing a restricted use tag shall only be shipped to a certified dealer and shall include specific language detailing the intended use of the shellstock.

Chapter XIV. Reshipping

.01 Critical Control Points

E. Shellstock Shipping Critical Control Point

(1) Shellstock that is received bearing a restricted use tag shall only be shipped to a certified dealer and shall include specific language detailing the intended use of the shellstock.

Section IV. Guidance Documents Chapter IV. Naturally Occurring Pathogens

.02 *Vibrio vulnificus* Management Plan

(l) Shellstock Harvested in Source States
Harvesters must include on the tag of all product harvested for restricted use the statement “for shucking by a certified dealer” and/or "For PHP Only." Harvesting controls must be provided by the authority to ensure that restricted use shellstock is not diverted to retail or food service. Dealers must establish a restricted use shellstock Critical Limit as part of their HACCP Plan for receiving. A shipping Critical Control Point must include a restricted use shellstock disposition step. Restricted use shellstock is not intended for retail or food service.

.03 *Vibrio parahaemolyticus* Control Plan Guidance

B. *Vibrio parahaemolyticus* Control Plan

(3) Plan Effectiveness as Demonstrated by:

(d) The authority must notify harvesters and dealers of those areas restricted to harvest for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing or "For PHP Only." Harvesters must include on the tag of all product harvested in these areas the statement for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing or "For PHP Only." Harvesting controls must be provided by the Authority to ensure that restricted use shellstock is not diverted to retail or food service. Dealers must establish a for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing. or "For PHP Only" labeling Critical Limit as part of their HACCP plan for receiving. A shipping Critical Control Point must include for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing. or "For PHP Only" labeling requirement. Restricted Use Shellstock is not intended for retail or food service.

Section V. NSSP Approved Forms

Approve forms for:

1. Restricted Use Shellstock (Shucking or PHP) Shellfish Harvest/Purchase Record

2. Restricted Use Shellstock (Shucking or PHP) Sales/Disposition Record

Public Health Significance:

Cost Information (if available):

Restricted Use Shellstock (Shucking or PHP)______________________ Certified Dealer #____________

SHELLFISH HARVEST/PURCHASE RECORD

Lot #	Quantity	Species	Harvest Area	Harvest Date	Purchase Date	Received from (Harvester or Certified Dealer ID)

Restricted Use Shellstock (Shucking or PHP) ________________ Certified Dealer # ______________

SALES/DISPOSITION RECORD

Lot #	Date Sold or Processed	Sold To Dealer Cert # (N/A if Processed)	Quantity Sold Unprocessed	Quantity Processed

Action by 2011 Task Force II

Recommended approval of Proposal 11-200 as amended.

Restricted Use Shellstock means shellstock that is harvested from growing areas classified as approved or conditionally approved in the open status and under conditions that do not allow the sale of the shellstock for direct marketing for raw consumption. Restricted use shellstock is identified with a tag indicating that the shellstock is intended for further processing prior to distribution to retail or food service.

Model Ordinance

Chapter X. General Requirements for Dealers

.01 General HACCP Requirements

C. Contents of the HACCP Plan

(2) List the critical control points…

(c) Critical control points shall be designed to ensure that shellstock received with restricted use tags is processed consistent with the stated purpose. For Shellstock tagged for restricted use, critical control points shall be included in the Certified Dealer's HACCP plan to ensure that the shellstock is shipped to another Certified Dealer with the restricted use tag or processed consistent with the stated purpose

.05 Shellstock Identification

B. Tags

(4) If the shellstock is removed from the original container, the tag on the new container shall meet the requirements in §.05 B. If the shellstock is received bearing a restricted use tag all specific use language shall be transferred to the new shipping tag until processed consistent with the stated purpose.

E. All restricted use shellstock shall include a tag containing all information required in § .05 of Model Ordinance Chapter X. In addition the tag will include specific language detailing the intended use of the shellstock until processed consistent with the stated purpose.

F. Transaction Record. If shellstock are sold in bulk, the dealer shall provide a transaction record prior to shipment. This transaction record shall contain all the information required in §.05 B. with the addition of the name of the consignee.

Chapter XI. Shucking and Packing

.01 Critical Control Points

E. Shellstock Shipping Critical Control Point

(1) The dealer shall ensure that Shellstock that is received bearing a restricted use tag shall only be shipped to a certified dealer and shall include specific language detailing the intended use of the shellstock. The transaction record shall indicate the quantity of restricted use shellstock containers.

Chapter XIII. Shellstock Shipping

.01 Critical Control Points

E. Shellstock Shipping Critical Control Point

(1) Shellstock that is received bearing a restricted use tag shall only be shipped to a certified dealer and shall include specific language detailing the intended use of the shellstock. The transaction record shall indicate the quantity of restricted use shellstock containers.

Chapter XIV. Reshipping

.01 Critical Control Points

E. Shellstock Shipping Critical Control Point

(1) Shellstock that is received bearing a restricted use tag shall only be shipped to a certified dealer and shall include specific language detailing the intended use of the shellstock. The transaction record shall indicate the quantity of restricted use shellstock containers.

Section IV. Guidance Documents Chapter IV. Naturally Occurring Pathogens

.02 *Vibrio vulnificus* Management Plan

(l) Shellstock Harvested in Source States
Harvesters must include on the tag of all product harvested for restricted use the statement “for shucking by a certified dealer” and/or "For PHP Only." Harvesting controls must be provided by the authority to ensure that restricted use shellstock is not diverted to retail or food service. Dealers must establish a restricted use shellstock Critical Limit as part of their HACCP Plan for receiving. A shipping Critical Control Point must include a restricted use shellstock disposition step. Restricted use shellstock is not intended for retail or food service.

.03 *Vibrio parahaemolyticus* Control Plan Guidance

B. *Vibrio parahaemolyticus* Control Plan

(3) Plan Effectiveness as Demonstrated by:

(d) The authority must notify harvesters and dealers of those areas restricted to harvest for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing or "For PHP Only." Harvesters must include on the tag of all product harvested in

these areas the statement for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing or "For PHP Only." Harvesting controls must be provided by the Authority to ensure that restricted use shellstock is not diverted to retail or food service. Dealers must establish a for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing. or "For PHP Only" labeling Critical Limit as part of their HACCP plan for receiving. A shipping Critical Control Point must include for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing. or "For PHP Only" labeling requirement. Restricted Use Shellstock is not intended for retail or food service.

Section V. NSSP Approved Forms

Approve forms for:

1. Restricted Use Shellstock (Shucking or PHP) Shellfish Harvest/Purchase Record

2. Restricted Use Shellstock (Shucking or PHP) Sales/Disposition Record

Action by 2011 General Assembly Adopted recommendation of 2011 Task Force II on Proposal 11-200.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-200.

Proposal No. 11-200

Restricted Use Shellstock (Shucking or PHP) **Certified Dealer # ___________**

SHELLFISH HARVEST/PURCHASE RECORD

Lot #	Quantity	Species	Harvest Area	Harvest Date	Purchase Date	Received from (Harvester or Certified Dealer ID)

Restricted Use Shellstock (Shucking or PHP) Certified Dealer # ____________

SALES/DISPOSITION RECORD

Lot #	Date Sold or Processed	Sold To Dealer Cert # (N/A if Processed)	Quantity Sold Unprocessed	Quantity Processed

Proposal Subject: *Vibrio vulnificus* Controls

Specific NSSP Guide Reference: Section II Model Ordinance Chapter II Risk Assessment and Risk Management
@.04 *Vibrio vulnificus* Risk Management for Oysters

Section IV Guidance Documents Chapter IV Naturally Occurring Pathogens
.04 Naturally Occurring Pathogens

Text of Proposal/ Requested Action: During the January 2011 VMC meeting the Committee conducted an assessment of the effects of the 2010 *Vv* controls implemented by the *Vv* source states. The Committee also reviewed the *Vv* illness rate reductions for 2009 and 2010. The Committee concluded that the 60% goal had not been achieved for 2009, 2010 or 2009 and 2010 average. After a lengthy discussion which is described below, The VMC recommended, with Executive Board approval, the appointment of a workgroup to develop other *Vv* control options which would be included in a VMC proposal to the ISSC. The workgroup has been appointed and is working to develop new concepts. The workgroup will include Proposal 09-207, which was adopted in 2009, as a part of their discussions. The purpose of the proposal is to provide notice to the ISSC membership of this activity. The ISSC membership will be provided the full details of final recommendations when available.

Points of Discussion by the VMC during the January 2011 Meeting:

Chapter II @.04 includes requirements for States that have had two (2) or more etiologically confirmed shellfish borne *Vv* illnesses since 1995. Section IV Guidance Documents Chapter IV Naturally Occurring Pathogens includes guidance for implementation of the Chapter II Model Ordinance requirements. The ISSC adopted these requirements after years of encouragement by the USFDA. The very controversial *Vv* debate began in 1994 and after much resistance the ISSC adopted Proposal 00-201 in 2001. The controls of Proposal 00-201 were premised around illness rate reduction to be achieved by 2008.

Proposal 00-201 included the following three (3) major components:

(1) Consumer education: Each State *Vv* Management Plan was required to include a consumer education program.
(2) The development of PHP capacity to treat 50% of Gulf oysters intended for raw half-shall consumption. The capacity was to be available should the 60% goals not be achieved.
(3) Control strategies that could be implemented if the 40% and 60% goals were not met.

The implementation of Proposal 00-201 has been very controversial and problematic since 2001. The problems include:

(1) Our efforts to count cases for determining goal compliance has proven that illness reporting as it presently exists does not provide an adequate tool for determining the effectiveness of controls to lower risk for *Vv*.
(2) The use of four (4) states, especially California, has been publicly controversial. The FDA has stated that national illnesses should be used.
(3) In October 2009 FDA publicly announced that the agency no longer supported ISSC efforts to address *Vv*. The FDA stated its intent to reformulate policy and use the Fish and Fishery Product Hazards and Control Guidance 4th Edition to regulate *Vv* in raw oysters.

(4) States have had difficulty enforcing industry compliance.
(5) Restricted use shellstock has been diverted to restaurants and sold raw. Two (2) deaths have been attributed.
(6) FDA and ISSC have had disagreements regarding the responsibility for evaluating State compliance with *Vv* controls.
(7) The goal is a collective five (5) State goal. Determining compliance by individual States is problematic. The *Vibrio* Management Committee (VMC) concluded at the January 2011 meeting that the 60% goal has not been achieved.
(8) Results of Consumer Acceptance Study suggest consumers prefer traditional raw oysters at seven (7) days and PHP oysters at fourteen (14) days. Report indicates that most consumers would be unwilling to pay higher price for PHP oysters. RTI report suggests FDA should slow its efforts to mandate PHP.
(9) Congress passed the Food Safety Modernization Act which specifically addresses PHP in Section 114. The Senate authors of Section 114 of the Food Safety Modernization Act (FSMA) correspond with ISSC providing clarification of the intent of Congress and the Administration.
(10) The present goal approach for measuring success is not consistent with the other elements of the National Shellfish Sanitation Program (NSSP).

The Committee recommended additional time/temperature controls for April and November and recognized serious noncompliance issues in one Gulf State.

Public Health Significance:

Vibrio vulnificus is a naturally occurring bacterium found in seawater along the Gulf, Atlantic, and Pacific coasts, although it is most prevalent in the warm waters of the Gulf of Mexico. *Vibrio vulnificus* can be transmitted to humans through the consumption of raw shellfish harvested from waters containing the organism. Oysters from the Gulf of Mexico have been recognized as the primary species of molluscan shellfish associated with *Vibrio vulnificus* illnesses in consumers. *Vibrio vulnificus* does not normally affect healthy individuals, but persons who are immunocompromised, especially those with chronic liver disease, are at greater risk for contracting *Vibrio vulnificus* from oyster consumption. In immunocompromised individuals, there is a risk for the organism to invade the bloodstream, resulting in potentially fatal septicemia. Although the annual number in the US of reported *Vibrio vulnificus* illnesses associated with oyster consumption is low, generally in the range of 30 to 35, the incidence of death among those individuals who contract the disease is high. Between 2001 and 2010 (10 years) there were 335 cases of illnesses with 157 deaths reported to CDC.

Prior to 2001 the NSSP controls did not offer a strategy for controlling *Vibrio vulnificus*. In an effort to better control *Vibrio vulnificus* in oysters, in 2001 the Interstate Shellfish Sanitation Conference (ISSC) developed a *Vibrio vulnificus* Control Plan for inclusion in the NSSP.

The Plan adopted by the ISSC included a 60% illness rate reduction goal that was to be achieved by the end of 2008. To present the goal has not been achieved. The Plan also included several mandatory controls which could be implemented if necessary to achieve the 60% goal. Recognizing the potential economic damage of these controls to the industry the ISSC has continued to investigate other controls that could potentially assist the Gulf States in achieving the 60% goal. Very stringent time to

temperature controls were implemented in 2010. However, the implementation of these controls did not result in goal attainment.

The identified mandatory requirements included Post Harvest Processing (PHP) and closures. To evaluate the impact of requiring PHP, FDA contracted with RTI to conduct an economic assessment. The report entitled "Analysis of How Post-Harvest Processing Technologies for Controlling *Vibrio vulnificus* Can Be Implemented" suggest that it would take a minimum of 3 years and significant financial investment both by private and public sectors to prepare the industry for a PHP requirement. The other listed mandatory control which would likely result in 60% illness rate reduction was closure. Those supported the inclusion of closures thought that PHP would be a viable option by 2008.

Concerns for the economic impact of *Vibrio vulnificus* control prompted Congress and the Administration to include inclusion of Section 114 in the Food Safety Modernization Act. Although Section 114 is directed to FDA, the authors of the Section have communicated that they expect ISSC to consider economic effects in addressing *Vibrio vulnificus*. These directives make it very difficult to impose mandatory PHP or closures should the present expanded time to temperature approach prove ineffective in meeting the intended goals of 00-201. The VMC Proposal Workgroup will use the guidance of Procedure XIV and the ISSC Policy Statement on Consumption of Raw Molluscan Shellfish in characterizing the *Vibrio vulnificus* problem. From this characterization the workgroup will develop *Vibrio vulnificus* recommendations for VMC consideration.

Cost Information (if available):

Action by 2011 Task Force II

Recommended adoption of *Vibrio* Management Committee Substitute Proposal 11-201-A as amended.

Additionally, Task Force II recommended:

1. That a committee be established to consider options for water temperature determinations which can be used in the implementation of Proposal 11-201-A.

2. That a Committee be established to develop criteria for verifying reduction in harvest for raw consumption and the percentage of post harvest processed product on a monthly basis for those States required to have a *Vibrio vulnificus* Control Plan.

3. An implementation date of January 1, 2012 for Proposal 11-201-A.

Recommended referral of Proposal 11-201-B to an appropriate committee with representation from all regions to develop Model Ordinance language changes to support the time temperature requirements of the State's *Vibrio* Management Plans. This committee will be appointed and approved by the Executive Board at its closing Board meeting. The committee will be expected to meet within two (2) weeks of the close of the Conference. After its initial meeting, the committee shall meet by teleconference biweekly prior to an Executive Board meeting until the proposal is completed and at least once subsequent to the dissemination of the proposal and prior to an Executive Board meeting. The draft proposal that is to be considered by the Executive Board shall be disseminated to the ISSC membership a minimum of three

(3) weeks prior to the next Executive Board meeting and posted on the ISSC web site.

The Committee is directed to make recommendations to the Executive Board for interim approval with an effective date prior to the 2012 *Vibrio* season. The State's Authorities are requested to begin advising and educating their industries of these changes. Additionally, the committee will develop guidance for implementation of these controls.

Action by 2011 General Assembly

Adopted recommendation of 2011 Task Force II on Proposal 11-201 Part A.
Adopted recommendation of 2011 Task Force II on Proposal 11-201 Part B.

Action by FDA February 26, 2012

FDA concurred with Conference action on Proposal 11-201Part B but did not concur with Conference action on Proposal 11-201 part A.

FDA comments and recommendations in response to Proposal 11-201 Part A:

In October of 2009, the Food and Drug Administration (FDA) informed the Interstate Shellfish Sanitation Conference (ISSC) of its intention to reformulate the Agency's policy regarding implementation of the Seafood HACCP Regulation with the intent that post harvest processing (PHP) or equivalent measures be implemented for the control of *Vibrio vulnificus (Vv).* The new policy would require that oysters harvested from the Gulf of Mexico and intended for the raw half shell market be post harvest processed during those months when illness from *Vv* is reasonably likely to occur. Given that PHP can largely eliminate *Vv* while preserving the sensory qualities of raw untreated product FDA remains committed to this approach as the most prudent means of reducing the risk of illness from *Vv.* The efficacy of PHP is evidenced by the fact that since 2003, when the State of California banned the sale of untreated Gulf oysters harvested between April and October, there has been only one *Vv* illness in the State. Prior to 2003 California reported on average six *Vv* related illnesses per year.

In November 2009, having heard from elected State and Federal representatives, the oyster industry and State regulatory officials regarding the feasibility of implementing PHP or other equivalent controls, FDA acknowledged the need to further examine the process and timing of industry adoption of PHP technology and placed in abeyance the Agency's intent to change its policy for controlling *Vv* while taking steps to complete an independent study to assess how PHP controls can be implemented. In the interim, FDA has expressed its intention to continue working cooperatively with the ISSC to implement alternate controls which would reduce illnesses and meet the goals adopted by the ISSC in Proposal 00-201. Since adoption of Proposal 00-201 FDA has repeatedly expressed concerns relative to its implementation by the ISSC, including failure to consider national illness numbers and the lack of success in achieving the 60% illness rate reduction goal. FDA reiterated its concerns during ISSC deliberation of Proposal 11-201 at the October 2011 biennial meeting and those concerns were not adequately addressed by Conference action on Proposal 11-201. It is the position of FDA that Proposa111-201 deviates from current FDA policy in that it weakens the control measures adopted by the ISSC in Proposal 00-201. Therefore, FDA cannot concur with Proposal11-201 without further Conference action. FDA requests that the ISSC address the following issues and concerns.

1. ISSC adoption of Proposal 00-201 in 2001 established a 60% illness rate reduction goal. Although FDA no longer considers this the most appropriate goal given the efficacy of PHP, FDA has continued to recognize and support

ISSC efforts to achieve this level of illness reduction. However, the level of reduction reported by the ISSC *Vibrio* Management Committee (VMC) indicates only marginal success in moving toward that goal.

a. Proposal 00-201 included specific control measures to be taken by the *Vv* Source States if the 60% goal was not met. Those measures, intended for all oysters harvested during periods of risk included; closing shellfish growing areas to harvest, labeling oysters for shucking by a certified dealer, and subjecting oysters to PHP. Although the 60% illness rate reduction goal has not been achieved, none of these control measures have been implemented. Disagreement by States and the ISSC to pursue these more effective control measures has been a significant concern to FDA. That concern is further exacerbated by the fact that Source States, with ISSC support, have now adopted a policy that focuses control efforts toward more stringent time to temperature controls, for which compliance by industry is proving difficult. Section @.05 E. (1) (b) (iii) ofProposal11-201 establishes risk per serving standards for States using time/temperature controls and Section @.05 E. (1) (b) (iv) allows for alternative controls that achieve those same risk per servings standards. The risk per serving standards in Proposal11-201 are based on controls that were derived from the FDA developed *Vv* calculator. These controls have not yet been demonstrated to achieve a 60% illness rate reduction. The FDA maintains that until these risk per serving standards are demonstrated to achieve the intended 60% illness rate reduction, evaluation of their effectiveness is imperative. Guidance needs to be developed for how to evaluate State programs to determine if risk per serving standards are being achieved. Section @.05 E. (2) (a) ofProposal11-201 States that the State Authority in conjunction with FDA will evaluate the implementation and effectiveness of these controls. As written, FDA would consider a State to be in non-compliance when there is ineffective implementation due to industry noncompliance or when the controls are determined ineffective in achieving the risk per serving standards. FDA would expect a State to discontinue the use of the time/temperature control measures and implement other control options outlined in @.05 E. (1) (b) should the State evaluation indicate that the State is not meeting the risk per serving standards.

b. Proposal 11-201, based on temperature modeling using the *Vv* calculator, establishes risk per serving standards that are intended to achieve a 60% illness rate reduction. Determining the ability of the ISSC control strategy, based on implementing risk per serving standards, will focus on the number of nationally reported illnesses associated with oysters from the Source States. FDA expects that if the risk per serving standards established in Proposal 11-201 prove to be effective, the number of nationally reported *Vv* illnesses associated with Gulf oysters will be reduced by 60%.

c. The Source States have generically incorporated as part of their risk reduction measurement a 10% reduction in harvest attributed to stricter time/ temperature controls and a 15% reduction attributed to product diversion to PHP. Actual percentages are certain to vary from State to State and year to year, making it necessary that each State provide data supporting the use of these assumptions.

2. FDA is concerned that efforts to assess the effectiveness of time/temperature

controls in achieving risk per serving standards will be difficult. Given the small number of illnesses associated with oysters from an individual State, annual fluctuation of those numbers, and fluctuations in oyster production from year to year, calculating achievement of risk per serving numbers using national illness data and oyster production data from each *Vv* Source State will be challenging.

3. Beginning with the April2012 *Vv* season, FDA will be evaluating State *Vv* Control Plans, industry compliance, and State enforcement. While FDA is developing guidance regarding what Shellfish Specialists should consider when conducting *Vv* evaluations, presently neither FDA nor the ISSC has developed specific criteria for determining compliance with State *Vv* plan goals. FDA requests that an ISSC committee be appointed to work with FDA to develop State evaluation criteria. FDA requests development of:
    a. Evaluation criteria for determining proper and effective use of the *Vv* calculator;
    b. Evaluation criteria for determining State *Vv* control plan compliance with NSSP requirements;
    c. Evaluation criteria for determining the effectiveness of State regulatory efforts to ensure industry compliance with State *Vv* Control Plan requirements;
    d. A formula for calculating State compliance with risk per serving standards; and
    e. Actions and sanctions should a State be found out of compliance. In this regard FDA envisions that the established ISSC noncompliance process would be followed, which could result in advising receiving States of issues of noncompliance and recommending that shipments of oysters intended for raw consumption from non-compliant States not be accepted.

FDA remains committed to addressing *Vv* illnesses associated with consumption of raw Gulf oysters. As stated, FDA considers these illnesses to be preventable utilizing PHP technology. FDA will continue to support ISSC efforts to better control the risk of *Vv* until the obstacles associated with full implementation of PHP are addressed. In the interim, however, FDA cannot support Conference action to change existing *Vv* control requirements in such a way that they are less likely to achieve the existing 60% illness rate reduction goal. As adopted, FDA considers Proposal 11-201 a less effective approach to preventing *Vv* illnesses.

Proposal Subject: *Vibro vulnificus* Risk Management of Oysters

Specific NSSP Guide Reference: ISSC Constitution, Bylaws, and Procedures Article IV.
Section II Model Ordinance, Chapter II Risk Assessment and Risk Management
@.01 Outbreaks of Shellfish Related Illnesses
@.04 *Vibrio vulnificus* Risk Management for Oysters
Section IV. Guidance Documents, Chapter IV. Naturally Occurring Pathogens

Key Words: *Vibro vulnificus*Risk Management

Text of Proposal/ Requested Action:

Article IV. Executive Board, Officers, Committees

Section 10. The Board may appoint committees from industry, educational institutions, research fields, or any other areas as needed to report to the Board and advise the Conference on proposals under consideration. Committee appointments will be made from the Conference membership by the Executive Board Chairman. The following committees shall be designated as standing committees and shall convene as needed or as directed by the Executive Board or Chairperson of the Conference: Education, Foreign Relations, Proposal Review, Patrol, Research Guidance, Resolutions, ~~and~~ Shellfish Restoration, and *Vibrio* Management Committee. The Vice-Chairperson of the Conference shall assist the Executive Director in encouraging development of committee work plans and completion of subcommittee assignments prior to convention of the Biennial Meeting.

Section 14. The Executive Board Chairperson shall appoint a sixteen (16) member *Vibrio* Management Committee. The Committee will be comprised of a Chairperson with at least two (2) industry members from the East, Gulf and West coasts and at least one (1) state regulatory from each of the ISSC regions. The Committee will also include one voting member from NOAA, one voting member from FDA, one voting member from EPA and one voting member from CDC. The Federal entities will appoint these members. Non voting advisors will be appointed as appropriate. The Committee will assess if additional changes are needed in the NSSP Guide for the Control of Molluscan Shellfish Model Ordinance to reduce the risk of *Vibrio* illnesses. The Committee will annually review trends in *Vibrio* illnesses.

Chapter II Risk Assessment and Risk Management

@.01 Outbreaks of Shellfish Related Illnesses

~~J. The Authority shall assess annually Vibrio parahaemolyticus illnesses associated with the consumption of molluscan shellfish. The assessment will include a record of all V. parahaemolyticus shellfish-associated illnesses reported within the state and from receiving states, the numbers of illnesses per event, and actions taken by the Authority in response to the illnesses.~~

@.02 Annual Assessment of *Vibrio vulnificus* and *Vibrio parahaemolyticus* Illnesses.

The Authority shall assess annually *Vibrio vulnificus* and *Vibrio parahaemolyticus* illnesses associated with the consumption of molluscan shellfish. The assessment will include a record of all *Vibrio vulnificus* and *Vibrio parahaemolyticus* shellfish-associated illnesses reported within the State and from receiving States, the numbers of illnesses per event, and actions taken by the Authority in response to the illnesses.

@. 03~~2~~ Presence of Human Pathogens in Shellfish Meats.

@.04~~3~~ Presence of Toxic Substances in Shellfish Meats.

~~**.04 *Vibrio vulnificus* Risk Management for Oysters.**~~

~~B.For states having 2 or more etiologically confirmed shellfish-borne *Vibrio vulnificus* illnesses since 1995 traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state (Source State), the Authority shall develop and implement *a Vibrio vulnificus* Management Plan.~~

~~C.The Source State's *Vibrio vulnificus* Management Plan shall define the administrative procedures and resources necessary to accomplish (i.e. establish and maintain) involvement by the state in a collective illness reduction program. The goal of the *Vibrio vulnificus* Management Plan will be to reduce the rate of etiologically confirmed shellfish-borne *Vibrio vulnificus* septicemia illnesses reported collectively by California, Florida, Louisiana, and Texas, from the consumption of commercially harvested raw or undercooked oysters by 40 percent for years 2005 and 2006 (average) and by 60 percent for years 2007 and 2008 (average) from the average illness rate for the years 1995 -1999 of 0.303/million. The list of states (California, Florida, Louisiana, Texas) used to calculate rate reduction may be adjusted if after a thorough review, epidemiological and statistical data demonstrates that it would be appropriate. The illness rate shall be calculated as the number of illnesses per unit of population. The goal may be reevaluated prior to the year 2006 and adjusted in the event that new science, data, or information becomes available. State's compliance with the Plan will require States to maintain a minimum of 60% reduction in years subsequent to 2008. Determination and compliance after 2008 will be based on two-year averages beginning in 2009.~~

~~D.The Source State's *Vibrio vulnificus* Management Plan shall include, at a minimum:~~

~~(1)The ISSC Consumer Education Program targeted toward individuals who consume raw oysters and whose health condition(s) increase their risk for *Vibrio vulnificus* illnesses;~~

~~(2)A process to collected standardized information for each *Vibrio vulnificus* illness: including underlying medical conditions; knowledge of disease status; prior counseling on avoidance of high risk foods, including raw oysters; existence of consumer advisories at point of purchase or consumption; and, if possible, whether consumer was aware and understood the advisories;~~

~~(3)A standardized process for tracking products implicated in *Vibrio vulnificus* illnesses;~~

~~(4)Identification and preparation for achieving a goal of post harvest processing capacity of 25 percent of all oysters intended for the raw, half-~~

~~shell market during the months of May through September harvested from a Source State by the end of the third year (December 31, 2004). The percentage of post harvest processing will include the capacity of all operational plants and the capacity of plants under construction;~~

~~(5)Identification and preparation for implementation of required post harvest processing capacity of 50% of all oysters intended for the raw, half-shell market during the months of May through September, harvested from a Source State, which shall be implemented should the 40 percent illness reduction goal not be achieved by December 31, 2006. The percentage of post harvest processing will include the capacity of all operational plants and the capacity of plants under construction. In the alternative, the state may utilize the control measures, or equivalent control measures, listed in @.04, (C), (6) (a), (b), (c), and (d) below for such periods of time which, in combination with post harvest processing, will provide equivalent outcomes. This portion of the plan shall be completed no later than December 31, 2005; and~~

~~(6)Identification and preparation for implementation of one or more of the following controls, or equivalent controls, which shall be implemented should the 60 percent rate of illness reduction goal not be achieved collectively by 2008. The control measures identified in the plan shall be appropriate to the state and reflect that state's contribution to the number of Vv illnesses and the controls that have been implemented by each state. This portion of the Plan shall be completed no later than December 2007. The temperature and month-of the-year parameters identified in the following controls may be adjusted by the ISSC Executive Board as recommended by the Vibrio Management Committee (VMC) on a state by state basis, as needed to achieve the established illness reduction goal. The adjustment to the State's plan can take into account the illness rate reduction that has occurred since the last review of the plan.~~

~~(a)Labeling all oysters, "For shucking by a certified dealer", when the Average Monthly Maximum Water Temperature exceeds 75°F;~~

~~(b)Subjecting all oysters intended for the raw, half-shell market to an Authority- approved post harvest processing that reduces the *Vibrio vulnificus* levels to <30 MPN/gram when the Average Monthly Maximum Water Temperature exceeds 75°F;~~

~~(c)Closing shellfish growing areas for the purpose of harvest of oysters intended for the raw, half-shell market when the Average Monthly Maximum Water Temperature exceeds 75°F;~~

~~(d)Labeling all oysters, "For shucking by a certified dealer", during the months of May through September, inclusive;~~

~~(e)Subjecting all oysters intended for the raw, half-shell market to a post harvest processing that is both approved by the Authority and reduces the *Vibrio vulnificus* levels to <30 MPN/gram during the months of May through September, inclusive; and~~

~~(f)Closing shellfish growing areas for the purpose of harvesting oysters intended for the raw, half-shell market during the months of May through September, inclusive.~~

~~Effective January 1, 2012:~~

~~@.04 *Vibrio vulnificus* Risk Management for Oysters~~

~~A.For states having 2 or more etiologically confirmed shellfish borne *Vibrio vulnificus* illnesses since 1995 traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state (Source State), the Authority shall develop and implement a *Vibrio vulnificus* Risk Management Plan.~~

~~B.The Source State's *Vibrio vulnificus* Risk Management Plan shall define the administrative procedures and resources necessary to accomplish (i.e. establish and maintain) involvement by the state in a collective illness risk reduction program. The goal of the *Vibrio vulnificus* Risk Management Plan will be to reduce the risk per serving to a 60% illness rate reduction for etiologically confirmed shellfish-borne *Vibrio vulnificus* septicemia illnesses reported collectively by California, Florida, Louisiana, and Texas, from the consumption of commercially harvested raw or undercooked oysters to a level equivalent to a 60% illness rate reduction from 1995 – 1999 baseline average illness rate of 0.278 per million.~~

~~C.The Source State's *Vibrio vulnificus* Risk Management Plan shall include, at a minimum:~~

~~(1)The ISSC Consumer Education Program targeted toward individuals who consume raw oysters and whose health condition(s) increase their risk for *Vibrio vulnificus* illnesses;~~

~~(2)A process to collect standardized information for each *Vibrio vulnificus* illness: including underlying medical conditions; knowledge of disease status; prior counseling on avoidance of high risk foods, including raw oysters; existence of consumer advisories at point of purchase or consumption; and, if possible, whether consumer was aware and understood the advisories;~~

~~(3)A standardized process for tracking products implicated in *Vibrio vulnificus* illnesses; and~~

~~(4)~~(1) ~~Identification and implementation of the controls, or equivalent controls, which produced an illness per serving equivalent to a 60% illness rate reduction in the core states.~~

@.05 *Vibrio vulnificus* Control Plan

A. Risk Evaluation

Each shellfish producing State that is not currently implementing a *Vibrio vulnificus* control plan shall conduct a *Vibrio vulnificus* risk evaluation annually. The evaluation shall consider each of the following factors, including seasonal variations in the factors, in determining the risk of *Vibrio vulnificus* infection from the consumption of shellfish harvested from the State's growing waters.

(1) In conducting the risk evaluation the State Authority will at a minimum consider the following:

(a) The number of *Vibrio vulnificus* cases etiologically confirmed and epidemiologically linked to the consumption of commercially harvested shellfish from the State; and

(b) Levels of *Vibrio vulnificus* in the growing waters and in shellfish, to the extent that such data exists; and

(c) The quantity of harvest from the area and its uses i.e.

shucking, half shell, PHP.

B. States which have previously met the illness threshold requiring a *Vibrio vulnificus* Control Plan will continue to maintain and implement a *Vibrio vulnificus* Control Plan.

C. All States not currently implementing a *Vibrio vulnificus* Control Plan shall develop and implement a *Vibrio vulnificus* Control Plan should the risk evaluation indicate two (2) or more etiologically confirmed, and epidemiologically linked *Vibrio vulnificus* septicemia illnesses from the consumption of commercially harvested raw or undercooked oysters that originated from the growing waters of that state within the previous ten (10) years

D. The State shall develop a *Vibrio vulnificus* Contingency Plan should the risk evaluation indicate:

(1) Any etiologically confirmed shellfish-borne *Vibrio vulnificus* illness from the growing waters of that State but the number of cases does not reach the threshold established in @.04 C; and

(2) Information on Levels of *Vibrio vulnificus, if available* in the growing waters or in shellfish that is reasonably likely to cause an illness;

E. Control Plan

(1) The *Vibrio vulnificus* Control Plan shall include the following:

(a) Identification of triggers which address factors that affect risks. The triggers will be used to indicate when control measures are needed. One or more of the following triggers will be used:

(i) The water temperatures in the area; and

(ii The air temperatures in the area; and

(iii) Salinity in the area; and

(iv) Harvesting techniques in the area; and

(v) Other factors which affect risk which can be used as a basis for reducing risk.

(b) Implementation of one or more of the following control measures to reduce the risk of *Vibrio vulnificus* illness:

(i) Labeling oysters, "For shucking by a certified dealer", when the Average Monthly Maximum Water Temperature exceeds 70~~5~~°F.

(ii) Subjecting all oysters intended for the raw, half-shell market to Authority approved post harvest processing when the Average Monthly Maximum Water Temperature exceeds 70~~5~~°F.

~~(iii) Labeling oysters, "For shucking by a certified dealer", during the months of April through November, inclusive.~~

~~(iv) Subjecting oysters intended for the raw, half-shell market to Authority approved post harvest processing during the months of April through November, inclusive.~~

(iii~~v~~) Reducing time of exposure to ambient air temperature

prior to delivery to the initial certified dealer based on modeling or sampling, as determined by the Authority in consultation with FDA. For the purpose of time to temperature control, time begins once the first shellstock harvested is no longer submerged. When this control measure is selected, State *Vv* plans will include controls when water temperature promotes *Vv* levels and risk of illness increases. The controls will minimize risk to less than three (3) illnesses per 100,000 servings when water temperature exceeds 80°F. Authority approved Best Management Practices (BMPs) will be applied to minimize *Vv* growth to the extent possible when water temperature exceeds 70°F but is less than 80°F. BMPs will ensure that when the water temperature exceeds 70°F but is less than 75°F risk is minimized to less than 1.75 illnesses per 100,000 servings and when water temperatures exceed 75°F but are less than 80°F the risk will not exceed 2.5 illnesses per 100,000 servings. These risks per serving will be determined using the FDA developed *Vibrio vulnificus* calculator.

(iv~~vi~~) The State Authority may implement other comparable to that will reduce the risk per servings alternative controls that will reduce the risk to a level comparable to the risk per serving identified above in @.05 E. (1) (b) (iii) when water temperatures exceed 70°F.

(2) Control Plan Evaluation

(a) In consultation with FDA the Authority will evaluate the implementation and effectiveness of their Control Plan.
(i) Changes in the annual number of *Vibrio vulnificus* cases associated with the State's growing waters.
(ii) Environmental changes which could affect total *Vibrio vulnificus* in shellfish pre and post harvest.
(iii) Industry compliance with existing controls.
(iv) The Authorities enforcement of industries implementation of the controls.

(b) The Control Plan shall be modified when the evaluation shows the Plan is ineffective, or when new information or more effective technology is available as determined by the Authority.

F. Contingency Plan

(1) The Contingency Plan shall include a detailed plan outlining the regulatory steps that will be implemented should the number of illnesses reach the threshold established for development and implementation of a *Vv* Control Plan.

(2) Contingency Plan Evaluation
In consultation with FDA the Authority will evaluate the adequacy of their Contingency Plan.

@.0~~6~~5 *Vibrio parahaemolyticus* Control Plan

Guidance Documents, Chapter IV. Naturally Occurring Pathogens

~~.01 *Vibrio* Risk Management for Oysters Background~~

~~Current information concerning *Vibrio vulnificus*, which is responsible for several shellfish associated illnesses and deaths each year can be found in Watkins and McCarthy (1994).~~

~~A small number of shellfish-borne illnesses have also been associated with bacteria of the genus *Vibrio* (Bonner, 1983; Blake *et al.*,1979; Morris, 1985; Joseph *et al.*,1982; Roderick, 1982). The *Vibrios* are free-living aquatic microorganisms, generally inhabiting marine and estuarine waters (Joseph *et al*, 1982: Spira, 1984; Colwell 1984; Bachman, 1983). Among the marine *Vibrios* classified as pathogenic are strains of non-01 *Vibrio cholerae*, *V. parahaemolyticus*, and *V. vulnificus* (Bachman, 1983; Desmarchelier, 1984; Blake, 1980). All three species have been recovered from coastal waters in the United States and other parts of the world (Joseph, 1982; Colwell, 1984; Blake, 1980; DePoala, 1981; Madden, 1982; Davey, 1982; Oliver, 1983; Tamplin, 1982; NIH, 1984). These and other *Vibrios* have been detected in some environmental samples recovered from areas free of overt sewage contamination and coliform (Bonner, 1983; Joseph, 1982; Spira, 1984).~~

~~In general, shellfish-borne vibrio infections have tended to occur in coastal areas in the summer and fall when the water was warmer and vibrio counts were higher (Bonner, 1983; Morris, 1985; Joseph, 1982). *V. parahaemolyticus* and non-01 *V. cholerae* are commonly reported as causing diarrhea illness associated with the consumption of seafood including shellfish (Bonner, 1983; Blake, 1979; Morris, 1985; Joseph, 1982; Baross and Liston, 1970; Morris, 1981). In contrast, *V. vulnificus* has been related to two distinct syndromes: wound infections, often with tissue necrosis and bacteria, and primary septicemia characterized by fulminant illness in individuals with severe chronic illnesses such as liver disease, hemochromatosis, thalassemia major, alcoholism or malignancy (Bonner *et al.*, 1983; Tacket, 1984). Increasing evidence shows that individuals with such chronic diseases are susceptible to septicemia and death from raw seafood, especially raw oysters (Bonner *et al.*, 1983; Blake, 1979; Morris, 1985; Rodrick, 1982; Bachman, 1983; Blake, 1980; Oliver, 1983; NIH, 1984; Tacket, 1984; Oliver 1982; FDA, 1985). Shellfish borne vibrio infections can be prevented by cooking seafood thoroughly, keeping them from cross contamination after cooking, and eating them promptly or storing them at hot (60°C or higher) or cold (4°C or lower) temperatures. If oysters and other seafood are to be eaten raw, consumers are probably at lower risk to vibrio infection during months when seawater is cold than when it is warm (Blake, 1983 and 1984).~~

~~.02 *Vibrio vulnificus* Management Plan~~

~~The voting delegates at the 1999 Annual Meeting in New Orleans created the Vibrio Management Committee (VMC). Subsequently, *Vibrio vulnificus* and *Vibrio parahaemolyticus* subcommittees have been charged to develop appropriate illness control measures for these two pathogens. The VMC provides guidance and oversight to the subcommittees. Subcommittee recommendations are reviewed by the VMC before submittal to Task Forces. At the 2001 annual meeting, Task Forces reviewed the VMC's recommendation of reducing the rate of etiologically confirmed shellfish-~~

~~borne *Vibrio vulnificus* septicemia with the intention to submit the recommendation to the voting delegates. The goal is to reduce the rate of illness reported in California, Florida, Louisiana and Texas due to the consumption of commercially harvested raw or undercooked oysters by 40 percent, for years 2005 and 2006 (average) and by 60 percent for years 2007 and 2008 (average) from the average illness rate for the years 1995 – 1999 of 0.306/million. The list of states may be adjusted if after a thorough review, epidemiological and statistical data demonstrates that it would be appropriate. The rate of illness shall be calculated as the number of illnesses adjusted for population. This adjustment will be performed in consultation with statisticians and epidemiologists from California, Florida, Louisiana and Texas and Federal agencies. The baseline data and all future data for measuring illness reduction shall be the reported illnesses in the California, Florida, Louisiana and Texas for the period 1995 to 1999, inclusive, as compiled by the Southeast Regional Office of the U.S. Food and Drug Administration. The data used for measuring goal attainment shall begin with 2002 data. For the purpose of maintaining an accurate count of the number of illnesses report by each state (California, Florida, Louisiana and Texas), the following will apply:~~

~~(a) Illness cases counted are those reported by California, Florida, Louisiana and Texas;~~
~~(b) Each illness case is recorded under the state that reports it;~~
~~(c) Each case is not counted more than once; and~~
~~(d) In the event more than one report per case is filed, the case is recorded under the state of diagnosis.~~

~~The formula for calculating the rate of illness is as follows:~~

$$\frac{\text{number of cases}}{\text{population}}$$

~~The *Vv* subcommittee members will include, at a minimum, balanced representation from industry and state shellfish control authorities from *Vibrio vulnificus* Illness Source States California, Florida, Louisiana and Texas, FDA, NOAA, EPA, CDC, state epidemiologists; as well as industry and shellfish control representatives from other regions. *Vibrio vulnificus* Illness Source States are those states reporting two (2) or more etiologically confirmed shellfish borne *Vibrio vulnificus* illnesses since 1995 traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state. Etiologically confirmed means those cases in which laboratory evidence of a specific agent is obtained and specified criteria are met.~~

~~Recognizing the increasing importance and roles for the Committee, leadership will be expanded and structured in a similar manner as stated in the ISSC By-Laws for Task Forces (reference: ISSC By-Law, Article I Task Forces). The VMC Chair shall alternately be selected from a state shellfish control authority and from industry. The Board Chairman, with approval of the Board, shall appoint a VMC Chair and Vice-Chair. If the VMC Chair represents a state shellfish control authority, the Vice-Chair shall be an industry representative. At the end of the VMC Chair's term of office, the Vice-Chair will become Chairman and a new Vice-Chair will be appointed who represents the same segment of the Conference as the outgoing VMC Chair. A VMC Chair and Vice-Chair should be appointed before October 1, 2001 in order to be consistent with plans for annual VMC meetings and with the effective date of *Vibrio*~~

~~*vulnificus* Risk Management Plans. Likewise, the term of office shall be for (2) years.~~

~~The VMC will meet at least annually to develop and approve annual VMC work plans for *Vibrio vulnificus* illness reduction and review progress. A series of work plans, each covering a one-year period shall be adopted. The first work plan and progress review period will cover a seventeen-month period from August 1, 2001 to December 31, 2003 followed subsequently by annual work plans. Work plans will include goals, tasks, performance measures and assessment methods to track and achieve progress towards the illness reduction goals. The work plans will be developed by the VMC and approved by the VMC membership. The chair of the VMC will deliver a written annual progress report, including a summary of the previous year's progress made in the education program, to the ISSC March executive board meeting. The report shall be made available to the general membership. The annual work plan structure, outlined below, provides adaptive management and assures consistent progress towards the illness reduction goals. If annual assessment of progress towards achieving the illness rate reduction goals show inadequate progress the VMC shall incorporate actions into current and subsequent work plans to assure success in achieving those goals. In addition, if annual review shows inadequate progress the VMC will develop issues for deliberation at the 2005 biennial meeting to consider actions such as:~~

- ~~increased educational efforts,~~
- ~~limited harvest restriction,~~
- ~~reduction in time from harvest to refrigeration,~~
- ~~phased-in post-harvest treatment requirements, or~~
- ~~other equivalent controls.~~

~~Work plans developed by the VMC shall include the following elements and shall define the administrative procedures and resources necessary for accomplishment (i.e. establishment and maintenance):~~

~~(a) An ISSC Consumer Education Program targeted toward individuals who consume raw oysters and whose health condition(s) increase their risk for *Vibrio vulnificus* infection. The Education Program's objectives will be 1) to increase the target audience's awareness that eating raw, untreated oysters can be life-threatening to them, and; 2) to change the at-risk group's oyster-eating behavior, i.e., to reduce or stop eating raw, untreated oysters. The ISSC Vibrio Management Committee and the *Vibrio vulnificus* Education Subcommittee will evaluate Year 2001 survey results and compare them with the Year 2003 or 2004 survey results to determine the effectiveness in meeting the two objectives of the *Vv* education effort: (1) Show 40% increase in awareness of risk from *Vv*; and (2) Show 15% increase in at-risk consumers no longer eating raw oysters while minimizing impacts to non-at-risk consumer raw oyster consumption.~~

~~(i) The Consumer Education Program will focus educational efforts in California, Florida, Louisiana and Texas. The Education Program will make educational materials available to additional states upon request.~~

~~(ii) Educational approaches will emphasize partnerships with health and advocacy organizations, and include dissemination of printed materials, posting materials on the Internet, broadcast of television~~

~~spots, press releases, and other measures deemed effective such as the USDA Physician Notification Program.~~

~~(iii) Survey assessments at the state level shall be used as a means of assessing the baseline knowledge and effectiveness of educational interventions.~~

~~(b) Administration of a survey to determine the current *Vibrio vulnificus* disease reporting and education in each state.~~

~~(c) Creation of a working group to work cooperatively with local, state, and federal agencies and programs to assist in the collection of environmental and epidemiological data to further expand on the current information available. A coordinator may be utilized to facilitate the activities of this working group to develop standardized collection of environmental and epidemiological information from harvest to consumer.~~

~~(d) The Voting Delegates at the 2007 Biennial Meeting in Albuquerque, New Mexico approved appointment of a committee that will consist of three (3) epidemiologists and advisors as appropriate. The Committee will use this form to screen cases for the purposes of determining if a case is attributable to a single source state as well as whether the case is includable in the Vv Illness Reduction Goals. In addition, to ensure uniformity, the form shall be used for screening 2007-2008 cases and that cases from the baseline will be screened using the same form.~~

~~**Criteria FOR INCLUDING *Vv* CASES IN ILLNESS REDUCTION CALCULATIONS and determining source states**~~

~~1.Each case that is considered must be reported on a Center for Disease Control and Prevention Cholera and Other *Vibrio* Illness Surveillance Report (COVIS) Form CDC 52.79.~~

~~2.Each case must also be listed be on the FDA database (NSSP Guide for the Control of Molluscan Shellfish Guidance Documents Chapter IV .02).~~

~~3.The ISSC committee to review reported *Vv* illnesses to determine the appropriateness of inclusion into the database used for illness reduction calculations must have access to the COVIS form for each case (patient names and other necessary information appropriately redacted). The ISSC addendum form is also provided, where available. This access to the COVIS form is critical for adequate interpretation of the data collected during the state epidemiological investigation.~~

~~4.The ISSC *Vv* Illness Review Committee will complete the following criteria table for each case. These tables serve as documentation.~~

~~5.For cases to be included in illness reduction calculations the following criteria must be met:~~

- ~~Item 1-4 and 5a must be answered yes.~~
- ~~Should the COVIS form include information that suggests other exposures that may be responsible for the Vv illness further investigation may occur. Consultation with State Shellfish Control Authorities and Epidemiologist from the state is encouraged to determine which exposure should be recorded as the cause of illness. Should oyster consumption not be determined to be the cause of~~

illness the case will not be counted. Should there be disagreements with the inclusion of a case; the disagreeing party may request a review. The request must include a rationale for the review and should be addressed to the Executive Board Chairman.

- If 5b is no, other exposures should be considered. If no other exposures exist, the case will not be counted.
- Should the only exposure be consumption of cooked oysters or unknown 5b will be checked yes.

Vibrio vulnificus Criteria Table			
Case Identifier / Number _____	**Criteria Status Determination**		
Criteria	**Yes**	**No**	**Unknown**
1. Etiologically Confirmed	☐	☐	☐
2. Septicemia Illness	☐	☐	☐
3. Reporting State (CA, FL, LA, TX)	☐	☐	☐
4. Commercial Harvest from US Production	☐	☐	☐
5. Exposures			
a. Onset Consistent with Consumption of Oysters	☐	☐	☐
b. Raw or undercooked oysters	☐	☐	☐
6. Traceback Information			
a. Were shipping tags available or was other traceback information reported	☐	☐	☐
b. State of harvest and harvest area (s)			☐
c. Harvest date (s)			☐
7. Case Determination			
a. Is case included in Vv illness reduction Calculations	☐	☐	
b. Is case attributed to a single source state	☐	☐	

Instructions for completing Criteria Table:

- Check YES if Criterion is confirmed from the COVIS form or addendum.
- Check NO if Criterion is not confirmed from the COVIS form or addendum.
- Check UNKNOWN if Criterion is not clear or absent from the COVIS form or addendum.
- No Criterion can have more than one check entered.
- Each Criterion must have one check entered (YES, NO, or UNKNOWN).

These criteria tables will be used to review reported *Vv* illnesses to determine the appropriateness of inclusion into the database used for illness reduction calculations and will also be used for identifying other source states.

(e) Industry-implemented post-harvest controls to reduce *Vibrio vulnificus* levels in oyster shellstock which may include: time-temperature, post harvest treatment (i.e. hydrostatic pressure, cool pasteurization, IQF, and irradiation--pending approval), rapid chilling and other emerging technologies.

(f) Pursuit of ISSC options such as industry education and communication; FDA label incentives; PHT specific growing area classifications; targeted time/temperature assessment by FDA during annual shellfish program evaluations; assistance, as necessary, for the further study and possible implementation of dockside icing to investigate its effects on shelf life and variations in the effectiveness of the method as a result of seasonal and regional differences and incentives to add refrigeration capacity to harvest vessels. The goal will be to provide incentives necessary to post-harvest treat 25 percent of all oysters intended for the raw, half-shell market during the

~~months of May through September harvested from a Source State by the end of the third year (December 31, 2004). The assessment will include the capacity of all operational plants and the capacity of plants under construction. Should the 25 percent goal not be accomplished, the VMC will investigate and report their findings as to why the goal was not reached.~~

~~(g) Development by the VMC of a list of issues relating to public health, various technologies including Post-harvest treatments; marketability; shelf -life and similar matters that lend themselves to investigation. The VMC will work with FDA, NOAA, CDC, EPA, the shellfish industry and other entities as appropriate to obtain or facilitate the investigation of the issues listed and take the results into account as it develops plans or recommended Issues for the ISSC.~~

~~(h) Provision for VMC compilation and review of the data on rates of illness, which will be made available to the ISSC at the ISSC Biennial meeting following the year in which the data was gathered. In the event that the data is not available at the time of the meeting, the VMC shall meet and review the data when it becomes available and issue a compilation report, which will be made available to the entire ISSC membership. In the event there is no Biennial meeting scheduled for a certain year, the VMC shall meet and review the data when it becomes available and issue a compilation report which will be made available to the entire membership.~~

~~(i) Provision for a VMC evaluation of the effectiveness of reduction efforts, which will be conducted at the end of the fifth year (December 31, 2006). The evaluation will determine whether the 40 percent, 5-year goal to reduce the rate of illness or education/consumer intervention or post harvest controls performance measures set forth in prior work plans have been achieved. Should the VMC evaluation indicate the 40 percent, 5 year goal has not been accomplished, the committee will identify additional harvest controls in the 2007 - 2008 work plan to assure achievement of the 60 percent reduction in the rate of illness goal by the close of the seventh year. In addition, the VMC will evaluate the requirements in Section 04.C. with the possibility of changing the controls to achieve remaining illness reduction goals.~~

~~(j) Should a disagreement arise between FDA and the Authority on the equivalency of a control as described in .04(C), the V.v. Subcommittee will be requested to provide guidance.~~

~~(k) In 2006 the Executive Board directed the elimination of the Vv & Vp subcommittees. The VMC assumed all responsibilities of the subcommittees as outlined in the Vibrio vulnificus Management Guidance Document. Representation on the VMC Committee will be consistent with all guidance (VMC and Vv subcommittee) outlined in the Vibrio vulnificus Management Guidance Document.~~

~~(l) Shellstock Harvested in Source States Harvesters must include on the tag of all product harvested for restricted use the statement "for shucking by a certified dealer" and/or "For PHP Only." Harvesting controls must be provided by the Authority to ensure that restricted use shellstock is not diverted to retail or food service. Dealers must establish a restricted use shellstock Critical Limit~~

~~as part of their HACCP Plan for receiving. A shipping Critical Control Point must include a restricted use shellstock disposition step. Restricted use shellstock is not intended for retail or food service.~~

~~Should a disagreement arise between FDA and the Authority on the equivalency of a control as described in .04(C), the V.v. Subcommittee will be requested to provide guidance.~~

~~In 2006 the Executive Board directed the elimination of the Vv & Vp subcommittees. The VMC assumed all responsibilities of the subcommittees as outlined in the Vibrio vulnificus Management Guidance Document. Representation on the VMC Committee will be consistent with all guidance (VMC and Vv subcommittee) outlined in the Vibrio vulnificus Management Guidance Document.~~

.0~~1~~3 *Vibrio parahaemolyticus* Control Plan
.0~~2~~4 Post Harvest Processing Validation Verification Interim Guidance for *Vibrio vulnificus* and *Vibrio parahaemolyticus*
.0~~3~~5 Guidance for Demonstrating the Effectiveness of Time to Temperature Reduction Criteria for *Vibrio vulnificus* and *Vibrio parahaemolyticus*

Public Health Significance:

The level of *Vv* in oysters at the time of harvest can cause illness in immuno compromised individuals with increased susceptibility. This risk ranges from approximately .06 to 3.33 illnesses per 100,000 servings depending upon water temperature. The controls presently required by State *Vibrio vulnificus* Control Plans, if properly implemented, can reduce growth and reduce *Vibrio vulnificus* levels after harvest.

Changes will provide additional options for managing the risks associated with *Vv*. These options will not require Post Harvest Processing (PHP) controls which are presently not economically feasible. The RTI Economic Study suggested that it would take 2 to 3 years to implement PHP and, even with that time for implementation, would create a significant economic burden.

References:
(1) VMC Committee Reports (Al Rainosek's updated illness rate Calculations);
(2) RTI International Report Project Number 0211460.008
(3) "Analysis of How Post-harvest processing Technologies for Controlling Vibrio vulnificus Can Be Implemented"; Dr. Steve Otwell, Laura Garrido,Victor Garrido and Dr.Charlie Sims report "Sensory Assessment Study for Post -Harvest Processed (PHP) Oysters

Cost Information: (if available)

Action by 2011 Task Force II

Refer to Proposal 11-201 Pages 328 and 329.

Action by 2011 General Assembly

Refer to Proposal 11-201 Pages 335 and 336.

Action by FDA February 26, 2012

Refer to Proposal 11-201 Pages 336 – 338.

Proposal Subject: Transportation and Critical Control Points

Specific NSSP Guide Reference:
Section II Model Ordinance, Chapter IX. Transportation
Section II Model Ordinance, Chapter XI. Shucking and Packing
Section II Model Ordinance, Chapter XII. Repacking of Shucked Shellfish
Section II Model Ordinance, Chapter XIII. Shellstock Shipping
Section II Model Ordinance, Chapter XIV. Reshipping

Key Words: *Vibro vulnificus*Risk Management

Text of Proposal/ Requested Action:

Recommended Changes to Chapter IX. Transportation

Requirements for the Harvester/Dealer.

.01 Trucks or Other Vehicles Used to Transport Shellstock to the Original Dealer.

A. The harvester, or dealer who transports shellstock from the harvester to the original dealer, shall assure that all trucks used to transport shellstock are properly constructed, operated, and maintained to prevent contamination, deterioration, and decomposition.
B. Storage bins on trucks or other vehicles used in the transport of shellstock for direct marketing shall be:
   (1) Kept clean with potable water or water from an approved area or conditionally approved area in the open status; and
   (2) Provided with effective drainage.
C. Shellstock shall be transported in adequately refrigerated trucks or iced when the shellstock have been previously refrigerated or when ambient air temperature and time of travel are such that unacceptable bacterial growth or deterioration may occur.
D. Prechilling trucks or other vehicles to 45º or below shall be required when ambient air temperatures are such that unacceptable bacterial growth or deterioration may occur.
E. When mechanical refrigeration units are used, the units shall be:
   (1) Equipped with automatic controls; and
   (2) Maintained at an ~~Capable of maintaining the~~ ambient air temperature in the storage area at temperatures of 45º Fahrenheit (7.2º Centigrade) or less in the storage area
F. Any ice used to cool shellstock during transport shall meet the requirements of Chapter XI.02A~~(~~. (2).
G. Cats, dogs, and other animals shall not be allowed in any part of the truck or other vehicle where shellstock is stored.

.02 Receiving Shellfish

A. The dealer shall reject or discard any shellfish shipments which:
   (1) Do not originate from a licensed harvester or dealer; and/or
   (2) Are unwholesome, inadequately protected, or whose source cannot be identified.
B. Transportation agents or common carriers used by a dealer are not required to be certified.

C. The dealer shall:
(1) Inspect incoming shellfish shipments to assure that the shipments are received under the conditions required in this Chapter;
(2) Place shellstock under temperature control within 2 hours after receipt from the harvester, or when the dealer is also the harvester, when shellstock reaches the dealer's facility;
(3) Ensure that shellstock are not permitted to remain without ice, mechanical refrigeration, or other approved means of lowering the internal body temperature of the shellstock to, or maintaining it at, 50° Fahrenheit (10° Centigrade) or less for more than 2 hours at points of transfer such as loading docks;
(4) Ensure that shucked shellfish and in-shell product are not permitted to remain without ice, mechanical refrigeration, or other approved means of maintaining shellfish temperature at 45° Fahrenheit (7.2° Centigrade) or less; and
(5) Ensure that frozen shellfish remain frozen.
D. For the purpose of this section, temperature control is defined as the management of the environmental temperature of the shellstock by means of ice, mechanical refrigeration or other means approved by the Authority.

.05 Shipping Times.

A. Shipping Time is No More Than Four Hours.
(1) When the shipping time is four hours or less, the dealer shall ship all shellfish:
(a) Well iced; or
(b) Using other acceptable means of refrigeration.
(2) When mechanical refrigeration units are used, the units shall be equipped with automatic controls and shall be ~~capable of~~ maintain~~ed~~ing ~~the ambient air in the storage area~~ at temperatures of 45° Fahrenheit (7.2°Centigrade) or less in the storage area.
(3) The dealer shall not be required to provide thermal recorders during shipment.
(4) Lack of ice or other acceptable types of refrigeration shall be considered an unsatisfactory shipping condition.
B. Shipping Time is Greater Than Four Hours.
(1) When the shipping time is greater than four hours, the dealer shall ship all shellfish in:
(a) Mechanically refrigerated conveyances ~~which are equipped with automatic controls and capable of maintaining the ambient air in the storage area~~ at temperatures of 45° Fahrenheit (7.2° Centigrade) or less in the storage area; or
(b) Containers with an internal ambient air temperature maintained at or below temperatures of 45° Fahrenheit (7.2° Centigrade) or less.
(2) Unless the dealer has an approved HACCP plan with an alternate means of monitoring time-temperature, the initial dealer shall assure that a suitable time-temperature recording device accompanies each shipment of shellfish.
(3) The initial dealer shall note the date and time on the temperature-indicating device, if appropriate.
(4) Each receiving dealer shall write the date and time on the temperature-indicating device, if appropriate, when the shipment is received and

the doors of the conveyance or the containers are opened.

(5) The final receiving dealer shall keep the time-temperature recording chart or other record of time and temperature in his files and shall make it available to the Authority upon request.

(6) An inoperative temperature-indicating device shall be considered as no recording device.

Recommended Changes to Chapters XI. Shucking and Packing

Requirements for Dealers.
.01 Critical Control Points.

A. Receiving Critical Control Point - Critical Limits. The dealer shall shuck and pack only:

(1) Shellstock obtained from a licensed harvester who has:

(a) Harvested the shellstock from an Approved or Conditionally Approved area in the open status as indicated by the tag; and [C]

(b) Identified the shellstock with a tag on each container or transaction record on each bulk shipment; or [C]

(2) Shellstock obtained from a dealer other than the original harvester who has:

(a) Shipped the shellstock adequately iced; or in a conveyance at or below 45°F (7.2°C) ambient air temperature; and~~or~~ 50°F (10°C) internal temperature or less; ~~or in a conveyance capable of lowering the temperature of the shellstock and will maintain it at 50°F (10°C) or less;~~ [C]; and

(b) Identified the shellstock with a tag on each container or transaction record with each bulk shipment. [C]

(3) In-shell product obtained from a dealer who has:

(a) Shipped the in-shell product adequately iced; or in a conveyance at or below 45°F (7.2°C) ambient air temperature; or 45°F (7.2°C) internal temperature or less; and [C]

(b) Identified the in-shell product with a tag on each container [C]

B. Shellstock Storage Critical Control Point - Critical Limits. The dealer shall ensure that:

(1) If wet storage in artificial bodies of water is practiced, water quality meets the requirements outlined in Chapter X.08; and **[C]**

(2) Once placed under temperature control and until sale to the processor or final consumer, shellstock shall be;

(a) Iced; or **[C]**

(b) Placed and stored in a storage area or conveyance maintained at 45° F (7.2° C) or less; and **[C]**

(c) Not permitted to remain without ice, mechanical refrigeration or other approved methods of refrigeration, as required in **§**B (1) or **§**B (2) for more than 2 hours at points of transfer such as loading docks. **[C]**

C. In-shell Product Storage Critical Control Point - Critical Limits. The dealer shall ensure that in-shell product shall be:

(1) Iced; or **[C]**

(2) Placed and stored in a storage area or conveyance maintained at 45°F (7.2°C) or less. **[C]**

D. Processing Critical Control Point - Critical Limits. The dealer shall ensure that:
   (1) For shellstock which has not been refrigerated prior to shucking, shucked meats are chilled to an internal temperature of 45° F (7.2° C) or less within three hours of shucking. **[C]**
   (2) For shellstock refrigerated prior to shucking, shucked meats are chilled to an internal temperature of 45° F (7.2° C) or less within four hours of removal from refrigeration. **[C]**
   (3) If heat shock is used, once heat shocked shellstock is shucked, the shucked shellfish meats shall be cooled to 45° F (7.2° C) or less within two hours after the heat shock process. **[C]**
   (4) When heat shock shellstock are cooled and held under refrigeration for later shucking, the heat shocked shellstock shall be cooled to an internal temperature of 45° F (7.2° C) within two hours from time of heat shock. **[C]**
   (5) For in-shell product the internal temperature of meats does not exceed 45°F (7.2°C) for more than 2 hours during processing. **[C]**

E. Shucked Meat Storage Critical Control Point - Critical Limit. The dealer shall store shucked and packed shellfish in covered containers at an ambient temperature of 45° F (7.2° C) or less or covered with ice. **[C]**

~~F. Shellstock Shipping Critical Control Point.~~
   ~~(1) The dealer shall ensure that Shellstock that is received bearing a restricted use tag shall only be shipped to a certified dealer and shall include specific language detailing the intended use of the shellstock.~~

Recommended Changes to Chapter XII. Repacking of Shucked Shellfish
.01 Critical Control Points.

A. Receiving Critical Control Point - Critical Limits. The dealer shall repack only shellfish which:
   (1) Originated from a dealer who has:
      (a) Shipped the shellfish iced, or in a conveyance at or below 45°F (7.2°C) ambient air temperature; **[C]** and
      (b) Identified the shellfish with a label as outlined in Chapter X.06. **[C]**

B. Processing Critical Control Point - Critical Limits. The dealer shall ensure that repacked shucked shellfish do not exceed an internal temperature of 45° F (7.2° C) for more than 2 hours. **[C]**

C. Shucked Meat Storage Critical Control Point - Critical Limit. The dealer shall store repacked shellfish in covered containers at an ambient temperature of 45° F (7.2° C) or less or covered in ice. **[C]**

D. Shellstock Shipping Critical Control Point Shellstock that is received bearing a restricted use tag shall only be shipped to a certified dealer and shall include specific language detailing the intended use of the shellstock.

Recommended Changes to Chapter XIII. Shellstock Shipping
.01 Critical Control Points.

A. Receiving Critical Control Point - Critical Limits. The dealer shall ship or repack only:
   (1) Shellstock obtained from a licensed harvester who has:
      (a) Harvested the shellstock from an Approved or Conditionally Approved area in the open status as identified by the tag; and **[C]**
      (b) Identified the shellstock with a tag on each container or transaction record on each bulk shipment; or **[C]**
   (2) Shellstock obtained from a dealer other than the original harvester who has:
      (a) Shipped the shellstock adequately iced, or in a conveyance at or below 45°F (7.2°C) ambient air temperature and~~or~~ 50°F (10°) internal temperature ~~or less; or in a conveyance capable of lowering the temperature of the shellstock and will maintain it at 50°F (10°) or less **[C]**;~~ and
      (b) Identified the shellstock with a tag on each container. **[C]**
   (3) In-shell product obtained from a dealer who has;
      (a) Shipped the in-shell product adequately iced; or in a conveyance or at or below 45°F (7.2°C) ambient air temperature; or 45°F (7.2°C) internal temperate or less; and **[C]**
      (b) Identified the in-shell product with a tag on each container. **[C]**

B. Receiving Critical Control Point - Critical Limits. The dealer shall ship or repack only:
   (1) Shellstock obtained from a licensed harvester who has:
      (a) Harvested the shellstock from an Approved or Conditionally Approved area in the open status as identified by the tag; and **[C]**
      (b) Identified the shellstock with a tag on each container or transaction record on each bulk shipment; or **[C]**
   (2) Shellstock obtained from a dealer other than the original harvester who has:
      (a)Shipped the shellstock adequately iced, or in a conveyance at or below 45°F (7.2°C) ambient air temperature or 50°F (10°) internal temperature or less; or in a conveyance capable of lowering the temperature of the shellstock and will maintain it at 50°F (10°) or less **[C]**; and
      (b)Identified the shellstock with a tag on each container. **[C]**
   (3) In-shell product obtained from a dealer who has:
      (a) Shipped the in-shell product adequately iced; or in a conveyance or at or below 45°F (7.2°C) ambient air temperature; or 45°F (7.2°C) internal temperate or less; and **[C]**
      (b) Identified the in-shell product with a tag on each container **[C]**

C. Shellstock Storage Critical Control Point - Critical Limits. The dealer shall ensure that:
   (1) If wet storage in artificial bodies of water is practiced, water quality meets the requirements outlined in Chapter X.08; and **[C]**
   (2) Once placed under temperature control and until sale to the processor or final consumer, shellstock shall be:
      (a) Iced; or **[C]**

(b) Placed in a storage area or conveyance maintained at 45° F (7.2° C) or less; and **[C]**

(c) Not permitted to remain without ice, mechanical refrigeration or other approved methods of refrigeration, as required in §~~B(~~B (1) or §B (2) for more than 2 hours at points of transfer such as loading docks. **[C]**

D. In-shell Product Storage Critical Control Point - Critical Limits. The dealer shall ensure that in-shell product shall be:

(1) Iced; or **[C]**

(2) Placed and stored in a storage area or conveyance maintained at 45°F (7.2°C) or less. **[C]**

E. Shellstock Shipping Critical Control Point

(1) Shellstock that is received bearing a restricted use tag shall only be shipped to a certified dealer and shall include specific language detailing the intended use of the shellstock.

(2) Should a State be implementing a *Vibrio parahaemolyticus* or *Vibrio vulnificus* Control Plan the dealer shall only ship shellstock that has been cooled to the temperature outlined in the State Plan.

Recommended Changes to Chapter XIV. Reshipping
.01 Critical Control Points.

A. Receiving Critical Control Point - Critical Limits. The dealer shall reship only shellfish which:

(1) Originated from a dealer other than the original harvester who has:

(a) Shipped the shellstock adequately iced; or in a conveyance at or below 45°F (7.2°C) ambient air temperature; and~~or~~ 50°F (10°C) internal temperature or less; ~~or in a conveyance capable of lowering the temperature of the shellstock and will maintain it at 50°F (10°C) or less; [C];~~ and/or

(b) Shipped the shucked shellfish and/or in-shell product iced or in a conveyance at or below 45°F (7.2°C) ambient air temperature; **[C]** and

(c) Identified the shellstock with a tag as outlined in Chapter X.05, identified the in-shell product with a tag as outlined in Chapter X .07, and/or identified the shucked shellfish with a label as outlined in Chapter X.06. **[C]**

Shellstock Shipping Critical Control Point Shellstock that is received bearing a restricted use tag shall only be shipped to a certified dealer and shall include specific language detailing the intended use of the shellstock.

Public Health Significance:

The present *Vv* and *Vp* Control Plans of the Model Ordinance include time to temperature controls which require that shellstock be cooled and maintained at specific temperatures to limit post harvest growth of *Vv* and *Vp*. For these controls to be effective it is imperative that the shellstock be maintained at the temperatures outlined in the Control Plans. The proposed changes to Chapter IX., XI., XIII., and XIV. are intended to modify present requirements to ensure that these temperatures are maintained. Recent FDA audits of *Vv* and *Vp* Control Plan compliance and reports from States and the industry suggest that these modifications are necessary.

Cost Information:
(if available)

Action by 2011 Task Force II	Refer to Proposal 11-201 Pages 335 and 336.
Action by 2011 General Assembly	Refer to Proposal 11-201 Pages 335 and 336.
Action by FDA February 26, 2012	Refer to Proposal 11-201 Pages 336 – 338.

Proposal Subject: *Vibrio vulnificus* and *Vibrio parahaemolyticus* Risk Management of Oysters

Specific NSSP Guide Reference: Section II Model Ordinance, Chapter II Risk Assessment and Risk Management
@.01 Outbreaks of Shellfish Related Illnesses
@.04 *Vibrio vulnificus* Risk Management for Oysters

Text of Proposal/ Requested Action: Chapter II Risk Assessment and Risk Management

@.01 Outbreaks of Shellfish Related Illnesses

J. The Authority shall assess annually *Vibrio vulnificus* and *Vibrio parahaemolyticus* illnesses associated with the consumption of molluscan shellfish. The assessment will include a record of all *V. vulnificus* and/or *V. parahaemolyticus* shellfish-associated illnesses reported within the state and from receiving states, the numbers of illnesses per event, and actions taken by the Authority in response to the illnesses.

Effective January 1, 2012:

@.04 *Vibrio vulnificus* and *Vibrio parahaemolyticus* Risk Management for Oysters

A. For states having 2 or more etiologically confirmed shellfish-borne *Vibrio vulnificus* or *Vibrio parahaemolyticus* illnesses ~~since 1995~~ within ~~a~~the prior five (5) years traced to the consumption of commercially harvested raw or undercooked oysters that originated from ~~the waters~~ a growing area of that state (Source State), the Authority shall develop and implement *a Vibrio vulnificus* and/or *Vibrio parahaemolyticus* ~~Risk Management~~ Control Plan.

~~B. The Source State's *Vibrio vulnificus* Risk Management Plan shall define the administrative procedures and resources necessary to accomplish (i.e. establish and maintain) involvement by the state in a collective illness risk reduction program. The goal of the *Vibrio vulnificus* Risk Management Plan will be to reduce the risk per serving to a 60% illness rate reduction for etiologically confirmed shellfish-borne *Vibrio vulnificus* septicemia illnesses reported collectively by California, Florida, Louisiana, and Texas, from the consumption of commercially harvested raw or undercooked oysters to a level equivalent to a 60% illness rate reduction from 1995 – 1999 baseline average illness rate of 0.278 per million.~~

~~C.~~B. The goal of the Control Plan is to reduce the probability of occurrence of *Vibrio* illness during periods that have been historically associated with annual illnesses. The Plan is to be implemented as part of a comprehensive program which includes all the time and temperature requirements contained in the Model Ordinance. The Source State's *Vibrio ~~vulnificus~~* ~~Risk Management~~ Control Plan shall include, at a minimum:

(1) The ISSC Consumer Education Program targeted toward individuals who consume raw oysters and whose health condition(s) increase their risk for *Vibrio vulnificus* illnesses; and

~~(2)A process to collect standardized information for each *Vibrio vulnificus* illness: including underlying medical conditions; knowledge of disease status; prior counseling on avoidance of high risk foods, including raw oysters; existence of consumer advisories at point of purchase or consumption; and, if possible, whether consumer was aware and understood the advisories;~~

(2) A standardized process for tracking products implicated in ~~*Vibrio vulnificus*~~ illnesses.~~; and~~

~~(4) Identification and implementation of the controls, or equivalent controls, which produced an illness per serving equivalent to a 60% illness rate reduction in the core states.~~

~~@~~**~~.05 *Vibrio parahaemolyticus* Control Plan~~**

~~The goal of the Control Plan is to reduce the probability of occurrence of *Vibrio parahaemolyticus* illness during periods that have been historically associated with annual illnesses. The Plan is to be implemented as part of a comprehensive program which includes all the time and temperature requirements contained in the Model Ordinance.~~

~~A.~~C. Risk Evaluation.

Every State from which oysters are harvested shall conduct a *Vibrio vulnificus* and a *Vibrio parahaemolyticus* risk evaluation annually. The evaluation shall consider each of the following factors, including seasonal variations in the factors, in determining whether the risk of *Vibrio* ~~*parahaemolyticus*~~ infection from the consumption of oysters harvested from an area (hydrological, geographical, or growing) is reasonably likely to occur: (For the purposes of this section, "reasonably likely to occur" shall mean that the risk constitutes an annual occurrence)

(1) The number of *Vibrio vulnificus* and *Vibrio parahaemolyticus* cases epidemiologically linked to the consumption of oysters commercially harvested from the State; and
(2) Levels of total and tdh+ *Vibrio parahaemolyticus* in the area, to the extent that such data exists; and
(3) The water temperatures in the area; and
(4) The air temperatures in the area; and
(5) Salinity in the area; and
(6) Harvesting techniques in the area; and
(7) The quantity of harvest from the area and its uses i.e. shucking, halfshell, PHP.

~~B.~~D. Control Plan

(1) If a State's *Vibrio vulnificus* and/or *Vibrio parahaemolyticus* risk evaluation determines that the risk of *Vibrio* ~~*parahaemolyticus*~~ illness from the consumption of oysters harvested from a growing area is reasonably likely to occur, the State shall develop and implement a *Vibrio* ~~*parahaemolyticus*~~ Control Plan; or
(2) For *Vibrio parahaemolyticus*, ~~I~~if a State has a shellfish growing area in which harvesting occurs at a time when average monthly daytime water temperatures exceed those listed below, the State shall develop and implement a *Vibrio parahaemolyticus* Control Plan. The average water temperatures representative of harvesting conditions (for a period not to exceed thirty (30) days) that prompt the need for a Control Plan are:
(a) Waters bordering the Pacific Ocean - 60°F.
(b) Waters bordering the Gulf of Mexico and Atlantic Ocean (NJ and

south) - 81°F.

(c) However, development of a Plan is not necessary if the State conducts a risk evaluation, as described in §~~A~~C., that determines that it is not reasonably likely that a ~~–~~*Vibrio parahaemolyticus* illness will occur from the consumption of oysters harvested from those areas.

(i) In conducting the evaluation, the State shall evaluate the factors listed in §~~A~~C. for the area during periods when the temperatures exceed those listed in this section;

(ii) In concluding that the risk is not reasonably likely to occur, the State shall consider how the factors listed in §~~A~~C**.** differ in the area being assessed from other areas in the state and adjoining states that have been the source of shellfish that have been epidemiologically linked to cases of *Vibrio parahaemolyticus* illness.~~; or~~

~~(3) If a State has a shellfish growing area that was the source of oysters that were epidemiologically linked to an outbreak of *Vibrio parahaemolyticus* within the prior five (5) years, the State shall develop and implement a *Vibrio parahaemolyticus* Control Plan for the area.~~

~~(4)~~ (3) For States required to implement *Vibrio ~~parahaemolyticus~~* Control Plans, the Plan shall include the administrative procedures and resources necessary to accomplish the following:

(a) Establish one or more triggers for when control measures are needed. These triggers shall be the temperatures in §~~B~~D. (2) where they apply, or other triggers as determined by the risk evaluation.

(b) Implement one or more control measures to reduce the risk of *Vibrio ~~parahaemolyticus~~* illness at times when it is reasonably likely to occur.

The control measures for *Vibrio vulnificus* may include:

(i) Labeling all oysters, "For shucking by a certified dealer", when the Average Monthly Maximum Water Temperature exceeds 75°F;

(ii) Subjecting all oysters intended for the raw, half-shell market to an Authority- approved post harvest processing that reduces the *Vibrio vulnificus* levels to <30 MPN/gram when the Average Monthly Maximum Water Temperature exceeds 75°F;

(iii) Closing shellfish growing areas for the purpose of harvest of oysters intended for the raw, half-shell market when the Average Monthly Maximum Water Temperature exceeds 75°F;

(iv) Labeling all oysters, "For shucking by a certified dealer", during the months of May through September, inclusive;

(v) Subjecting all oysters intended for the raw, half-shell market to a post harvest processing that is both approved by the Authority and reduces the *Vibrio vulnificus* levels to <30 MPN/gram during the months of May through September,inclusive;

(vi) Closing shellfish growing areas for the purpose of harvesting oysters intended for the raw, half-shell market during the months of May through September, inclusive; and

(vii) Limiting time from harvest to refrigeration based on modeling or sampling, as determined by the Authority in

consultation with FDA;

~~2.~~ The control measures for *Vibrio parahaemolyticus* may include:

(i) Post harvest processing using a process that has been validated to achieve a 2 log reduction in the levels of total *Vibrio parahaemolyticus* for Gulf and Atlantic Coast oysters and a 3 log reduction for the Pacific Coast oysters;
(ii) Closing the area to oyster harvest;
(iii) Restricting oyster harvest to product that is labeled for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing;
(iv) Limiting time from harvest to refrigeration to no more than five hours, or other times based on modeling or sampling, as determined by the Authority in consultation with FDA;
(v) Limiting time from harvest to refrigeration such that the levels of total *Vibrio parahaemolyticus* after the completion of initial cooling to 60 °F (internal temperature of the oysters) do not exceed the average levels from the harvest water at time of harvest by more than 0.75 logarithms, based on sampling or modeling, as approved by the Authority;
(vi) Other control measures that based on appropriate scientific studies are designed to ensure that the risk of *Vp* illness is no longer reasonably likely to occur, as approved by the Authority.

(c) Require the original dealer to cool oysters to an internal temperature of 50°F (10°C) or below within 10 hours or less as determined by the Authority after placement into refrigeration during periods when the risk of *Vibrio ~~parahaemolyticus~~* illness is reasonably likely to occur. The dealer's HACCP Plan shall include controls necessary to ensure, document and verify that the internal temperature of oysters has reached 50°F (10°C) or below within 10 hours or less as determined by the Authority of being placed into refrigeration. Oysters without proper HACCP records demonstrating compliance with this cooling requirement shall be diverted to PHP or labeled *"for shucking only"*, or other means to allow the hazard to be addressed by further processing.

(d) Evaluate the effectiveness of the Plan.

(e) Modify the Control Plan when the evaluation shows the Plan is ineffective, or when new information is available or new technology makes this prudent as determined by the Authority.

(f) Optional cost benefit analysis of the ~~*Vibrio parahaemolyticus*~~ Control Plan.

~~C~~ E. The Time When Harvest Begins

For the purpose of time to temperature control, time begins once the first

shellstock harvested is no longer submerged.

F. Evaluating Effectiveness of Plans

In consultation with FDA the Authority will evaluate the implementation of their control plan based on effective management and enforcement of control measures to reduce the risk of illnesses.

Public Health Significance:

Changes will provide options and improve the ability for State Shellfish Control Authorities and the shellfish industry to achieve realistic risk management related to naturally occurring *Vibrio* bacteria. It was clearly stated at the VMC meeting held in January 2011 that because of the low incidence of *Vv* illness the 60% reduction of *Vv* illnesses from the Gulf States is not attainable without post harvest processing (PHP) (1). The cost of having all product from the Gulf of Mexico post harvest processed is economically prohibitive to the industry (2) and PHP product is not desired by the oyster consuming public (3).

References: (1)VMC Committee Reports (Al Rainosek's updated illness rate Calculations); (2) RTI International Report Project Number 0211460.008 (3)"Analysis of How Post-harvest processing Technologies for Controlling Vibrio vulnificus Can Be Implemented"; Dr. Steve Otwell, Laura Garrido,Victor Garrido and Dr.Charlie Sims report "Sensory Assessment Study for Post -Harvest Processed (PHP) Oysters

Cost Information (if available):

Neutral

Action by 2011 Task Force II

Recommended no action on Proposal 11-202.

Rationale: Proposal was adequately addressed by Task Force II action on Proposal 11-201-A.

Action by 2011 General Assembly

Adopted recommendation of 2011 Task Force II on Proposal 11-202.

Action by FDA February 26, 2012

Concurred with Conference action on Proposal 11-202.

Al Rainosek

This will be discussed with GAO
Illness Rate Reduction

From: "Al Rainosek" <rainosek@jaguar1.usouthal.edu>
To: "alr" <Al.Rainosek@noaa.gov>
Sent: Monday, January 10, 2011 9:45 AM
Attach: Numbers!.eml
Subject: Fw: Updated Revised Illness Rate Calculations

Based on updated population estimates provided by the Bureau of Census, and reviewed/confirmed Vv cases for 2007 throught 2010 from ISSC, the revised Illness Rate Calculations for the Core States (CA, FL, LA, TX) appear below.

Notes: (1) Population numbers from the Bureau of Census Annual Estimates of the Resident Population for the United States, Regions, States and Puerto Rico.
(2) Number of countable Vv cases provided by Marc Glatzer, FDA.

A. BASELINE (Core States): 1995 - 1999
Total Countable Cases = 98
Av Cases per year = 98 / 5 = 19.6
Av Population per year = 70,637,188
Av Baseline Illness Rate = 19.6 / 70,637,188 = 0.278 per million

B. Illness Rate Reductions from Baseline:

1. 2007: 17 countable (reviewed) cases
Est Population = 82,717,833
Est Illness Rate = 17 / 82,717,833 = 0.206 per million
Estimated Illness Rate Reduction from Baseline:
(0.278 - 0.206) / 0.278 = 25.9%

2. 2008: 13 countable (reviewed) cases
Est Population = 83,760,052
Est Illness Rate = 13 / 83,760,052 = 0.155 per million
Estimated Illness Rate Reduction from Baseline:
(0.278 - 0.155) / 0.278 = 44.2%

3. 2007/2008 Average Illness Rate Reduction from Baseline:
Average Cases = 15
Est Average Population = 83,238,942
Est Av Illness Rate = 15 / 83,238,942 = 0.180 per million
Est Average Illness Rate Reduction from Baseline:
(0.278 - 0.180) / 0.278 = 35.0%

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4. 2009: 15 cases (reviewed)
Est. Pop'n = 84,774,011
Est. Illness Rate = 15 / 84,774,011 = 0.177 per million
Est Illness Rate Reduction from Baseline:
(0.278 - 0.177) / 0.278 = 36.3%

5. 2010: 14 cases (reviewed)
Est. Pop'n = 86,065,142
Est. Illness Rate = 14 / 86,065,142 = 0.163 per million
Est Illness Rate Reduction from Baseline:
(0.278 - 0.163) / 0.278 = 41.4%

6. 2009/2010 Est. Av IRR = 38.8%

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7. 2007 - 2010 Est Av IRR = 37.0%

1/10/2011

Cases from Illness review Sub-committee

Core State Vv Cases - Baseline & Measurable Years

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
1995	0	0	0	2	4	1	0	2	3	3	1	0	16
1996	0	0	0	1	2	4	3	6	4	1	1	0	22
1997	0	0	0	0	4	1	1	2	4	1	1	0	14
1998	0	0	0	1	3	3	2	4	1	5	1	0	20
1999	0	0	1	4	3	2	4	2	7	2	0	1	26
2007	0	0	1	5	3	0	2	3	1	1	1	0	17
2008	0	0	0	2	2	1	3	0	3	1	1	0	13
2009	0	0	0	1	0	2	0	3	2	2	5	0	15
2010	0	0	0	3	1	1	2	3	1	0	3	0	14
Total	0	0	2	19	22	15	17	25	26	16	14	1	157

NOTE: 1995-1999 cases represent the Baseline Cases; 2007-2010 cases represent the cases used to measure the Illness Rate Reduction. The Baseline Cases and 2007-2008 Cases have been reviewed by the ISSC Vv Illness Case Review Subcommittee and accepted by the ISSC Vibrio Management Committee (VMC) as countable cases. The 2009-2010 cases have been reviewed by the ISSC Vv Illness Case Review Subcommittee and will be submitted to the VMC for acceptance at the VMC meeting in January, 2011. In addition, CDC has not closed out the 2010 Vv case submission period, and there may be additional cases reported that will require review and acceptance by the ISSC. Assignment of a case to a month is made using the date of consumption. If the date of consumption is not known, date of onset will be used.

Sensory Assessment Study

for

Post-Harvest Processed (PHP) Oysters

Dr. Steve Otwell, Laura Garrido, Victor Garrido

and Dr. Charlie Sims

Aquatic Food Products Lab

Food Science and Human Nutrition Department

University of Florida

November 22, 2010

SENSORY ASSESSMENT STUDY
for
POST- HARVEST PROCESSED (PHP) OYSTERS

ACKNOWLEDGEMENTS

This project would not have been possible without collaborative assistance by numerous individuals with special talents and shared concerns for the oyster industry and consumer health. Participants included:

Charlene Burke, University of Florida, Apalachicola, FL. Served as technical advisor and assisted in coordination and logistics of product.

David Heil, FL Department of Agriculture and Consumer Service, Tallahassee, FL. Served as a regulatory advisor to the project.

Rick Hunter, Food Technology Service, Inc. Provided plant time and personnel for Gamma Irradiation (GI) oyster process.

Jim Jones, Food Technology Service, Inc. Provided plant time and personnel for Gamma Irradiation (GI) oyster process.

Grady Leavins, Leavins Seafood, Inc., Apalachicola, FL. Served as an industry advisor to the project.

William T. Mahan, Sea Grant College Program, Apalachicola, FL. Served as technical advisor and local liaison for the project.

Michael Voisin, Motivatit Seafood, Inc. Houma, LA. Provided plant time and personnel for high pressure (HP) oyster processing.

Tommy Ward, Buddy Ward and Sons Seafood, Inc. Apalachicola, FL. Provided access to the company's oyster lease and harvest vessel necessary for the product procurement.

T.J. Ward and Jordan Todd, Apalachicola, FL. Served as expert shuckers for the project.

Robert Webb, Webb's Seafood, Inc. Youngstown, FL. Provided plant time and personnel for Heat Intervention (HI) and Low Temperature Freezing (LTF) oyster processing.

SENSORY ASSESSMENT STUDY
for
POST- HARVEST PROCESSED (PHP) OYSTERS

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SENSORY ASSESSMENT STUDY
for
POST- HARVEST PROCESSED (PHP) OYSTERS

INTRODUCTION

Persistent concerns for illnesses associated with certain consumers eating raw oysters harvested during the warmer months about the Gulf of Mexico are calling for more use of post-harvesting processing (PHP) methods that reduce or eliminate the microbial culprits, *Vibrio vulnificus and Vibrio parahaemolyticus*. The PHP methods in question include validated operations involving the application of mild temperatures, gamma irradiation, high pressure, or low temperature freezing. These methods are in various stages of commercial use and they have been validated in accordance with required analytical protocols to verify the reduction and/or elimination of the naturally occurring yet potentially pathogenic *Vibrio* bacteria. The traditional processing methods for untreated oysters do not incorporate a similar bacteria kill step.

While the PHP methods can provide reduction of the bacterial concerns they can also introduce changes in the sensory attributes of the raw oysters that could influence consumer acceptance. The successful implementation of PHP methods will depend on consumer preferences and acceptance. This situation calls for a non-biased, science-based study to determine consumer preferences and acceptance for PHP versus traditional processed oysters destine for raw consumption.

RESEARCH OBJECTIVES

The objectives of this project were to measure consumer preference and acceptance for raw oysters from untreated, traditional (T) processing compared with each of the four PHP methods (MH- mild heat intervention, HP-high pressure, GI-gamma irradiation, and LTF-low temperature freezing) using live oysters from the same harvest based on paired comparison tests and acceptability ratings. The intent was to assess preferences and acceptance for traditional verses PHP oysters rather than comparisons amongst PHP products. Procedures incorporated shelf-life considerations during the consumer sensory testing to account for any changes in PHP product attributes versus the traditional raw oysters through two separate periods of storage common in summer commerce. In addition, the same traditional and respective PHP oyster products were formally evaluated for sensory characteristics (i.e., taste, aroma, color, and texture) based on the established oyster sensory profiling system developed for ISSC (http://fshn.ifas.ufl.edu/seafood). Sensory profiling can provide some explanation for any differences measured for consumer preference and acceptance.

METHODOLOGY

All project work depended on industry cooperation in procurement and processing of the raw oysters. The work was conducted in a manner to exemplify typical oyster processing and marketing practices through existing commercial operations. All oyster products and processing were subject to a chain of custody arrangement that included continuous participation and monitoring of all products by the project investigators from the moment of harvest through processing, distribution, storage and preparation for consumer testing and sensory profiling.

All oysters (*Crassosteria virginica*) were harvested from approved waters with existing commercial procedures (small vessel dredging) from one selected site in Apalachicola Bay, Florida. The site was a private lease maintained by owner, Tommy Ward, in Apalachicola, Florida and denoted by the official Florida state designation, FL-1632 L-525. Site selection involved pre-monitoring of weather conditions and on-site prescreening by the experienced project investigators to assure the live oysters had a consistent salt flavor not subject to freshwater exposure that tends to dilute flavors. The site selection was critical in terms of uniform product condition and quality. All oysters used in this study were from the same harvest site and could not be distinguished or culled by any quality differences at the moment of harvest.

The oysters were harvested in two installments, one on September 6, 2010 and one on September 7th, 2010 (Table 1). Each harvest consisted of 15 bushels (900 lbs) that were collected in the morning and delivered to an approved processing plant (Tommy Ward's; 13 Miles) for an initial wash to remove external mud and debris. The washing procedure was a simple, short time rinse through a typical stainless-steel tumbling unit that applied a water spray on the surface of the oysters. Product post-harvest handling achieved an internal product temperature below 50°F within 2 hours of delivery in accordance with harvest regulations stipulated by the Florida Department of Agriculture and Consumer Services regulations (FL DOACS, 2009).

The quantity of harvest from the selected site and time was determined by the required amount of product for testing and to assure a simultaneous period of processing through the traditional and all PHP procedures within 48 hours post-harvest. The anticipate product volume and flow of work allowed two periods for sensory assessments for shelf-life consequences after 7 and 14 days post-harvest.
The work plan is illustrated in Table 1.

Table 1. Illustration of the work schedule from harvest through respective processing methods and eventual consumer and expert assessments. Oysters for traditional (T) processing were collected simultaneously during each harvest September 6 and 7 (Harvest site – Apalachicola Bay, Florida site designation FL-1632 L-525)

<table>
<tr><th rowspan="2">Days in storage</th><th colspan="4">Post-Harvest Processing (PHP) Methods</th></tr>
<tr><th>HP</th><th>LTF</th><th>GI</th><th>MH</th></tr>
<tr><td>0</td><td colspan="2">Harvest and refrigeration</td><td>Harvest & transported to Panama City, FL - iced and processed (stored at 0°F)</td><td>Harvest & transported to Panama City, FL - iced</td></tr>
<tr><td>1</td><td>Transport to Houma, LA - iced</td><td>Transported to Mulberry, FL - iced</td><td>Transported to Gainesville, FL – Dry ice</td><td>Processed and transported to Gainesville, FL - iced</td></tr>
<tr><td rowspan="2">2</td><td>Processed and transported to Gainesville, FL - iced</td><td>Processed and transported to Gainesville, FL - iced</td><td rowspan="2">Frozen storage (0°F)</td><td rowspan="2">Refrigerated storage (35°F)</td></tr>
<tr><td colspan="2">Refrigerated Storage (35°F)</td></tr>
<tr><td>7</td><td colspan="2">Day 7 Sensory Evaluations & Expert Evaluations</td><td colspan="2">Day 7 Sensory Evaluations & Expert Evaluations</td></tr>
<tr><td>14</td><td colspan="2">Day 14 Sensory Evaluations & Expert Evaluations</td><td colspan="2">Day 14 Sensory Evaluations & Expert Evaluations</td></tr>
</table>

Oyster Processing Methods

The traditional (T) processing involved simple refrigerated storage in customary burlap oyster bags stored in refrigeration (35°F). Each PHP method was conducted in accordance with prior validated and published procedures currently available for commercial use. The protocol for PHP validation is specified and maintained by the National Shellfish Sanitation Conference (NSSP 2007a and 2007b). Each State Shellfish Certification Authority is responsible for the evaluation and approval of the PHP methods with concurrence from Food and Drug Administration (FDA).

High Pressure (HP): Refrigerated oysters were initially banded with plastic strips to maintain closure before placing in a cylindrical metal container that was filled with potable water and pressurized to approximately 36,000 PSI for 3 minutes. The treated oysters were then unloaded on a table for visual sorting and final packaging in an igloo cooler with ice, then transported to the University of Florida in Gainesville for storage

refrigerated (35°F) and sensory evaluations. These procedures were conducted at the validated and approved HP processing facilities of Motivatit Seafood in Houma, Louisiana.

Gamma Irradiation (GI): Oysters were banded with rubber bands to maintain closure while being held in 30 lbs waxed carton boxes that were placed on metal racks that carried the product into the irradiation chamber. The oysters were exposed to gamma radiation for a period of time necessary to achieve a minimum absorbed dose of 0.82 kGy as indicated by dosimeters placed on the waxed cartons. Treated product was then placed into an igloo cooler with ice, then transported to the University of Florida in Gainesville for refrigerated storage (35°F) and sensory evaluations. The irradiation procedures were conducted at processing facilitates maintained by Food Technology Services, Inc based in Mulberry, Florida. This is the same operation where the gamma irradiation procedures were validated for raw oyster PHP in December 2008.

Low Temperature Freezing (LTF): Oysters were manually shucked by removing the top shell, leaving the meat attached to the bottom shell. The half-shell product was placed on a conveyor belt that traveled through a nitrogen freezing tunnel set at an ambient temperature of – 170°F with a belt speed of 3 ft/minute. Product exiting the nitrogen tunnel was solidly frozen. A potable, cold-water glaze was applied on the top of each frozen oyster to provide protection against freezer burn and dehydration. All frozen oysters were placed in an igloo cooler with dry ice, then transported to the University of Florida in Gainesville for frozen storage (0°F) and sensory evaluations. The validated low temperature freezing operations were conducted at Webb's Seafood, Inc in Youngstown, Florida.

Mild Heat Treatment (MH): Oysters were banded with rubber bands to assure closure during submersion in a tank of water maintained at 150°F +/- 2°F. Product was kept in the warm water for approximately 5 minutes to achieve an internal temperature of 122°F for 1 - 2 minutes, then immediately placed in an ice slush for 2 minutes. The treated product was drained and placed in igloo coolers with ice, then transported to the University of Florida in Gainesville for refrigerated storage (35°F) and sensory evaluations. The mild heat interventions were conducted at Webb's Seafood, Inc in Youngstown, Florida. The mild heat interventions were based on prior work by Hesselman et al 1999.

Sample Preparation

The preparation of all oysters for sensory assessments was conducted in the Aquatic Food Products Lab at the University of Florida under supervision of the project investigators. All samples were presented in half-shell product form. Shucking was performed by professional oyster shuckers hired to assure the task was done correctly so as not to damage the oyster tissues and to present a whole edible oyster product with some accompanying "liquor" or product fluids that are commonly associated with the consumption of half-shell oysters. Shucking involved carefully severing of the adductor muscles to remove the top shell followed by careful severing of the adductor muscles from the bottom shell that provided a container for the product. In order to maintain a uniform, cold product temperature and to prevent dehydration, the oysters were shucked 20 minutes prior to each sensory session and the half-shell products were placed on ice until served. The frozen, LTF half-shell oysters were thawed in containers held at room temperature for less than one hour then placed on ice until served. After thawing, the adductor muscle was severed from the bottom shell which served as a product container. All oysters used for the consumer and the expert panels were served at an average temperature of 45°F or less which is the temperature customarily used for serving raw oysters in restaurants.

Note, during the shucking and thawing process any defective products were discarded. Defects included dead oysters for traditional products, excessive mud or debris, or damaged for PHP products. At 7 days post-harvest, the traditional oysters were the sample with the highest amount of rejects (49) followed by LTF (11), MH (11) and GI (4). After 14 days post-harvest, again the traditional oysters resulted in the highest rate of rejects (68), followed by HP (16), MH (10), GI (3) and LTF (3). The higher rate of rejects for the traditional product was due to mortality which is not an issue with PHP and banded oysters.

Consumer Sensory Assessments

Consumer preferences and acceptance were determined based on paired comparison tests and acceptability ratings. The tests were conducted with a group of consumers prescreened to assure familiarity with oyster consumption and a balance for various demographics (Table 3). Although the participants were recruited from one location, Gainesville, Florida, this college location included individuals from across the United States. Total participants ranged from 84 to 90 consumers per session. In each session the consumers were presented with a set of two different, unidentified oyster products served in the same manner at the same time with instructions to direct their responses. There were four possible

sets for each consumer; T vs. HP; T vs. MH; T vs. GI; or T vs. LTF. All sets compared traditional (T) oysters to one of the PHP methods. Two sets were presented per session and there were two sessions per each period of storage, 7 and 14 days post-harvest. This approach allowed comparison tests for all possible sets at both 7 and 14 days post-harvest.

The sets were presented in a random order per consumer so as not to introduce any unintended bias by order of presentation. During each session the consumers were asked to examine and consume at least two oysters from each oyster product presented. Thus the consumers ate at least 4 oysters for each set presented. To avoid sensory exhaustion only two sets are presented during one session and consumers were only allowed to participate in one session per day (two sets and 8 oysters per session). The same consumers were used in two sessions through two consecutive days to assure the same consumers responded to all possible sets of oyster products. The sample procurement and processing schedule (Table 1) were arranged to provide sessions for all the oyster products after 7 and 14 days post-harvest. There were no intermittent questions, discussions or interviews with the consumers between sessions or the separate periods of shelf-life that would have influenced their ratings or identity of the products.

All oyster products were presented utilizing blind codes so that the consumers were not aware of traditional or PHP products. The panelists were first asked to examine then taste both products per set and select the product they preferred. Then, they were asked to rate the acceptability of each product in the set. Acceptability ratings included measures for overall likeability, appearance, flavor and texture. A 9-point hedonic scale (1=dislike extremely, 5=neither like nor dislike, 9=like extremely) was used for all acceptability ratings (Attachment #1).

All consumer paired comparison tests were conducted in the Food Science and Human Nutrition Department's sensory laboratory equipped with sensory booths and computer data entry for real-time results. Coaching was limited to only assure consumer understood of procedures. Water and un-salted crackers were provided to panelists to cleanse the palate between samples. Their responses were recorded via computer entry using the program *Compusense*. The number of responses required to distinguish a significant preference was based on reference to the established paired comparison table number 17-12 in Meilgarrd et al. 2007. The acceptability ratings were subjected to analysis of variance and mean separations (Tukey's HSD, 0.05).

It is important to note that the preferences and acceptable ratings are strictly based on sets of comparisons between traditional and each individual PHP oyster product. There were no measures or ratings based on comparisons amongst any PHP products.

Table 3. Demographics for consumers prescreened for participation in the preference and acceptance tests.

Age Range		Sex		Raw Oyster Consumption	
20-40 yrs.	66%	Female	49%	> Once /month	43%
40-60 yrs.	34%	Male	51%	< Once/month but >twice /year	41%
				Twice /year or less	16%

Expert Sensory Assessments

The trained expert panel evaluated the oyster products using standard sensory profiling concurrently (same day) with the consumer sensory assessments for both periods of shelf-life, days 7 and 14 post-harvest. Expert profiling involved an established Oyster Sensory Panel that was trained and developed for ISSC. The expert panel has been maintained with continuous raw oyster assessments since 2008 (http://fshn.ifas.ufl.edu/seafood). The expert panel involved 10 screened and trained adults using standard protocol for sensory profiling stipulated in Meilgarrd et al. 2007. This panel has developed a full slate of lexicons and respective standards for a multitude of oyster product characteristics involving appearance, flavor, aroma, texture, mouthfeel and other sensory attributes. They rated or scored the various raw oyster products relative to the established standards and score sheets (http://fshn.ifas.ufl.edu/seafood; (Attachment #2 and Attachment #3). The expert panel scores were subjected to analysis of variance and mean separations (Tukey's HSD, 0.05).

RESULTS

Consumer Sensory Assessments

Consumer preference was influenced by oyster processing methods and duration of storage after processing (Table 2). The majority of consumers preferred traditional (T) oysters at the initial 7 days post-harvest. This initial preference for traditional oysters was significant at the 95% confidence level in comparisons with MH, HP and GI oysters. The difference in preference for traditional oysters was less distinct in comparisons with LTF oysters. These initial

preferences shifted as the product was held in refrigeration. After 14 days post-harvest there were no significant differences in consumer preferences at 95% confidence levels. Although the totaled preferences per comparisons on day 14 appeared to favor traditional and GI oysters, the differences in preference ratings were not significant. The loss in distinct preference can be partially explained by changes in the sensory attributes as the products aged in refrigeration (see Expert Panel results). Likewise, the preference comparisons involving LTF oysters were less subject to sensory changes during the short period of frozen storage.

Table 2. Results of the paired comparison preference tests through 7 and 14 days post-harvest storage. The number of consumers per session and the respective preferences per oyster process are tallied under each column.

PHP Oysters	7 days post-harvest			14 days post-harvest		
	No. Consumers	PHP	Traditional	No. Consumers	PHP	Traditional
Mild Heat (MH)	89	34	55**	84	34	50
Gamma Irradiation (GI)	89	26	63**	84	49	35
High Pressure (HP)	90	28	62**	86	38	48
Low Temp Freezing (LTF)	90	36	54	86	43	43

** indicates these values are significantly different at the p= 0.05 or 95% confidence level

Consumer acceptability ratings indicated general acceptance for all oyster products regardless of processing method (Figures 1-4). Average consumer ratings remained above scores of 5.0 which represents the median transition from unacceptable to acceptable products relative to overall likeability, appearance, texture and flavor. Ratings for overall likeability followed the pattern of consumer preference that was influenced by post-harvest refrigeration of the products (Figure 1). Likeability was scored significantly higher at the 95% confidence level for traditional oysters in comparisons with all PHP products after 7 days post-harvest, but there was no difference in likeability for any of the various processed oysters after 14 days post-harvest. Appearance after 7 days post-harvest was not a significant factor in acceptability except in comparisons with the LTF oysters (Figure 2), but the significantly higher ratings for acceptable texture and flavor explain the acceptability differences and preferences scored for

traditional products after 7 days post-harvest (Figures 3 and 4). Most acceptability ratings were not significantly different in comparison for all oyster products after 14 days post-harvest which explains the lack of difference in preference. In general, the acceptability ratings slightly decreased as the products aged in refrigeration and the appearance and texture of the LFT oysters still rated significantly lower than the traditional oysters after 14 days post-harvest.

Figure 1. Acceptance ratings for Overall Likeability of each PHP oyster in comparison with the traditional oysters. Significant differences (p=0.05 or 95% confidence levels) in ratings per comparisons are denoted by different letters 'a and b'.

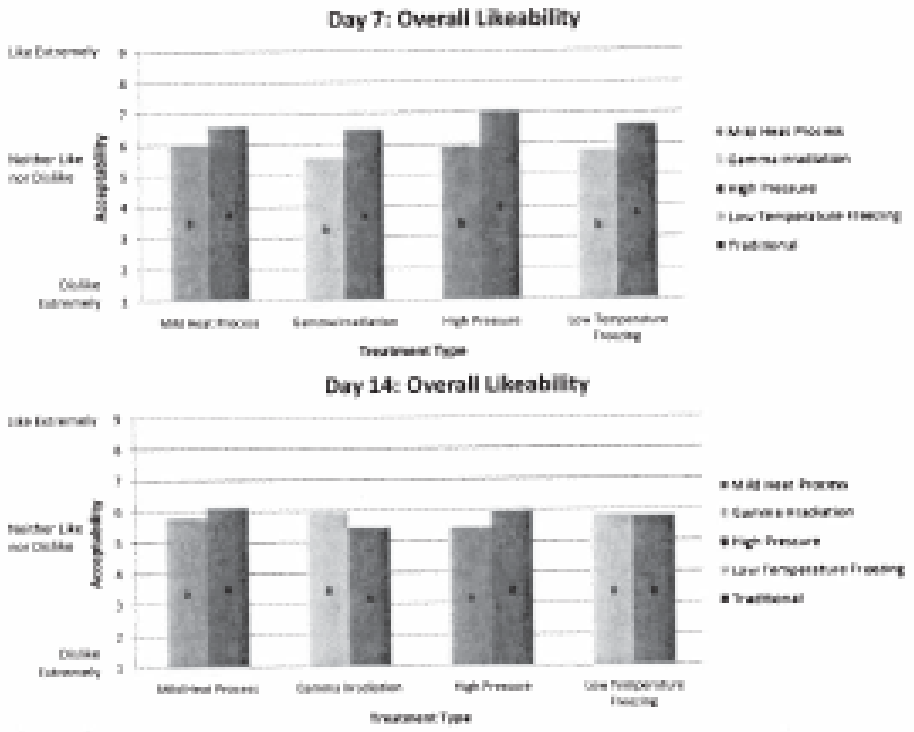


Figure 2. Acceptance ratings for Appearance of each PHP oyster in comparison with the traditional oysters. Significant differences (p=0.05 or 95% confidence levels) in ratings per comparisons are denoted by different letters 'a and b'.

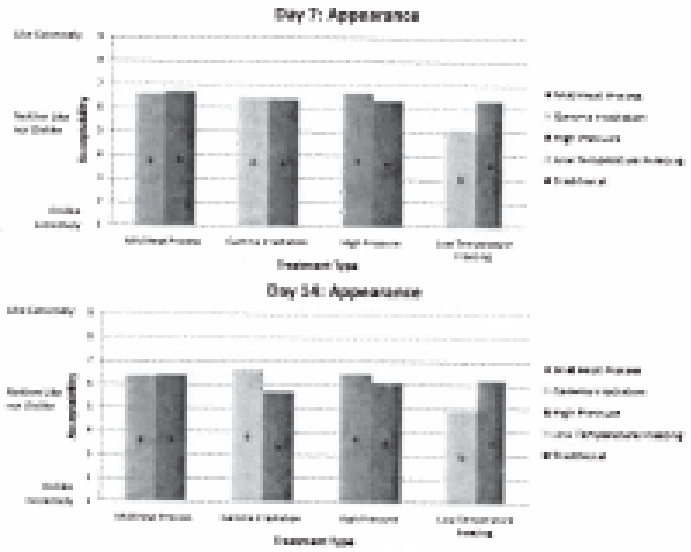


Figure 3. Acceptance ratings for Texture of each PHP oyster in comparison with the traditional oysters. Significant differences (p=0.05 or 95% confidence levels) in ratings per comparisons are denoted by different letters 'a and b'.

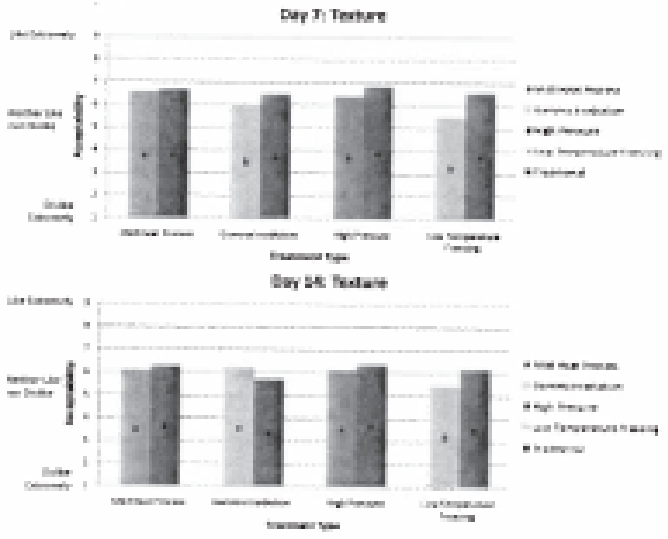
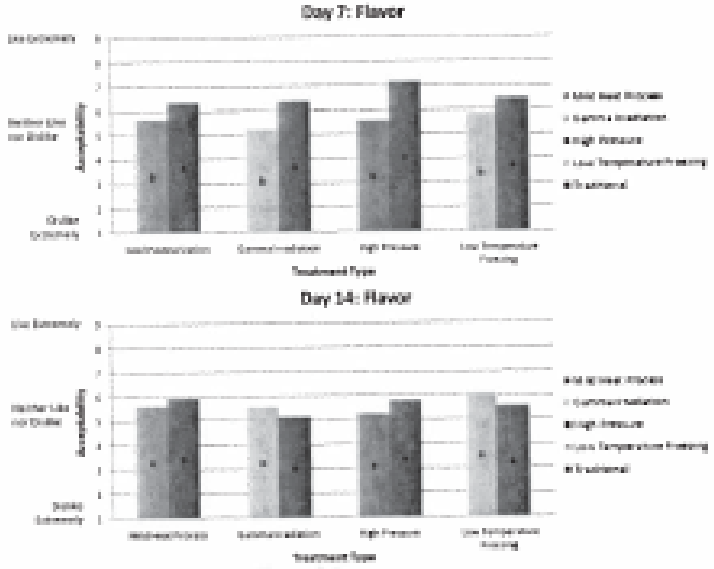


Figure 4. Acceptance ratings for Texture of each PHP oyster in comparison with the traditional oysters. Significant differences (p=0.05 or 95% confidence levels) in ratings per comparisons are denoted by different letters 'a and b'.



Expert Sensory Assessments

The sensory profiles developed by the expert panel provide some explanation for the consumer preferences and acceptability ratings (Figures 5-10). For example, the higher ratings for the traditional oysters after 7 days post-harvest can be partially explained by the higher perceived salty taste (Figure 8) and less earthy tones in flavor (Figure 9). Despite the low ratings, earthy tones are objectionable. The earthy tones noted in the flavor of the MH oysters reduced the preference for MH oysters in comparison with traditional oysters which had a similar salty taste rating. All PHP oysters had slightly higher earthy tones in aroma and flavor which persisted through 14 days shelf-life (Figure 9). Likewise, the PHP product aromas were initially scored as more briny and seaweed-like than the traditional oysters after 7 days post-harvest (Figure 7). These sensory attributes were not rated during the consumer comparison tests but they may play a role in influencing preference and acceptance. Additionally, the appearance and texture of all oyster products were similar across both periods of shelf-life,

with the exception of a drier and less plumb LTF product and the firmer more prominent textured HP product. The firmer texture attributes were persistent and more obvious for HP oysters through 14 days storage. Plump appearance and firm mouth feel or bites can influence consumer preferences.

Interestingly, the LTF oysters had the lowest score for salty taste (Figure 8) due to the use of the fresh water glaze to protect the product during frozen storage. This sensory attribute could be influenced by use of salt water glazes.

The shift in preferences and acceptance after 14 day post-harvest is distinctly obvious due to the perceived decreases in oyster liquor color (Figure 5), product aromas (Figure 7), and salty taste which was accompanied by a slight decrease in sweet and umami tastes (Figure 8). Overall, the sensory attributes became more similar as the oyster products aged in refrigerated storage. Likewise, an adverse aftertaste began to increase (Figure 10) and actual bitter flavors were noted as side observations with the standard sensory profiling. These negative attributes decrease preference and acceptance.

The various expert color ratings for shell and meats were more variable within individual oyster products than in comparisons between the various oyster products. This is not unexpected since the oysters were harvested from the same location and were similar in size and season of harvest. Likewise, the variation in color ratings did not change during storage such that color was not a useful attribute to distinguish differences between traditional and PHP products.

Figure 5. Expert sensory profiles for the volume, viscosity and color of the liquor that accompanies the oyster products are represented by bars for the average ratings based on 10 expert scores. Any bars marked by the same letter are not significantly different at the p = 0.05 or 95% confidence level.

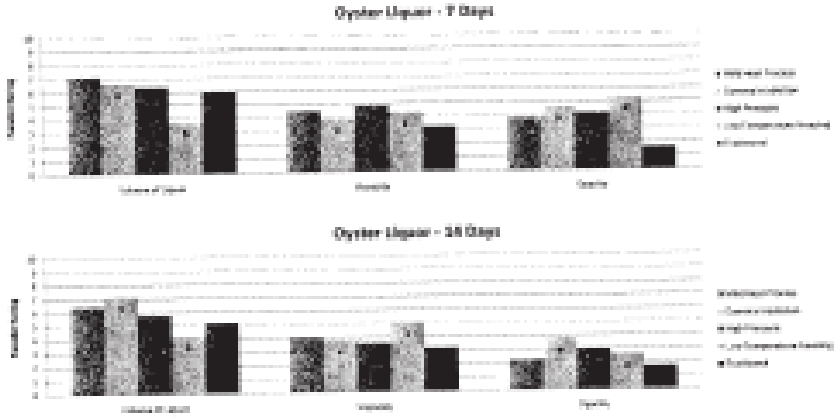


Figure 6. Expert sensory profiles for the volume, plumpness and various texture measures for the edible oyster meats are represented by bars for the average ratings based on 10 expert scores. Any bars marked by the same letter are not significantly different at the p = 0.05 or 95% confidence level.

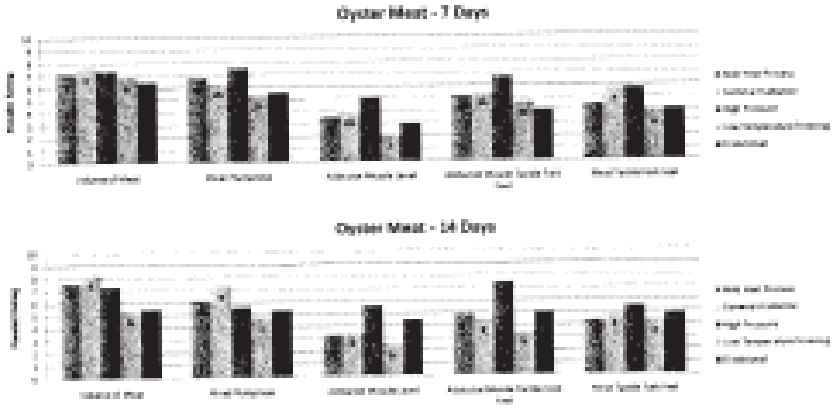


Figure 7. Expert sensory profiles for the briny, seaweed, earthy and metallic aromas associated with the oyster products are represented by bars for the average ratings based on 10 expert scores. Any bars marked by the same letter are not significantly different at the p = 0.05 or 95% confidence level.

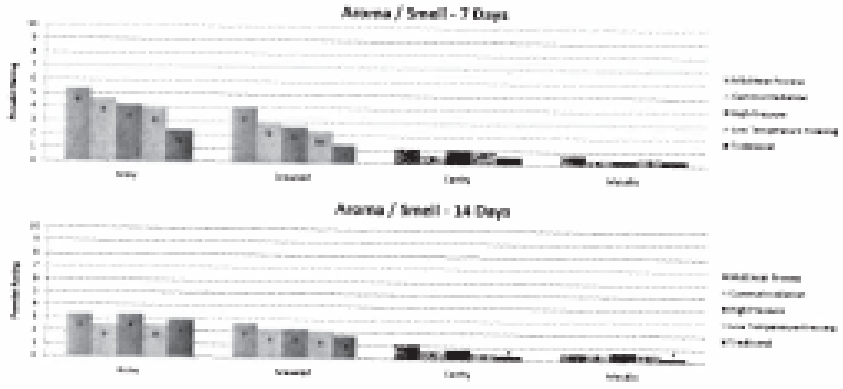


Figure 8. Expert sensory profiles for the salty, sweet and umami tastes associated with the oyster products are represented by bars for the average ratings based on 10 expert scores. Any bars marked by the same letter are not significantly different at the p = 0.05 or 95% confidence level.

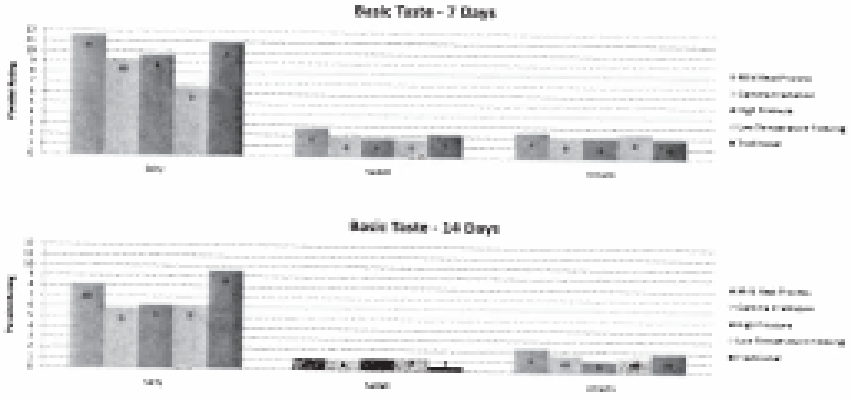


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Figure 9. Expert sensory profiles for the seaweed, chick-liver-like, earthy and green-leafy flavors associated with the oyster products are represented by bars for the average ratings based on 10 expert scores. The term CLL represents chick-like-liver flavor. Any bars marked by the same letter are not significantly different at the p = 0.05 or 95% confidence level.

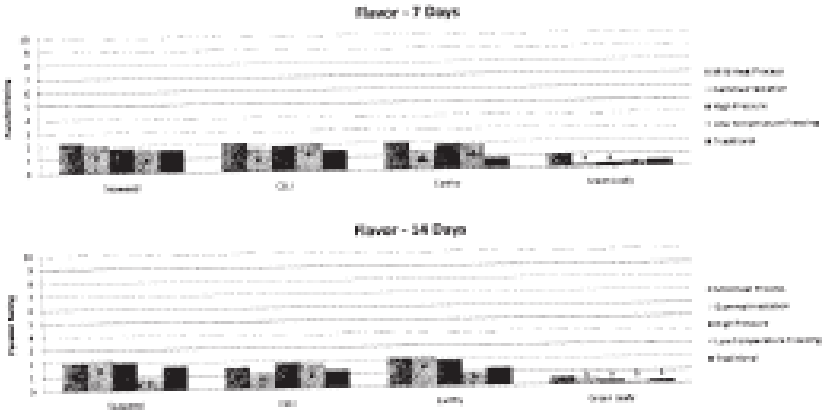


Figure 10. Expert sensory profiles for metallic and astringent aftertastes associated with the oyster products are represented by bars for the average ratings based on 10 expert scores. Any bars marked by the same letter are not significantly different at the p = 0.05 or 95% confidence level.

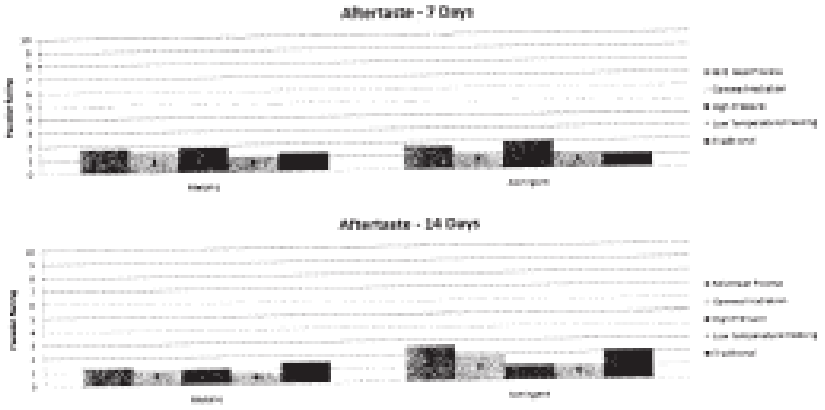
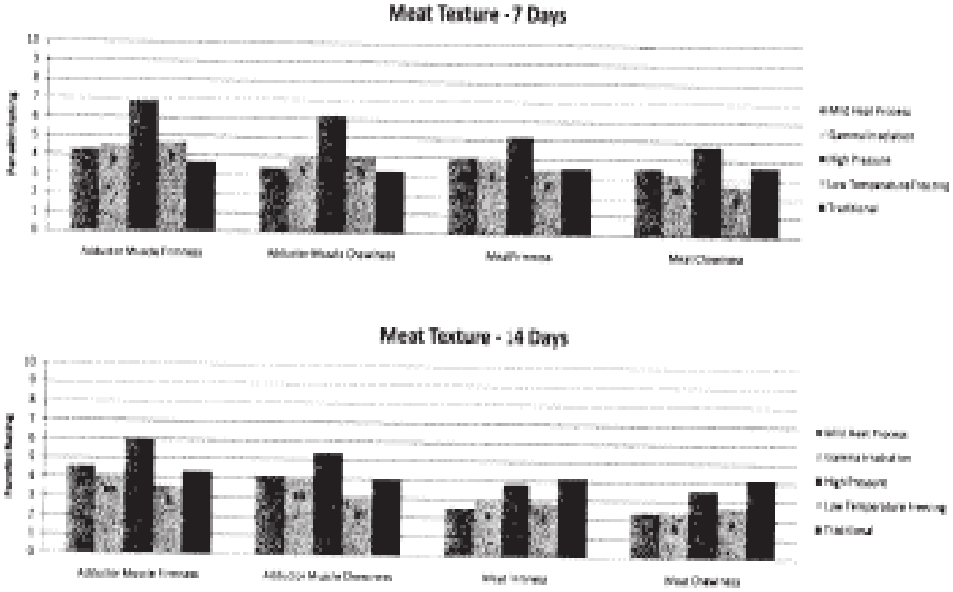


Figure 11. Expert sensory profiles for meat texture in the adductor muscle and general body or oyster meat for the oyster products are represented by bars for the average ratings based on 10 expert scores. Any bars marked by the same letter are not significantly different at the p = 0.05 or 95% confidence level.



CONCLUSIONS

Typical raw oyster consumers preferred the traditional raw oysters in comparisons with PHP oysters prepared from the same harvest during September from a typical Gulf of Mexico source (Apalachicola Bay, FL), yet this preference is diminished during prolonged refrigerated storage. The primary sensory attributes affecting preference were flavor and texture. These attributes are less distinguishable in comparisons between traditional and PHP oysters as the products aged in refrigeration. As a perishable product, the refrigerated oysters progressively change during storage. Apparently the changes caused a shift in product preference. In contrast, the preference for traditional oysters in comparisons with frozen PHP oysters (LFT) remained similar during storage as the frozen state preserves the oysters.

Despite the consumer preference expressed for traditional raw oysters during initial storage, the consumers rated all oyster products, both traditional and PHP, as acceptable. The acceptability ratings initially favored traditional raw oysters, as noted by the preference, but acceptability ratings became similar for all oyster products during more prolonged storage. Based on expert sensory profiling of the respective oyster products, the dominant sensory attributes affecting favorable acceptance were salty taste and less earthy tones in flavor and aroma.

These conclusions are based on a warm month harvest from the Gulf of Mexico. Harvest during other months with differing water temperatures that are known to influence the composition and sensory character of oysters could alter the results.

RECOMMENDATIONS

Interest for implementation of PHP methods for raw oysters harvested during warm months about the Gulf of Mexico should recognize a distinct and demonstrated consumer preference for traditional, fresh (non-frozen) products, yet a clear acceptance for both traditional and PHP oysters. This situation provides opportunities to market oysters in both traditional and PHP forms to suit particular markets relative to consumer demand, cost, convenience, and regulatory guidance.

REFERENCES

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Meilgaard, M., Civille, G., and Carr, B. 2007. Sensory Evaluation Techniques. Fourth Edition. Boca Raton, FL: CRC Press.

NSSP. 2007a. National Shellfish Sanitation Program. Guide for the Control of Molluscan Shellfish. 2007a. Model Ordinance Section II Chapter XVI. Post Harvest Processing. www.issc.org

NSSP. 2007b. National Shellfish Sanitation Program. Guide for the Control of Molluscan Shellfish 2007b. Section IV Chapter IV. Naturaly Occurring Pathogens. www.issc.org

ATTACHMENT #1

Excerpt of the questionnaire presented to the consumers for each raw oyster product evaluated during the consumer acceptability ratings

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Please indicate how much you like or dislike the following attributes in **sample A**

Sample A

Overall Likeability

dislike extremely	dislike very much	dislike moderately	dislike slightly	neither like nor dislike	like slightly	like moderately	like very much	like extremely
1	2	3	4	5	6	7	8	9

Appearance

dislike extremely	dislike very much	dislike moderately	dislike slightly	neither like nor dislike	like slightly	like moderately	like very much	like extremely
1	2	3	4	5	6	7	8	9

Texture

dislike extremely	dislike very much	dislike moderately	dislike slightly	neither like nor dislike	like slightly	like moderately	like very much	like extremely
1	2	3	4	5	6	7	8	9

Flavor

dislike extremely	dislike very much	dislike moderately	dislike slightly	neither like nor dislike	like slightly	like moderately	like very much	like extremely
1	2	3	4	5	6	7	8	9

Please indicate how much you like or dislike the following attributes in **sample B.**

ATTACHMENT #2

Sensory Standards for the Evaluation of Raw Oyster Products

Contains standards for the various sensory attributes use in the profiling of raw oysters by expert panel. This document aligns with the score sheets (Attachment 3).

Source:

http://fshn.ifas.ufl.edu/seafood/oysters/sensory%20school/services.shtml#tools

Contact: Laura Garrido
University of Florida
shrimp@ufl.edu

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Sensory Standards for the Evaluation of Raw Oyster Products

The following attributes are rated using a scale 0-10; 0 typically represents absence , 1 represents very low, 5 represents either moderate or intermediate while10 represents either very high or extreme. For each of the attributes one or more standards were developed to help guide the panelists. The rating of the standards for each attribute can be identified by the number (i.e. Std 4,Std 5, etc) and the triangle(s) placed on each respective rating scale (attachment 3). For example a standard 4 represents the 4 in the scale 1-10.

Lexicon	Description	Scale
APPEARANCE		
Color	Color (s) of the oyster parts captured by human eye	Figures 1 & 2
APPEARANCE OF OYSTER LIQUOR		
Milkiness	Presence of a milky-like substance more noticeable in the oyster liquor. This is related to reproduction not to processing.	Figure 3- Presence or absence
Air Bubbles	Presence of small air bubbles trapped in the oyster's liquor, most likely around the meat.	Figure 4 - Presence or absence
Volume of Liquor	Quantity of oyster liquor in the shell.	Figure 5
Viscosity	How freely the liquor flows on the shell (watery vs. gluey).	Actual samples
Opacity	How clear/translucent or how cloudy/opaque the oyster liquor is.	Figure 6
APPEARANCE OF OYSTER MEAT		
Shattered Meat	If the meat appears and/or is broken into pieces.	Actual samples - Presence or absence
Volume of the Meat	Refers to how much of the oyster shell is covered by the meat.	Figure 7
Plumpness	How well-rounded and full in form the oyster meat.	Figure 8
Adductor muscle	How raised the adductor muscle is when compared to the meat.	Figure 9
Adductor muscle tactile-fork feel	How the adductor muscle feels when touch by a plastic fork.	Std 2 - Soft Gelatine (Knox)*** Std 5 - Canned Peaches-Diced-4oz pull top cup (Del Monte) Std 8 – Hard Gelatine Knox****
Meat tactile-fork feel	How the meat feels when touch by a plastic fork.	

AROMA		
Briny	Related to or resembling saltiness or the sea	Std 5* &10** - Ito-Wakame dried seaweed imported by Rhee Bros, Inc - Columbia, MD
Seaweed	Related to the aroma of seaweed.	
Earthy	Refers to the characteristics of damp soil, and wet plants.	Std 10 - Whole White Mushrooms with soil - cut in half and smell.
Metallic	Relating to, or having the characteristic of a metal.	Std 10 - 2 capsules of Sundown Iron 28 mg capsules in 440 ml of water. Rub on skin and smell; or shake bottle, open and smell.
UNDESIRABLE/OBJECTIONABLE AROMAS		
Agar	Related to the odor of agar.	Std 10 - Difco Bacto Agar (Fisher Scientific Catalog)
Ammonia	Related to ammonia.	Std 10 - Ammonia for household cleaning.
Boiled potato	Refers to earthy/dirty aroma in the internal portion of a boiled potato.	Std 10 - Canned Potato (Del Monte fresh cut whole new potatoes)
Fecal	Aroma associated with feces.	Std 10 - Past experiences
Fishy	Refers to the aroma associated with strong fish odors.	Std 10 - Can of Sardines in water(King Oscar) Std 10 - Clam Juice (Doxsee/Snows Clam juice)
Garlic	Refers to the aroma of garlic.	Std 5 - Garlic Butter Papa John's Std 10 - Kalsec Garlic Oil
Sour	The aroma stimulated by acids, such as citric, malic, phosphoric, etc. (Meilgaard, Civille et al.)	Std 10 - shucked oyster placed in the refrigerator for about 21-28 days will produce a maximum sour odor.
Wet dog	Refers to smell of a wet dog.	Std 8 - Canned of shrimp (Chicken of the sea or bumble bee)
Wet burlap sack	Refers to the smell of a wet burlap sack used in the oysters business to transport oysters	Std 10 - wet burlap sacks (cream, brown, or beige) from Wal-Mart.

BASIC TASTES		
Salty	Taste stimulated by sodium salts, such as sodium chloride and sodium glutamate and in part by other salts such as potassium chloride. (Meilgaard, Civille et al.)	Std 5 - 0.3% salt Std 10 - 0.55% salt Std 15 - 0.7 % salt (Meilgaard, Civille et al.)
Sweet	Taste stimulated by sucrose and other sugars, such as fructose, glucose, etc. and by other sweet substances.(Meilgaard, Civille et al.)	Std 4 - Ritz crackers (Meilgaar Civille et al.)
Umami	Taste produced by substances such as Monosodium Glutamate (MSG). A meaty, savory, or mouth filling sensation (Codex).	Std 5 - 1/4 tsp Accent in 500 ml of water Std 10 - ½ tsp Accent in 500 ml of water
UNDESIRABLE/OBJECTIONABLE BASIC TASTES		
Sour	The taste stimulated by acids, such as citric, malic, phosphoric, etc. (Meilgaard, Civille et al.)	Std 5 - 0.1% citric acid; Presence or absence (Meilgaard, Civille et al.)
Bitter	The taste stimulated by substances such as caffeine, and hop bitters (Meilgaard, Civille et al.).	Std 5 - 0.08% caffeine solution Presence or absence (Meilgaard, Civille et al.)
FLAVOR		
Seaweed	Relating to or having the characteristic to a flavor like seaweed.	Std 10- Ito-Wakame dried seaweed imported by Rhee Bros, Inc Columbia,MD
Chicken liver like / iron-	Relating to the iron flavor of cooked liver (organ) meat.	Std 4 - Chicken liver (Tyson's) Add to boiling water and keep boiling for 10 minutes
Earthy	Refers to the characteristics of damp soil, and wet plants.	Std 10 – mushrooms, white and whole with soil - cut and taste.
Green Leafy (spinach)-	Relating to or having the characteristic flavor of spinach.	Std 5- Fresh spinach (ready pac)
UNDESIRABLE/OBJECTIONABLE FLAVORS		
Boiled Potato	Refers to earthy/dirty flavor in the internal portion of a boiled potato.	Std 6 - Potato (Del Monte fresh cut whole new potatoes)
Fishy	Refers to a fishy flavor.	Std 10 - Can of sardines in water (any brand)
Garlic	Relating to or having the characteristic flavor of garlic.	Std 4- Garlic butter Papa John's Std 10 - Kalsec garlic oil
Raw Cabbage	Relating to or having the characteristic to the flavor of raw cabbage.	Std 7 - Red cabbage Std 8 - Green cabbage

Wet Burlap Sac	Relating to or having the characteristic of the flavor imparted by a wet.	Std 10- wet burlap sacks (cream, brown, and beige) from Wal-Mart.
Aftertastes		
Metallic	Relating to or having the characteristic of a metal.	Std 5 - 1 capsules of Sundown Iron 28 mg capsules in 440 ml of water. Std 6 - Canned oysters - Chicken of the Sea whole oysters juice only (strain juice though fine wire strainer).
Astringency	The chemical feeling factor combining three different aspects: drying of the mouth, roughing of oral tissues and drawing (shrinking) sensation felt in the cheeks and the muscles of the face.	Std 5- 1/8 teaspoon (0.5g) of alum (McCormick) in 500 ml of water. Std 5- Fresh Spinach (Ready Pac).
Chalkiness	In reference to texture, a product which is composed of small particles which imparts a drying sensation in the mouth (Codex).	Std10- 14 ml of milk of magnesia in 400 ml of water or Std 10 - 3/4 teaspoon of Tricalcium phosphate food grade –Budenheim, Germany in 400 ml of water.

Texture & Mouth feels		
Firmness Chewiness	Refers to consistency of how soft versus how firm in resistance the oysters flesh holds. Amount of maceration required to comfortably swallow the oyster.	Std 1- Soft gelatin (Knox) *** Std 3 -Tofu – Nasoya soft Std 5 -Canned peaches-diced- 4oz pull top cups (Del Monte) Std 6 – Hard gelatine (Knox)**** Std 8 – Cooked chicken breast-salad topping (Pilgrim-Purdue) Std 10 - Dried apricots (Sunmaid – Mediterranean)
Grittiness	Presence of sand	Actual samples

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*Briny Std 5

Use approximately 1 to 1 1/2 cups of water for 2 to 3 strands of seaweed. Bring water to boil or close to boil. Break dried seaweed into 2 to 3 inch pieces and put in hot water. Allow to soak overnight and cool. Use seaweed for strips needed and liquid for briny standard. For a strong briny solution use more seaweed (about 6 - 8 strands) per cup of hot water.

**Briny Std 10

For a strong briny (standard 10), leave the seaweed for48 hours or more at refrigerated temperature after warm liquid as the soaked seaweed cools down.

***Soft Gelatin

4 cups of water
2 envelopes KNOX Gelatine unflavored

Measure 4 cups of water.
Put 1 to 2 cups of the measured water in a container, (big enough for about 5 cups)
Doesn't have to be exact. Sprinkle 2 KNOX envelopes on top of the water, let it stand for 2 minutes or until the gelatin is hydrated.
(DO NOT mix it or stir it it will be a mess!)
Meanwhile heat the rest of the water for 2 minutes in the microwave.
When hot pour the water into the hydrated gelatin and stir until it is completely dissolved. Pour the liquid gelatin in the little containers and let it stand in the refrigerator for about 5 hours.

****Hard Gelatin

3 cups of water
6 envelopes KNOX Gelatine unflavored

Measure 3 cups of water.
Put 1 to 1 1/2 cups of the measured water in a container, (big enough for about 5 cups)
Doesn't have to be exact. Sprinkle 6 KNOX envelopes on top of the water, let it stand for 2 minutes or until the gelatin is hydrated.
(DO NOT mix it or stir it it will be a mess!)
Meanwhile heat the rest of the water for 2 minutes in the microwave.
When hot pour the water into the hydrated gelatin and stir until it is completely dissolved. Pour the liquid gelatin in the little containers and let it stand in the refrigerator for about 5 hours.

Figure 1. Diagram of an oyster for color assessment

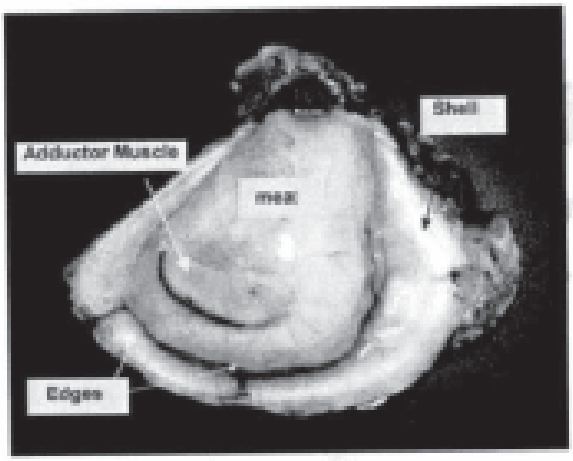

Figure 2. Color Scales

White

	1	2	3	4	5
Colors					
Munsell					
RGB					
Glidden Name	Nature's Whisper	Natural White	Fencepost	White High	White Swan
Glidden Code	49YY 78/053	50YY 85/029	81YY 87/033	58YY 82/022	60YY 83/052

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Pink

	1 Lightest	2	3	4	6 Darkest
Colors					
Munsell					
RGB	241/232/233	211/200/172	220/202/215	229/167/190	206/109/137
Glidden Name	Whimsical	Carnation Pink	Saltmarsh Pink	Checkerberry	Fiesta Pink
Glidden Code	30RR 83/040	41RR 79/079	29RR 66/154	32RR 50/260	53RR 27/417

Gray to Black

	1 Lightest	2	3	4	5	6 Darkest
Colors						
Munsell						
RGB	217/219/217	204/205/204	188/188/187	160/160/159	94/96/94	62/62/61
Glidden Name	Snowfield	Universal Grey	Veil	Granite Grey	Obsidian Glass	Dark Secret
Glidden Code	00NN 72/000	00NN 62/000	00NN 53/000	00NN 37/000	00NN 15/000	00NN 05/000

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Gray / Brown

	1 Lightest	2	3	4	5	6 Darkest
Colors						
Munsell						
RGB	231/228/219	221/215/205	203/196/185	182/171/157	135/115/104	91/80/69
Glidden Name	Kitten White	Carolina Strand	Fossil Grey	Scroll Beige	Fauna	Pebble Mosaic
Glidden Code	30YY 78/035	30YY 69/048	30YY 55/060	30YY 43/083	10YY 18/074	10YY 08/093

Gray/Green

	1 Lightest	2	3	4	5	6 Darkest
Colors						
Munsell	5Y/8.5/2	5Y/8/2	7.5Y/7/2	2.5Y/6/4	5Y/4/4	5Y/3/4
RGB	222/214/183	208/201/171	175/174/146	166/145/100	110/96/62	85/72/55
Glidden Name	Wishes	Autumn Haze	Chatham Green	Sunny Beige	Calm Water	Oak Alley
Glidden Code	45YY 75/110	45YY 67/120	60YY53/110	30YY 36/185	30YY 20/193	30YY/09/175

Green scale

	1 Lightest	2	3	4	5	6 Darkest
Colors						
Munsell	10Y/9/2	10Y/8.5/2	10Y/8/4	2.5GY/7/4	10Y/5/4	10Y/4/2
RGB	231/230/194	217/216/182	206/203/144	190/184/151	126/126/73	99/98/75
Glidden Name	Brocade Cream	Hint of Gold	Mount Olive	Pennyroyal	Retro Green	Laurentian
Glidden Code	60YY 70/189	60YY 64/211	60YY 54/252	60YY 48/243	60YY23/227	70YY15/160

Emerald Green

	1 Lightest	2	3	4	5	6 Darkest
Colors						
Munsell						
RGB	228/236/223	212/228/205	198/222/190	154/185/141	108/160/96	86/117/74
Glidden Name	Boudoir	Nature Mist	Sea Scent	Summer Picnic	Frog Pad	Splendor
Glidden Code	50GY 83/060	50GY 75/122	50GY 69/165	50GY 44/248	50GY 23/289	50GY 15/289

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Blue/Green (Teal)

	1 Lightest	2	3	4	5	6 Darkest
Colors						
Munsell						
RGB	221/237/250	206/228/221	186/220/211	160/199/189	91/149/136	13/113/99
Glidden Name	Bubbling Brook	Annabell	Warm Meadow	Country Cottage	Kelly's Island	Forest Hush
Glidden Code	50GG 83/057	50GG 74/077	50GY 69/165	50GG 53/144	50GG 36/228	50GG 13/314

Maroon

	1 Lightest	2	3	4	5	6 Darkest
Colors						
Munsell						
RGB	217/207/210	196/180/185	166/142/151	135/107/117	108/83/95	88/71/77
Glidden Name	Whisper	Soft Wine	Sonata	Mystic	Alabaster	Black Currant
Glidden Code	30RR 64/043	30RR 49/067	30RR 30/103	30RR 17/140	30RR 10/131	30RR 07/094

Purple

	1 Lightest	2	3	4	5	6 Darkest
Colors						
Munsell						
RGB	224/222/228	216/211/226	165/142/153	159/154/184	135/115/153	92/86/121
Glidden Name	Mystic Purple	Touch of Violet	Giggles	Elevator	Coat of Arms	Purple Polka
Glidden Code	30BB 74/038	30BB 68/083	30BB 30/103	30BB 35/187	30BB 16/131	30BB 10/219

Tan

	1 Lightest	2	3	4	5	6 Darkest
Colors						
Munsell	2.5Y/9/2	2.5Y/8/4	10YR/6/6	10YR/7/8	10YR/6/10	10YR/6/8
RGB	240/227/158	220/198/148	236/194/120	216/165/81	194/137/24	188/135/57
Glidden Name	Lit Creme	Stucco	Light Topaz	Honeysweet	Golden Gate	Ovation
Glidden Code	30YY 77/160	30YY65/150	30YY58/166	30YY45/378	30YY 38/501	30YY 30/438

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Brown/Yellow

	1 Lightest	2	3	4	5	6 Darkest
Colors						
Munsell	2.5Y/8.5/2	2.5Y/8/2	2.5Y/7/4	10YR/6/6	2.5Y/5/4	2.5Y/4/6
RGB	225/218/185	211/196/172	194/171/123	181/143/81	140/119/76	120/93/52
Glidden Name	Oyster White	Ivory Sampler	Cookie Crumb	First Anniversary	New Suede	American Bronze
Glidden Code	30YY 64/149	30YY 58/178	30YY 46/236	10YY34268	10YY 26/239	10YY 15/280

Brown

	1 Lightest	2	3	4	5	6 Darkest
Colors						
Munsell	10YR/9/2	10YR/8/2	10YR/7/4	10YR/6/4	10YR/4/4	10YR/3/6
RGB	242/23680	214/199/175	198/169/127	171/143/104	115/92/57	98/87/16
Glidden Name	Desert floor	Dapper Tan	Family Legacy	Golden Pond	Side saddle	Timbre Trail
Glidden Code	20YY 69/320	10YY 55/353	00YY63/106	00YY 33/244	90YR 17/245	90YR 10/244

Figure # 3

Standard for presence of milkiness (Std 10)

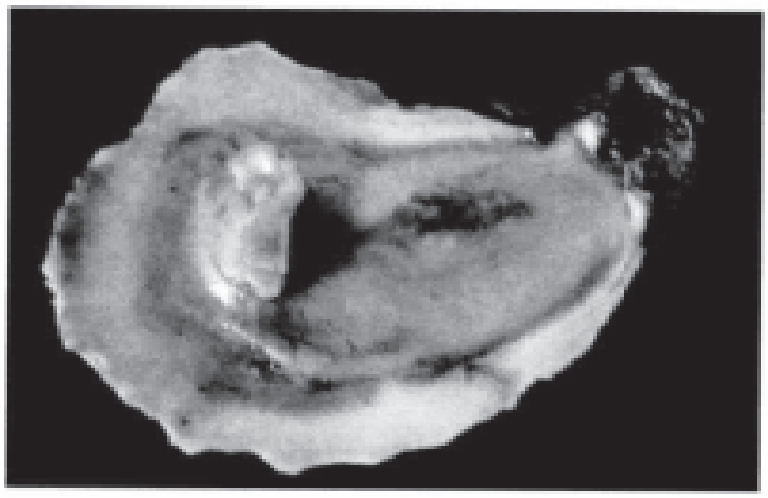


Figure 4. Standards for presence of bubbles

Presence

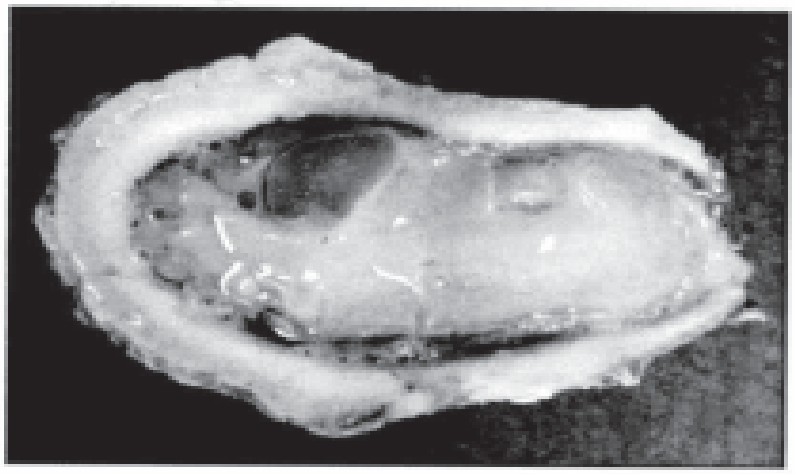
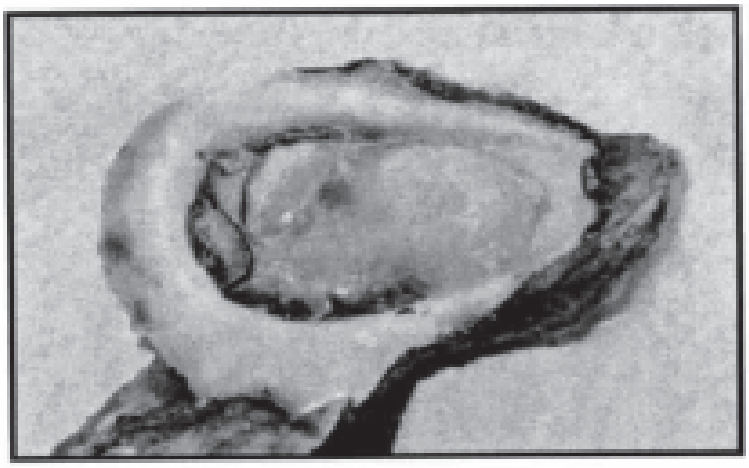
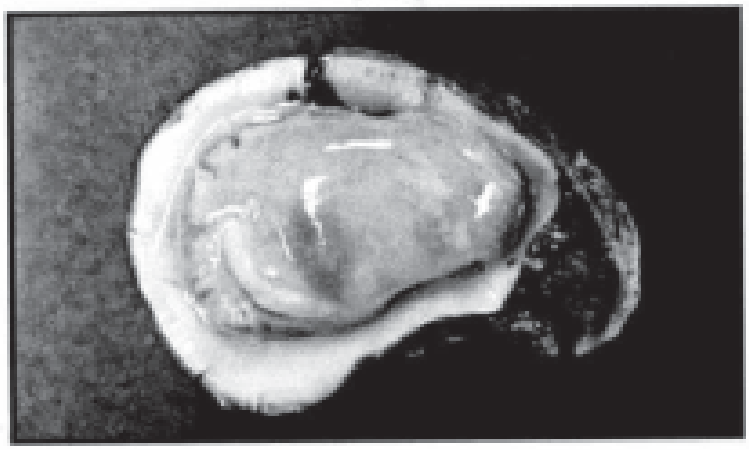


Figure 5. Standards for volume of liquor

Low (2)



High (10)



35

Figure 6. Standards for liquor opacity

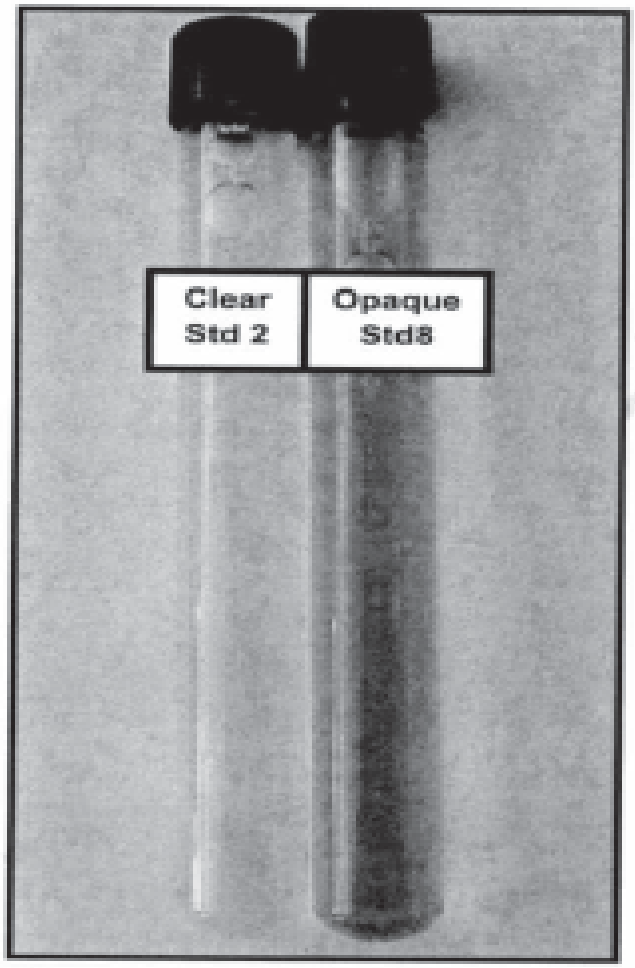


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Figure 7. Standards for volume of meat

Hardly
Covered (Std 2)

Fully
Covered (Std 10)

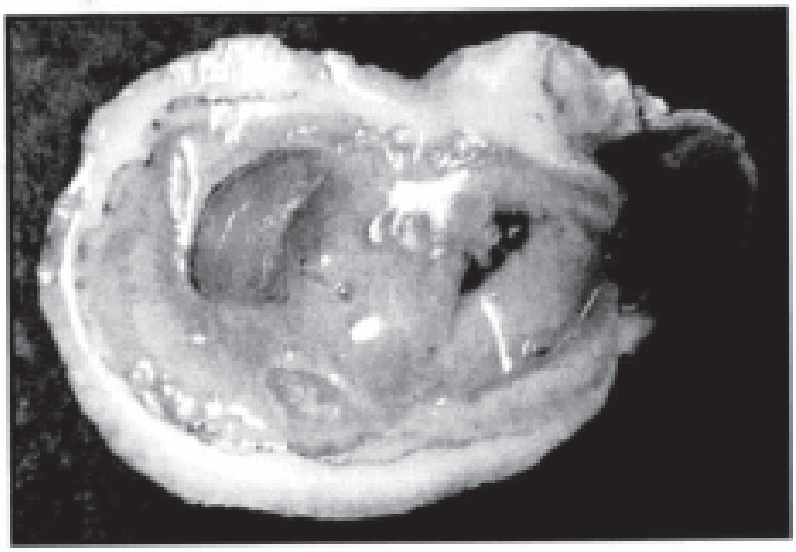

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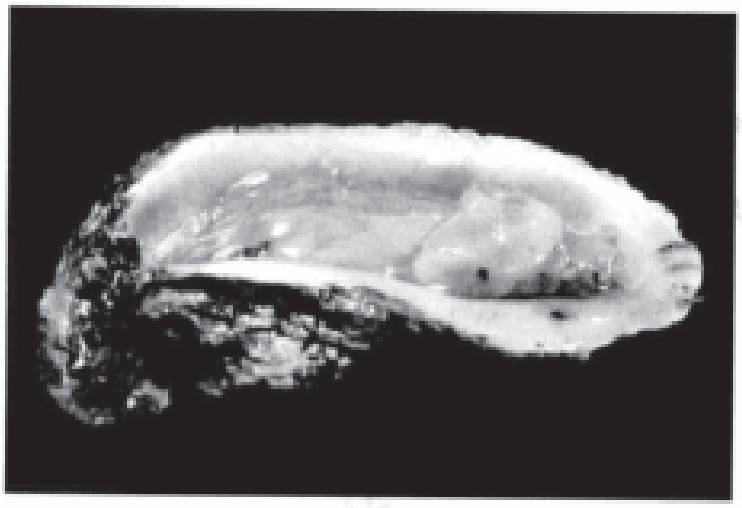
Figure 8. Standards for plumpness

Flaccid (Std 2)



Plump (Std 8)

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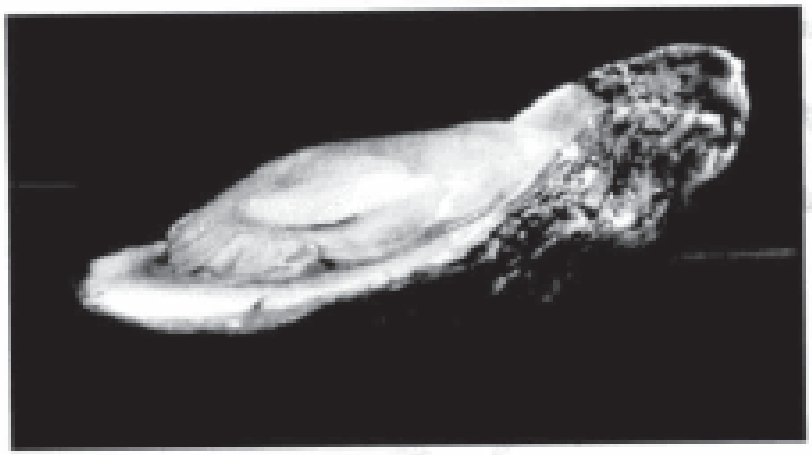
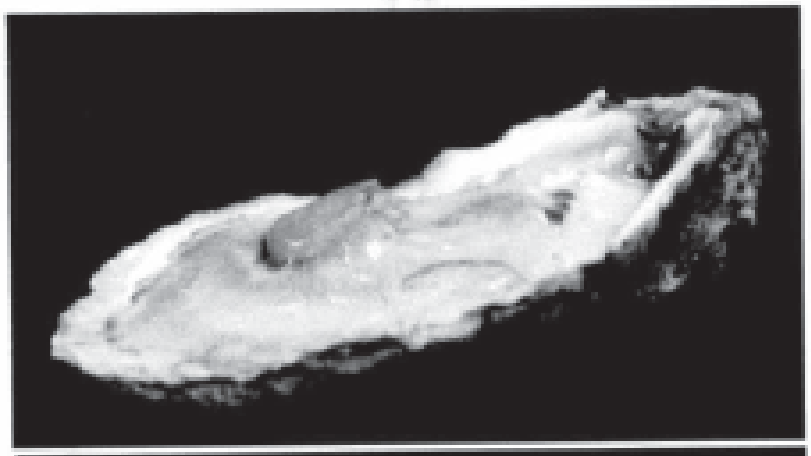
Figure 9. Standards for adductor muscle

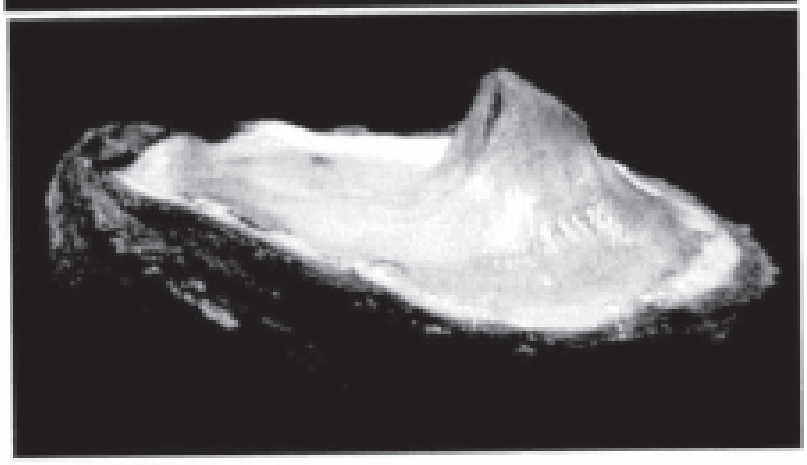
Level (Std 1)



Raised (Std 5)



Very Raised (Std 8)



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ATTACHMENT #3

Score Sheet for Raw Oyster Products

Contains the score sheet use to profile the sensory attributes raw oysters by expert panel. This document aligns with the list of standards (Attachment 2).

Source:
http://fshn.ifas.ufl.edu/seafood/oysters/sensory%20school/services.shtml#tools

Contact: Laura Garrido
University of Florida
shrimp@ufl.edu

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Oyster Product Characterization Form

Sample Code: _____
Date: _____
Panelist: _____

Appearance

Color Break-down - Circle all that apply

Oyster Meat:

1. White		1	2	3	4	5	
2. Pink		1	2	3	4	5	
		Light					Dark
3. Gray to Black		1	2	3	4	5	6
4. Gray/Brown		1	2	3	4	5	6
5. Grey/Green	1	2	3	4	5	6	
6. Green		1	2	3	4	5	6
7. Emerald Green		1	2	3	4	5	6
8. Blue/Green (Teal)		1	2	3	4	5	6
9. Maroon		1	2	3	4	5	6
10. Purple		1	2	3	4	5	6
11. Tan		1	2	3	4	5	6
12. Brow/Yellow		1	2	3	4	5	6
14. Brown		1	2	3	4	5	6

Edges:

1. White		1	2	3	4	5	
2. Pink		1	2	3	4	5	
		Light					Dark
3. Gray to Black		1	2	3	4	5	6
4. Gray/Brown		1	2	3	4	5	6
5. Grey/Green	1	2	3	4	5	6	
6. Green		1	2	3	4	5	6
7. Emerald Green		1	2	3	4	5	6
8. Blue/Green (Teal)		1	2	3	4	5	6
9. Maroon		1	2	3	4	5	6
10. Purple		1	2	3	4	5	6
11. Tan		1	2	3	4	5	6
12. Brow/Yellow		1	2	3	4	5	6

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14. Brown		1	2	3	4	5	6

Inner Rim of Shell:

1. White		1	2	3	4	5	
2. Pink		1	2	3	4	5	
		Light					*Dark*
3. Gray to Black		1	2	3	4	5	6
4. Gray/Brown		1	2	3	4	5	6
5. Grey/Green	1	2	3	4	5	6	
6. Green		1	2	3	4	5	6
7. Emerald Green		1	2	3	4	5	6
8. Blue/Green (Teal)		1	2	3	4	5	6
9. Maroon		1	2	3	4	5	6
10. Purple		1	2	3	4	5	6
11. Tan		1	2	3	4	5	6
12. Brow/Yellow		1	2	3	4	5	6
14. Brown		1	2	3	4	5	6

Oyster Liquor

Please circle appropriate descriptor(s):

Milkiness: Not Milky Milky

Air Bubbles: Absent Present

Volume of Liquor

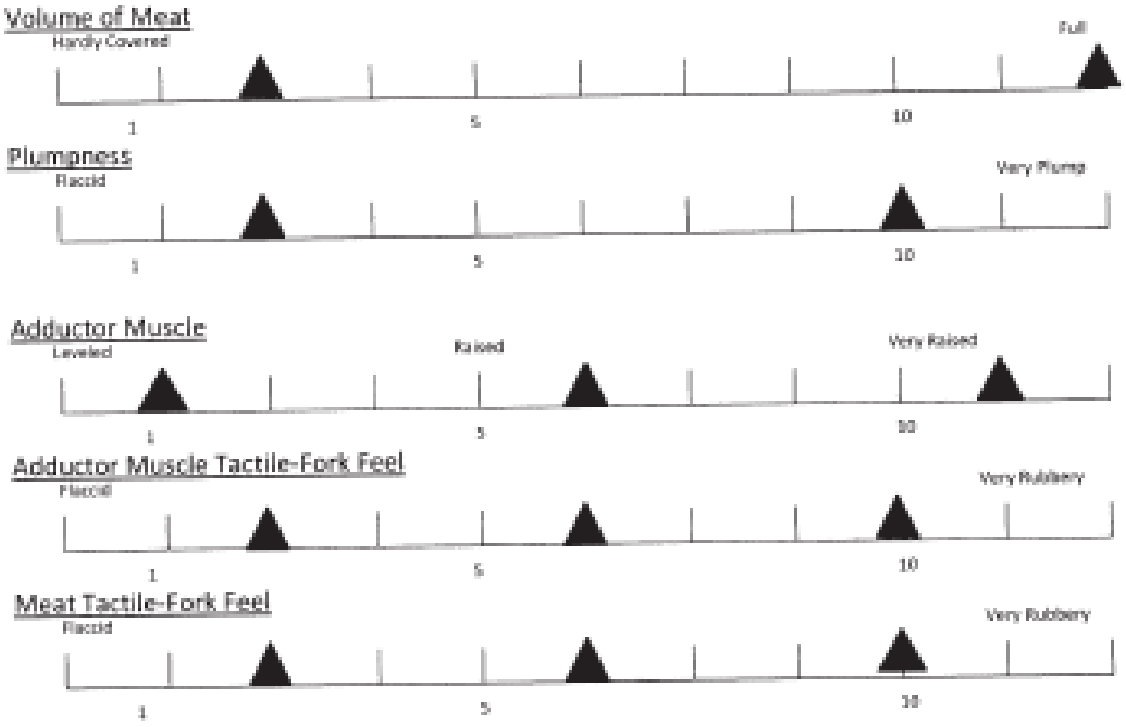
Viscosity

Opacity

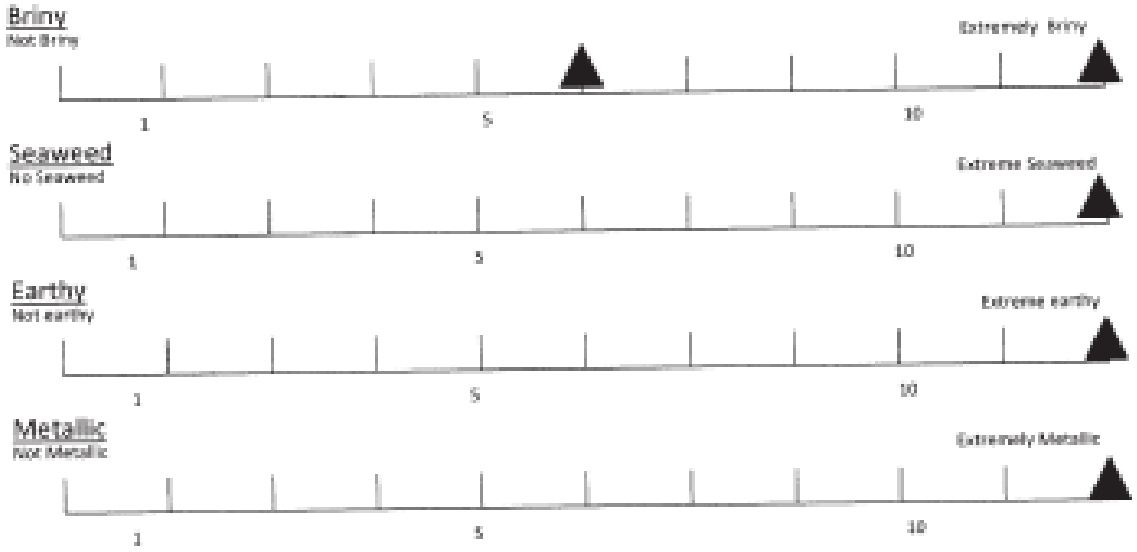
Oyster Meat

Please circle appropriate descriptor(s):

Shattered Meat: Yes No



Aroma/Smell



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If any objectionable odors are detected, please circle the appropriate descriptor(s):

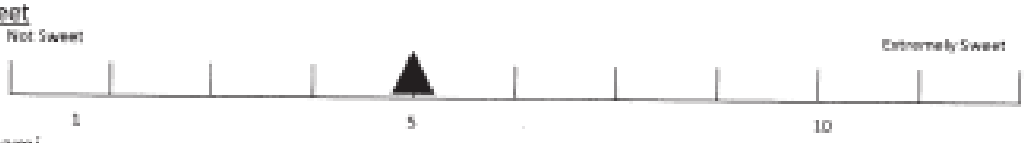
Agar	Ammonia	Boiled Potato	Fecal	Fishy
Garlic	Sour	Wet Burlap Sac	Wet Dog	Other:_____

Basic Tastes

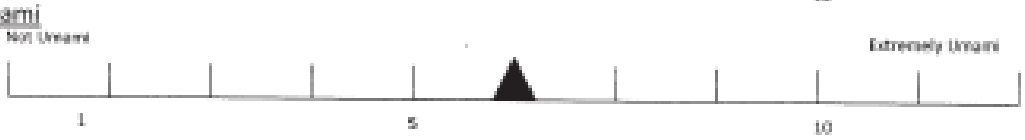
Salty

If saltiness is higher than 10, please specify: _____

Sweet



Umami



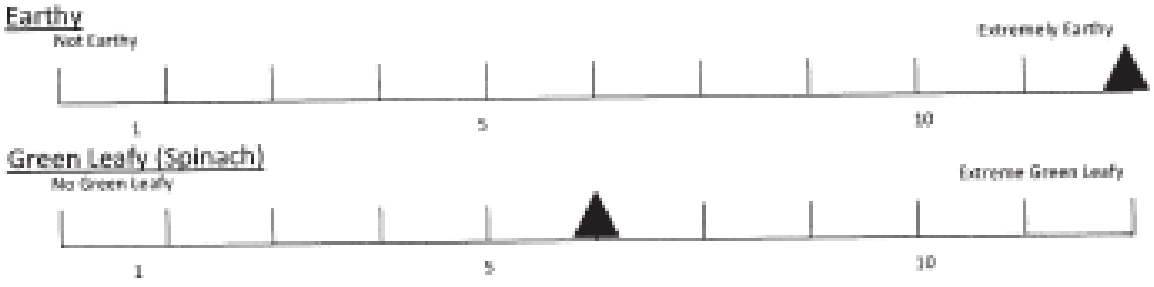
If any objectionable basic tastes are detected, please circle the appropriate descriptor(s):

Sour Bitter

Flavor

Seaweed

Chicken-Liver-Like/Iron(CLLI)

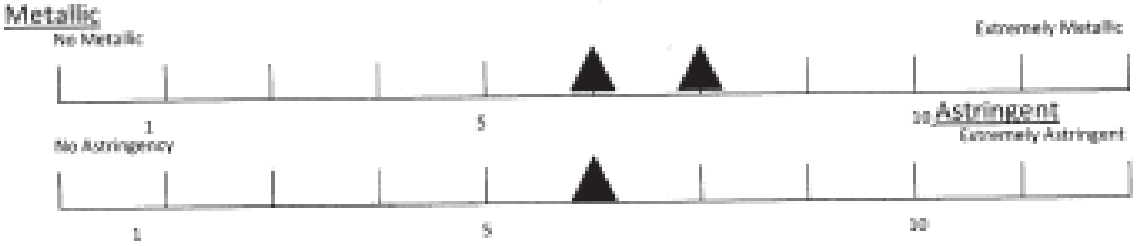


If any objectionable flavors are detected, please circle the appropriate descriptor(s):

Boiled Potato-Like Fishy Garlic (Oil) Raw Cabbage

Wet Burlap Sac Other:_____

Aftertastes

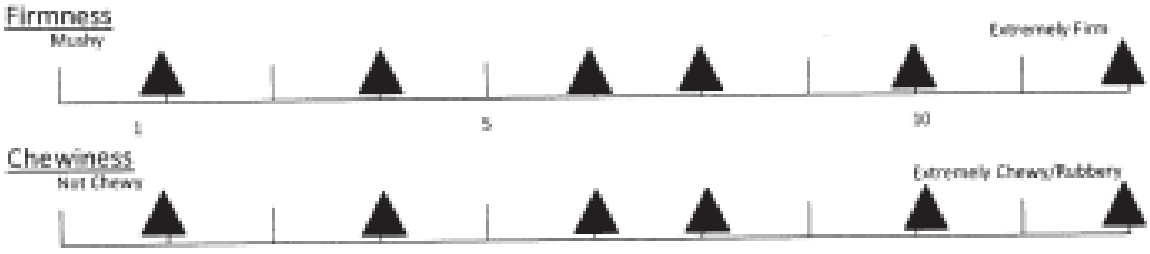


If any objectionable aftertastes are detected, please circle the appropriate descriptor(s):

Chalky Other:_____

Texture & Mouth feels

Adductor Muscle



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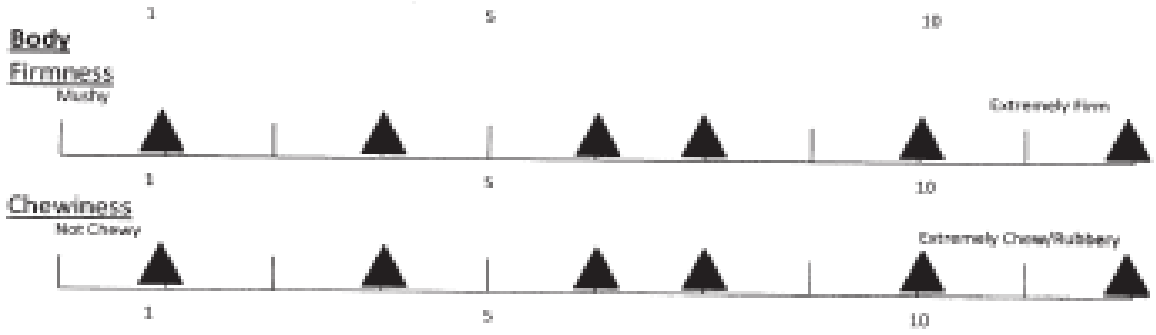

If any objectionable mouth feels are detected, please circle the appropriate descriptor(s):

Grittiness from sand Grittiness from shell

Other:_____

Proposal Subject: *Vibrio vulnificus* Management

Specific NSSP Guide Reference: Section II Model Ordinance Chapter II Risk Assessment and Risk Management @04 *Vibrio vulnificus* Risk Management for Oysters

Text of Proposal/ Requested Action: Effective January 1, 2012:

@.04 *Vibrio vulnificus* Risk Management for Oysters

A. For states having 2 or more etiologically confirmed shellfish-borne *Vibrio vulnificus* illnesses since 1995 traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state (Source State), the Authority shall develop and implement *a Vibrio vulnificus* Risk Management Plan.

B. The Source State's *Vibrio vulnificus* Risk Management Plan shall define the administrative procedures and resources necessary to accomplish (i.e. establish and maintain) involvement by the state in a collective illness risk reduction program. The goal of the *Vibrio vulnificus* Risk Management Plan will be to reduce the risk per serving to a 60% illness rate reduction for etiologically confirmed shellfish-borne *Vibrio vulnificus* septicemia illnesses reported collectively by California, Florida, Louisiana, and Texas, from the consumption of commercially harvested raw or undercooked oysters to a level equivalent to a 60% illness rate reduction from 1995 – 1999 baseline average illness rate of 0.278 per million.

C. The Source State's *Vibrio vulnificus* Risk Management Plan shall include, at a minimum:

(1) The ISSC Consumer Education Program targeted toward individuals who consume raw oysters and whose health condition(s) increase their risk for *Vibrio vulnificus* illnesses;

(2) A process to collect standardized information for each *Vibrio vulnificus* illness: including underlying medical conditions; knowledge of disease status; prior counseling on avoidance of high risk foods, including raw oysters; existence of consumer advisories at point of purchase or consumption; and, if possible, whether consumer was aware and understood the advisories;

(3) A standardized process for tracking products implicated in *Vibrio vulnificus* illnesses; and

(4) Identification and implementation of the controls, or equivalent controls, which produced an illness per serving equivalent to a 60% illness rate reduction in the core states. These controls include:

(a) Labeling all oysters, "For shucking by a certified dealer", when the Average Monthly Maximum Water Temperature exceeds 75°F;

(b) Subjecting all oysters intended for the raw, half-shell market to an Authority- approved post harvest processing that reduces the *Vibrio vulnificus* levels to <1000 MPN/gram when the Average Monthly Maximum Water Temperature exceeds 75°F;

(c) Closing shellfish growing areas for the purpose of harvest of oysters intended for the raw, half-shell market when the Average Monthly Maximum Water Temperature exceeds 75°F;

(d) Labeling all oysters, "For shucking by a certified dealer", during the months of May through September, inclusive;
(e) Subjecting all oysters intended for the raw, half-shell market to an Authority-approved post harvest processing that reduces the *Vibrio vulnificus* levels to <1000 MPN/gram when the Average Monthly Maximum Water Temperature exceeds 75°F; and
(f) Closing shellfish growing areas for the purpose of harvesting oysters intended for the raw, half-shell market during the months of May through September, inclusive.

Public Health Significance: A control standard that is easier to achieve will encourage industry acceptance by allowing for more PHP options (ie. high-salinity relay, and depuration). This would still very likely have a significant impact on reducing illnesses (considering the quagmire that the conference is in when dealing with V.v management). For the scientists: The <1000 MPN/gram level of V.v. may not be proven to reduce all risk of V.v. illness, but it is not disproven, either, that such a control level would help to significantly reduce the number of illnesses.

Cost Information (if available): None

Action by 2011 Task Force II Recommended adoption of Proposal 11-203 as amended.

Task Force II further recommended:

1. That the language of this proposal be incorporated into @.04 E. (1) (b) should Proposal 11-201-A be adopted.

2. The Executive Board appoint a committee to review the definition of post harvest processing to incorporate processing to achieve reductions to levels other than <30 MPN/g.

3. An effective date of January 1, 2012 be established for the Proposal.

Effective January 1, 2012:

@.04 *Vibrio vulnificus* Risk Management for Oysters

A. For states having 2 or more etiologically confirmed shellfish-borne *Vibrio vulnificus* illnesses since 1995 traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state (Source State), the Authority shall develop and implement *a Vibrio vulnificus* Risk Management Plan.

B. The Source State's *Vibrio vulnificus* Risk Management Plan shall define the administrative procedures and resources necessary to accomplish (i.e. establish and maintain) involvement by the state in a collective illness risk reduction program. The goal of the *Vibrio vulnificus* Risk Management Plan will be to reduce the risk per serving to a 60% illness rate reduction for etiologically confirmed shellfish-borne *Vibrio vulnificus* septicemia illnesses reported collectively by California, Florida, Louisiana, and Texas, from the

consumption of commercially harvested raw or undercooked oysters to a level equivalent to a 60% illness rate reduction from 1995 – 1999 baseline average illness rate of 0.278 per million.

C. The Source State's *Vibrio vulnificus* Risk Management Plan shall include, at a minimum:

(1) The ISSC Consumer Education Program targeted toward individuals who consume raw oysters and whose health condition(s) increase their risk for *Vibrio vulnificus* illnesses;
(2) A process to collect standardized information for each *Vibrio vulnificus* illness: including underlying medical conditions; knowledge of disease status; prior counseling on avoidance of high risk foods, including raw oysters; existence of consumer advisories at point of purchase or consumption; and, if possible, whether consumer was aware and understood the advisories;
(3) A standardized process for tracking products implicated in *Vibrio vulnificus* illnesses; and
(4) Identification and implementation of the controls, or equivalent controls, which produced an illness per serving equivalent to a 60% illness rate reduction in the core states. These ~~controls~~ include one or more of the following control measures:
   (a) Labeling all oysters, "For shucking by a certified dealer", when the Average Monthly Maximum Water Temperature exceeds 75°F;
   (b) Subjecting all oysters intended for the raw, half-shell market to an Authority- approved post harvest processing that reduces the *Vibrio vulnificus* levels to <0 MPN/gram when the Average Monthly Maximum Water Temperature exceeds 75°F;
   (c) Closing shellfish growing areas for the purpose of harvest of oysters intended for the raw, half-shell market when the Average Monthly Maximum Water Temperature exceeds 75°F;
   (d) Labeling all oysters, "For shucking by a certified dealer", during the months of May through September, inclusive;
   (e) Subjecting all oysters intended for the raw, half-shell market to an Authority-approved post harvest processing that reduces the *Vibrio vulnificus* levels to <1000 MPN/gram when the Average Monthly Maximum Water Temperature exceeds 70~~5~~°F (product meeting this requirement does not meet the minimum requirements for labeling claims); ~~and~~
   (f) Closing shellfish growing areas for the purpose of harvesting oysters intended for the raw, half-shell market during the months of May through September, inclusive.

Action by 2011 General Assembly

Adopted Section @.04 C. (4) (e) of Proposal 11-203 as amended by Task Force II.

The remainder of Proposal 11-203 was addressed by General Assembly action on Proposal 11-201A.

Action by FDA February 26, 2012

Concurred with Conference action on Proposal 11-203.

Proposal Subject: *Vibrio vulnificus* Management Plan

Specific NSSP Guide Reference: Section IV. Guidance Documents Chapter IV. Naturally Occurring Pathogens .02 *Vibrio vulnificus* Management Plan

Key Words: *Vibrio vulnificus; Vibrio vulnificus* Management Plan; Source States

Text of Proposal/ Requested Action: *Vibrio vulnificus* source states are those states reporting two (2) or more etiologically confirmed shellfish-borne *Vibrio vulnificus* illnesses in the previous five (5) years ~~since 1995~~ traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state.

Public Health Significance: Currently there is no path for a state to be removed from the list of Illness Source States. The proposed change would alter the definition of *Vibrio vulnificus* Source State to remove states that have not had an illness for five (5) years.

Cost Information (if available): None available.

Action by 2011 Task Force II Recommended adoption of Proposal 11-204 as amended contingent upon Proposal 11-201A being voted no action or referred to committee. Add in NSSP Guide reference that this be included in Chapter II. @ .04 (effective January 1, 2012) A.

~~*Vibrio vulnificus* source states are those states reporting two (2) or more etiologically confirmed shellfish-borne *Vibrio vulnificus* illnesses in the previous five (5) years since 1995 traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state.~~

Vibrio vulnificus Source States are those states reporting two (2) or more etiologically confirmed, and epidemiologically linked *Vibrio vulnificus* septicemia illnesses from the consumption of commercially harvested raw or undercooked oysters that originated from the growing waters of that state within the previous ten (10) years.

Action by 2011 General Assembly No action was taken on Proposal 11-204.

Rationale: This proposal was addressed by General Assembly action on Proposal 11-201A.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-204.

Proposal Subject: *Vibro* Management Committee Membership

Specific NSSP Guide Reference: Section IV. Guidance Documents Chapter IV. Naturally Occurring Pathogens .02 *Vibrio vulnificus* Management Plan

Text of Proposal/ Requested Action: The ~~V.v. subcommittee~~ *Vibrio* Management Committee members will include, at a minimum, balanced representation from industry and state shellfish control authorities from *Vibrio vulnificus* Illness Source States California, Florida, Louisiana and Texas, FDA, NOAA, EPA, CDC, state epidemiologists; as well as industry and shellfish control representatives from other regions. *Vibrio vulnificus* Illness Source States are those states reporting two (2) or more etiologically confirmed shellfish-borne *Vibrio vulnificus* illnesses since 1995 traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state. Etiologically confirmed means those cases in which laboratory evidence of a specific agent is obtained and specified criteria are met.

Recognizing the increasing importance and roles for the Committee, leadership will be expanded and structured in a similar manner as stated in the ISSC By-Laws for Task Forces (reference: ISSC By-Law, Article I Task Forces). The VMC Chair shall alternately be selected from a state shellfish control authority and from industry. The Board Chairman, with approval of the Board, shall appoint a VMC Chair and Vice-Chair. If the VMC Chair represents a state shellfish control authority, the Vice-Chair shall be an industry representative. At the end of the VMC Chair's term of office, the Vice Chair will become Chairman and a new Vice Chair will be appointed who represents the same segment of the Conference as the outgoing VMC Chair. A VMC Chair and Vice Chair should be appointed before October 1, 2001 in order to be consistent with plans for annual VMC meetings and with the effective date of *Vibrio vulnificus* Risk Management Plans. Likewise, the term of office shall be for (2) years.

Public Health Significance:

Cost Information (if available):

Action by 2011 Task Force II Recommended adoption of Proposal 11-205 as amended.

~~The V.v. subcommittee *Vibrio* Management Committee members will include, at a minimum, balanced representation from industry and state shellfish control authorities from *Vibrio vulnificus* Illness Source States California, Florida, Louisiana and Texas, FDA, NOAA, EPA, CDC, state epidemiologists; as well as industry and shellfish control representatives from other regions. *Vibrio vulnificus* Illness Source States are those states reporting two (2) or more etiologically confirmed shellfish-borne *Vibrio vulnificus* illnesses since 1995 traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state. Etiologically confirmed means those cases in which laboratory evidence of a specific agent is obtained and specified criteria are met.~~

~~Recognizing the increasing importance and roles for the Committee, leadership will be expanded and structured in a similar manner as stated in the ISSC By-Laws for Task Forces (reference: ISSC By-Law, Article I Task Forces). The VMC Chair shall alternately be selected from a state shellfish control authority and from industry. The~~

~~Board Chairman, with approval of the Board, shall appoint a VMC Chair and Vice-Chair. If the VMC Chair represents a state shellfish control authority, the Vice-Chair shall be an industry representative. At the end of the VMC Chair's term of office, the Vice Chair will become Chairman and a new Vice Chair will be appointed who represents the same segment of the Conference as the outgoing VMC Chair. A VMC Chair and Vice Chair should be appointed before October 1, 2001 in order to be consistent with plans for annual VMC meetings and with the effective date of *Vibrio vulnificus* Risk Management Plans. Likewise, the term of office shall be for (2) years.~~

The Executive Board Chairperson shall appoint a sixteen (16) member *Vibrio* Management Committee. The Committee will be comprised of a Chairperson with at least two (2) industry members from the East, Gulf and West coasts and at least one (1) state regulatory from each of the ISSC regions. The Committee will also include one voting member from NOAA, one voting member from FDA, one voting member from EPA and one voting member from CDC. The Federal entities will appoint these members. Non voting advisors will be appointed as appropriate. The Committee will assess if additional changes are needed in the NSSP Guide for the Control of Molluscan Shellfish Model Ordinance to reduce the risk of *Vibrio* illnesses. The Committee will annually review trends in *Vibrio* illnesses

Action by 2011 General Assembly

No action was taken on Proposal 11-205.

Rationale: This proposal was addressed was addressed by General Assembly action on Proposal 11-201A.

Action by FDA February 26, 2012

Concurred with Conference action on Proposal 11-205.

Proposal Subject: Review of CDC *Vp* Illness Information

Specific NSSP Guide Reference: Section II Model Ordinance Chapter II @.05

Text of Proposal/ Requested Action N/A

Public Health Significance: The number of cases of *Vp* associated with consumption of shellfish reported to the CDC by states in 2009 shows a significant increase from previous years. There were not any large outbreaks that occurred during the year, but the total number of reported cases was the second highest since 1998, which included cases from outbreaks associated with product from all three coasts. The large number of 2009 cases, in the absence of a large outbreak, suggests that the ISSC needs to review current CDC *Vp* illness information and determine the adequacy of current control strategies in the NSSP.

The VMC and the ISSC Executive Board briefly discussed the 2009 reported illnesses and agreed that a *Vp* subcommittee should discuss the CDC reported information and make appropriate recommendations for VMC review. The purpose of this proposal is to notify the interested parties that change to the controls of Chapter II @.05 may be discussed at the ISSC 2011 Biennial Meeting.

Cost Information (if available):

Action by 2011 Task Force II Recommended adoption of *Vibrio* Management Committee recommendation on Proposal 11-206 to refer to an appropriate committee as determined by the Conference Chairman.

Action by 2011 General Assembly Adopted the recommendation of Task Force II on Proposal 11-206.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-206.

Proposal Subject: *Vibrio cholera*

Specific NSSP Guide Reference: Section II Model Ordinance Chapter II Risk Assessment and Risk Management

Text of Proposal/ Requested Action

Public Health Significance: In April of 2011, the State of Florida reported a shellfish related illness outbreak associated with a toxigenic strain of *Vibrio cholera* O75. Current knowledge of *Vibrio cholera* O75 suggests that this toxigenic strain can be pollution oriented or naturally occurring. The National Shellfish Sanitation Program (NSSP) requirements for addressing outbreaks are different for pollution related hazards and naturally occurring hazards. The determination of whether an outbreak of *Vibrio cholera* O75 is pollution related or naturally occurring is difficult and creates management problems for public health officials and shellfish control authorities.

Procedure XIV of the ISSC Constitution, Bylaws, and Procedures outlines steps for addressing pathogens and deleterious substances newly recognized in shellfish. The purpose of this proposal is to provide notice to the membership that FDA and the ISSC will be discussing appropriate steps to address the *Vibrio cholera* situation. If recommendations for NSSP controls are developed for consideration at the 2011 Biennial Meeting, the ISSC membership will be notified.

Cost Information (if available):

Action by 2011 Task Force II Recommended adoption of the Pathogen Review Committee recommendation to refer Proposal 11-207 to an appropriate committee as determined by the Conference Chairman.

Action by 2011 General Assembly Adopted the recommendation of Task Force II on Proposal 11-207.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-207.

Proposal Subject:	Aquaculture Facility Inspection Frequency
Specific NSSP Guide Reference:	Section II Model Ordinance Chapter VI. Shellfish Aquaculture @.01 General C.
Text of Proposal/ Requested Action:	The Authority shall inspect commercial aquaculture systems at least annually.
Public Health Significance:	Moving to a lesser number of inspections per year will not impact public health.
Cost Information (if available):	States are facing serious budget restrictions. Some find the current requirement for semi annual inspections to be excessive and not in furtherance of public health. State may maintain a higher frequency of inspection if they choose while allowing other states to decrease the frequency. States should, within limits, be able to determine priorities and allocate resources accordingly.
Action by 2011 Task Force III	Recommended referral of Proposal 11-208 to the appropriate committee as determined by the Conference Chairman.
Action by 2011 General Assembly	Adopted the recommendation of Task Force III on Proposal 11-208.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 11-208.

Proposal Subject: Certification Requirements for Retail Distribution Centers

Specific NSSP Guide Reference: Section II. Model Ordinance Chapter X. General Requirements for Dealers

Text of Proposal/ Requested Action:

.04 Certification Requirements.

A. General.

(1) Except as specified in (4) below, ~~N~~no person shall act as a dealer prior to obtaining certification.

(2) Any person who wants to be a dealer shall:

(a) Make application to the Authority for certification;

(b) Have and implement a HACCP Plan, and have a program of sanitation monitoring and record keeping in compliance with 21 CFR 123 as it appears in the *Federal Register* of December 18, 1995, except for the requirement for harvester identification on a dealer's tag.

(3) Each dealer shall have a business address at which inspections of facilities, activities, or equipment can be conducted.

(4) A Retailer that operates a Distribution Center that receives and distributes molluscan shellfish is not required to obtain certification as a shellfish dealer if:

(a) the Distribution Center ships shellfish only to retail outlets that are owned and operated by the same company that owns and operates the Distribution Center;

(b) the Distribution Center receives the shellfish from a source listed on the ICSSL and distributes the product to the retail stores in the original containers in which it was received; and

(c) from the time the shellfish is received at the distribution center to the time of sale or service to the consumer, the shellfish is maintained under the ownership and control of the company that owns and operates the Distribution Center and the retail stores.

Public Health Significance:

FDA considers retail food stores that receive molluscan shellfish from company-owned Distribution Centers that operate in the manner described above to be in compliance with 3-201.15 of the FDA Food Code, even if those Distribution Centers are not listed in the ICSSL. State and local regulatory authorities that license food stores may wish to take additional steps to be assured that the Distribution Centers can be considered an acceptable source, such as verifying that the Distribution Center maintains the shellfish as 45 deg F or below during storage and transit to the retail store.

This exception to II.X.04 applies regardless of whether one or more of the retail stores to which the product is shipped is located in a different State from where the distribution center is located and regardless of whether the distribution center and/or the stores are located in a State that has a program for certifying shellfish dealers.

If all three conditions listed in the proposed X.04.A. (4) are not met, then a Distribution Center that receives and ships shellfish in interstate commerce should seek certification and listing on the ICSSL.

Food safety concerns related to this policy should be minimal since no breakdown or repacking of shellfish is taking place and safe distribution and receiving is the responsibility of a single retail company and their own stores. Also the ability to effective conduct a product traceback should not be compromised by this because

ownership of the product isn't being transferred if the stores and Distribution Center are part of same company.

Cost Information (if available):

Action by 2011 Task Force II Recommended adoption of Proposal 11-209 as submitted.

Action by 2011 General Assembly Voted no action on Proposal 11-209.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-209.

Proposal Subject: In-Shell Product Labeling and the Use of Shellstock Tags

Specific NSSP Guide Reference: Section II. Model Ordinance Chapter X. General Requirements for Dealers
.07 In-Shell Product or Post Harvest Processed In-Shell Labeling

Text of Proposal/ Requested Action:

A. The dealer shall label all in-shell product~~, with tags meeting the requirements of Chapter X .05. B. (1)~~.

B. In-Shell Product ~~Tags~~ Labels.

(1) The dealer ~~tag~~ label on in-shell product shall contain the following indelible, legible information ~~in the order specified~~ below:
(a) The dealer's name and address;
(b) The dealer's certification number as assigned by the Authority;
(c) The original shellstock shipper's certification number. ~~If depurated the original shellstock shipper's certification number is not required;~~
(d) A "SELL BY DATE" which is a reasonable subsequent shelf-life or the words "BEST IF USED BY" followed by a date when the product would be expected to reach the end of its shelf-life. The date shall include, month, day and year;
~~(e)If depurated, the depuration cycle number or lot number;~~
~~(f)~~(e) The most precise identification of the harvest location as is practicable including the initials of the state of harvest, and the Authority's designation of the growing area by indexing, administrative or geographic designation. If the Authority has not indexed growing areas, then an appropriate geographical or administrative designation must be used (e.g. Long Bay, Decadent County, lease number, bed, or lot number).
~~(g)When the in-shell product has been transported across state lines and placed in wet storage in a dealer's operation, the statement: **"THIS PRODUCT IS A PRODUCT OF (NAME AND STATE) AND WAS WET STORED AT (FACILITY CERTIFICATION NUMBER) FROM (DATE) TO (DATE)";**~~
~~(h)~~(f) The type and quantity of in-shell product; and
~~(i)The following statement in bold capitalized type on each tag or label: **"THIS TAG IS REQUIRED TO BE ATTACHED UNTIL CONTAINER IS EMPTY OR IS RETAGGED AND THEREAFTER KEPT ON FILE FOR 90 DAYS**~~
~~(j)~~(g) All in-shell product intended for raw consumption shall include a consumer advisory. The following statement, from Section 3-603.11 of the Current Food Code, or an equivalent statement, shall be included on all shellstock: "Consuming raw or undercooked meats, poultry, seafood, shellfish or eggs may increase your risk of foodborne illness, especially if you have certain medical conditions."
~~(k)The statement "Keep Refrigerated" or an equivalent statement must be included on the tag or label.~~
(h) At a minimum the dealer shall tag or label each individual container in a legible and indelible form in accordance with CFR 21, Part 101; Part 161. Subpart B (161.30 and 161.136) and the Federal Fair Packaging and Labeling Act.

~~(2) If the in-shell product is removed from the original container, the tag on the new container shall meet the requirements in §.07B.~~

~~(3)~~(2) Country of origin information (USDA 2004) may be included on the ~~shucker-packer or reshipper tag~~ label.

~~(4)~~(3) When in-shell product intended for retail sale are packed in containers of 5 pounds or less and shipped in a master container which includes a tag in compliance with Chapter X .05 B. (1), the individual containers of 5 pounds or less shall not require tags as specified in Chapter X .05 B. (1) but may be labeled in some other manner with indelible, legible, information which at a minimum is adequate to trace the in-shell shellfish back to the lot of in-shell product it is part of. Consumer advisory information identified in Chapter X .07 B. (1) (j) shall be included on each retail package.

NOTE: A transition period of up to twelve (12) months should be allowed to allow dealer to utilize their current inventory of shellfish and supplies before the new labeling requirements must be met.

~~NOTE: The Consumer Advisory shall be required for both A and B.~~

Public Health Significance:

Shellfish dealers are required by the NSSP to tag or label shellfish to ensure that shellfish are from an approved source and in the event of a shellfish related illness, tags, labels and records provide for trace ability. In-shell product is defined as "non-living, processed shellfish with one or both shells present." In 2007 the ISSC amended the Model Ordinance to require dealers to label in-shell product with shellstock tags. In-shell product is packaged differently than live shellstock and is often individually quick frozen (IQF), and packed in sealed containers. Since the inception of this requirement in 2007, the Virginia Division of Shellfish Sanitation has routinely found in-shell oysters from Texas and Mississippi and in-shell mussels from New Zealand at Reshipper and Shellstock Shipper facilities without tags. The labels provided on these containers have had varying degrees of the required information. The Texas and Mississippi Authorities were notified as well as the ISSC Executive Office and the FDA. As a result of notifying the FDA, the Virginia Division of Shellfish Sanitation received a response via email from Paul DiStefano stating, "FDA does not consider it necessary to oppose the fact that the labeling is on the box and not a tag. As long as all the labeling information is there FDA would consider that acceptable." In light of this correspondence and interpretation by the FDA, Virginia Division of Shellfish Sanitation proposes to allow for labels to be used on in-shell product.

Cost Information (if available):

Action by 2011 Task Force II

Recommended adoption of Proposal 11-210 as amended.

A. The dealer shall tag or label all in-shell product, with tags meeting the requirements of Chapter X .05 B.(1).

B. In-Shell Product Tags or Labels.

(1) The dealer tag or label on in-shell product shall contain the following indelible, legible information in the order specified below:

(a) The dealer's name and address;

(b) The dealer's certification number as assigned by the Authority;

(c) The original shellstock shipper's certification number. If depurated the original shellstock shipper's certification number is not required;

(d) A "SELL BY DATE" which is a reasonable subsequent shelf-life or the words "BEST IF USED BY" followed by a date when the product would be expected to reach the end of its shelf-life. The date shall include, month, day and year;

(e) If depurated, the depuration cycle number or lot number;

(f) The most precise identification of the harvest location as is practicable including the initials of the state of harvest, and the Authority's designation of the growing area by indexing, administrative or geographic designation. If the Authority has not indexed growing areas, then an appropriate geographical or administrative designation must be used (e.g. Long Bay, Decadent County, lease number, bed, or lot number).

~~(g)When the in-shell product has been transported across state lines and placed in wet storage in a dealer's operation, the statement: **"THIS PRODUCT IS A PRODUCT OF (NAME AND STATE) AND WAS WET STORED AT (FACILITY CERTIFICATION NUMBER) FROM (DATE) TO (DATE)";**~~

~~(h)~~(g) The type and quantity of in-shell product; and

~~(i)~~(h) The following statement in bold capitalized type on each tag or label: **"THIS TAG IS REQUIRED TO BE ATTACHED UNTIL CONTAINER IS EMPTY OR IS RETAGGED AND THEREAFTER KEPT ON FILE FOR 90 DAYS." OR "THIS LABEL IS REQUIRED TO BE ATTACHED UNTIL CONTAINER IS EMPTY OR IS RELABLED AND THEREAFTER KEPT ON FILE FOR 90 DAYS."**

~~(j)~~(i) All in-shell product intended for raw consumption shall include a consumer advisory. The following statement, from Section 3-603.11 of the Current Food Code, or an equivalent statement, shall be included on all shellstock: "Consuming raw or undercooked meats, poultry, seafood, shellfish or eggs may increase your risk of foodborne illness, especially if you have certain medical conditions."

~~(k)~~(j) The statement "Keep Refrigerated" or an equivalent statement must be included on the tag or label.

(k) At a minimum the dealer shall tag or label each individual container in a legible and indelible form in accordance with CFR 21, Part 101; Part 161. Subpart B (161.30 and 161.136) and the Federal Fair Packaging and Labeling Act.

~~(2)~~If the in-shell product is removed from the original container, the tag or label on the new container shall meet the requirements in §.07B.

~~(3)~~(2) Country of origin information (USDA 2004) may be included on the shucker-packer or reshipper tag or label.

~~(4)~~(3) When in-shell product intended for retail sale are packed in containers of 5 pounds or less and shipped in a master container which includes a tag in compliance with Chapter X .05 B. (1), the individual containers of 5 pounds or less shall not require tags as specified in Chapter X .05 B. (1) but may be labeled in some other manner with indelible, legible, information which at a minimum is adequate to trace the in-shell shellfish back to the lot of in-shell product it is part of. Consumer advisory information identified in Chapter X .07 B. (1) (j) shall be included on each retail package.

NOTE: A transition period of up to twelve (12) months should be allowed to allow dealer to utilize their current inventory of shellfish and supplies before the new labeling requirements must be met.

NOTE: The Consumer Advisory shall be required for both A and B.

Action by 2011 General Assembly Adopted the recommendation of Task Force II on Proposal 11-210.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-210.

Proposal Subject: Guidance Document for 2 and 3 Log Reduction Method

Specific NSSP Guide Reference: Section IV. Guidance Documents Chapter IV. Naturally Occurring Pathogens .06 Guidance for 2 or 3 Log Reduction of *Vibrio parahaemolyticus* PHP Validation as an Alternative for Rapid Cooling

Text of Proposal/ Requested Action

.06 Method for Validation and Verification of a Two or Three Log Reduction of *Vibrio parahaemolyticus* (*V.p.*) in Oysters.

A. VALIDATION

1. Introduction:

Rapid refrigeration can slow the growth of *Vibrio parahaemolyticus* (*V.p.*) in recently harvested oysters. An alternative to rapid refrigeration requirements under NSSP is a post harvest process (PHP) which requires at least a two log reduction in *V.p.* levels for the Gulf and a three log reduction for the Pacific. This document provides guidance for the validation of a PHP to achieve either the two or three log reduction of *V.p.* density as appropriate.

2. Overview:

Validation of the PHP to achieve a two or three log reduction in *V.p.* levels is conducted on three harvest lots, with one initial measurement prior to PHP, or "pre-process", and ten measurements after the PHP or "post-process". This process is divided into three basic parts: 1) the pre-process *V.p* density determination of the lot, 2) determination of tube number and concentration of oyster homogenate aliquoted (inoculum) to obtain post-process *V.p.* density 3) validation and/or verification of the two or three log reduction as prescribed. Samples must be taken from three independent harvest lots to test the efficacy of the PHP process with confidence.

Although the pre-process sampling protocol requires three dilutions from one sample, post-process sampling protocol requires only a single dilution as indicated for each of the ten samples. These ten samples for each of three lots make a total of thirty samples. The number of positive tubes in each post-processed sample determines whether the sample passes or fails. The PHP is validated if no more than five of the thirty samples collected after processing fail. The PHP must be verified in each month it is performed.

The method of analysis will be the same MPN method as is utilized in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, used for the regulatory analyses for *V.p.* in shellfish as approved under the NSSP and cited in the National Shellfish Sanitation Program Guide for the Control of Molluscan Shellfish 2009 Section IV. Guidance Document Chapter II. Growing Areas.10 Approved National Shellfish Sanitation Program Laboratory Tests. *Although a* Most Probable Number (MPN) *series will be performed, an MPN/g value will not be attained or used throughout the validation process. Instead, the information used to validate and verify, and the data generated, is based on the statistical analysis of probability.*

3. Initial *V.p.* Density Determination:
For each pre-process lot, a ten-tube decimal dilution MPN is performed. The

tube code obtained establishes initial *V.p.* density on the pre-processed lot to determine how to perform the post-process lot measurements. For confidence in the initial measurement at least three dilutions are necessary. (The amount of the original sample in each dilution is one tenth as much as in the previous dilution. For example, if the lowest dilution has x grams, the next dilution has x/10, then x/100, etc.)

For a lot to be included in the validation the dilutions selected for the analysis must not result in all positive or all negative tubes. *It should be noted that in the unlikely event that the pre-processed sample tube code is not listed in the attached table, a problem in the determination of the initial V.p. level likely occurred and that the initial V.p density of the lot will have to be retested before continuing the validation study.* If unsure of the initial *V.p.* density it may be necessary to use more than three dilutions in the initial analysis. When more than three dilutions are used, the results from only three contiguous dilutions are significant in determination of the outcome. To select the three dilutions to be used, the following guidance is provided. In each example the selected dilutions are underlined in bold.

(a) When more than one of the dilutions used has all ten tubes positive, select the highest dilution (most dilute sample portion) having all ten tubes positive and the two following dilutions (i.e. 10,10,6,0).
(b) When only one of the dilutions used has all ten tubes positive, select that dilution and the two following dilutions (i.e. 10,8,4,0)
(c) When a positive tube or tubes occur in dilutions higher than the three dilutions chosen, add the number of positive tubes in the higher dilutions to the third dilution chosen (i.e. 10,9,3,1 becomes 10,9,4).
(d) When the sum of the tubes in the third dilution would exceed ten, select the three highest consecutive dilutions having at least one positive tube among them (i.e. 10,9,9,2).

4. Post PHP Process *V.p.* density determination (see attached table):

The three dilutions so determined form a tube code for the initial density of *V.p.* in the pre-processed samples. This tube code, listed in column one of the attached table in Appendix A. (see *Appendix A: Tube Code Table for Validation and Verification*), determines both the number of tubes used and the amount of inoculum in each of the post-processed samples. Once the tube code from the initial pre-process *V.p.* density measurement is obtained from the first column of the attached table, the number of tubes to be used in each of the ten post-processed samples can be obtained from the same row in the third column. Directly adjacent to column three in this same row, column four, indicates the maximum number of tubes allowed to be positive for that sample to pass.

Column two of the table shows three possible dilutions of the original sample that could have been used in the initial *V.p.* density determination. If these dilutions were used to generate the tube codes in column one of the attached table, then the volume of sample to be inoculated into each of the post-process single dilution MPN tubes for the sample lot is given directly adjacent. Hence the amount to inoculate for *V.p.* density determination of post-process samples is in column five for the Gulf (2 log) and column six for the Pacific (3 log).

Since the initial density of *V.p.* may vary considerably, dilutions other than the dilutions given in column two of the table may be used. When this occurs an adjustment must be made in the volume of post-process sample inoculated into each of the single dilution MPN tubes used.

For example, the dilutions prescribed in column 2 for tube code 10, 1, 0 are 0.001, 0.0001, 0.00001. If the dilutions used were actually 0.01, 0.001, 0.0001, the amount in column five or six would be multiplied by ten. Thus, the nine tube post-process single dilution MPN would have an adjusted sample inoculum of 0.1 gram/mL (0.01 x 10) and must be used for each sample from the lot rather than the 0.01 gram/mL sample inoculum specified in column five of the table to validate the two log reduction. In the same example, to validate the three log reduction, the adjusted sample inoculum of 1.0 gram/mL (0.1 x 10) must be used for each post-process sample from the lot instead of the 0.1 gram/mL specified in column six of the table.

5. Determining validation of two or three log reduction post PHP process:
Individual post-process samples pass or fail based on the number of positive tubes which result from the single dilution MPN, as found in column four of the table. In the example above for a pre-process sample tube code of 10,1,0 using a nine tube, single dilution MPN for the analysis, column four directly across from the tube code indicates that no more than four of the nine tubes per sample may be positive for the sample to pass. For the three lots to pass and the PHP to be validated for a two or three long reduction in *V.p.* density, no more than five of the thirty individual samples from the three lots tested post-process can fail.

B. VERIFICATION

1. Initial V.p. density determination:
In each month that oysters are post harvest processed, the first lot for processing is selected for testing. The method of testing the lot is similar to the testing for validation. An initial measurement uses ten tubes at three dilution levels. This initial measurement determines the number of tubes, mass of homogenate, and number of allowed turbid growth (positive) tubes used to test the oysters after PHP processing. The table used for validation is also used for the verification process.

If the initial measurement has all negative (non turbid)tubes and the mass of inoculum in the least dilute tube contains at least 1 gram of the oyster homogenate, then the process is considered verified for that month. If the least dilute tube contains less than 1 gram of homogenate the process should be repeated with 1 gram of sample. If an all negative result is again obtained the process is considered verified for that month. If growth is observed post-process verification testing must be performed.

2. Post PHP Process *V.p.* density verification:
Post processed verification testing uses the first lot of the month. Three outcomes are possible;
(a) the process is verified for the month, or
(b) the process fails verification and the process must be revalidated, or
(c) additional testing using a subsequent lot is needed.

Four parameters determine the verification test and they are outlined in the following table. The first parameter is the number of samples taken from a lot. When the process is validated ten samples are selected from each lot; however, for verification seven samples are to be taken from the lot. The second parameter is the maximum number of growth tubes for the process to be verified with the first lot. The maximum number of samples allowed to be positive for the process to verify is 1. The third parameter is the minimum number of positive tubes that causes the process to require revalidation, which is three.

Table 1. Positive Sample Maximum and Minimum

Number of Samples	First Lot Maximum Positive for Pass	First Lot Minimum Positive for Fail	Second Lot Maximum Positive for Pass	Probability of Passing for Non-degenerate Process
7	1	3	1	96%

If the number of positive tubes in the testing of the first lot is 2, then a second lot is selected. The fourth parameter is the maximum number of positive tubes allowed for verification when the second lot is used. The following table outlines this scenario.

Table 2. Pass/Fail Schematic

Monthly Verification	
First Lot	Second Lot
7	7
6	6
5	5
Fail 4	4
3	Fail 3
Second Lot Needed 2	2
1	1
Pass 0	Pass 0

The process has a 96% probability of passing verification as long as it is working optimally; should the process degenerate in efficacy, the probability of passing significantly decreases.

Public Health Significance: In 2009, the ISSC adopted Proposal 09-208 which allows for processors to utilized shellstock that is harvested outside the *Vp* controls established as part of the States' *Vp* Plans. The proposal established a 2 log reduction requirement for the Gulf of Mexico and the Mid-Atlantic States and a 3 log reduction requirement for the Pacific Coast States. This proposal provides guidance for the validation and verification for processors choosing to use this processing option

Cost Information (if available):

Action by 2011 Task Force II: Recommended adoption of Proposal 11-211-L as submitted.

Action by 2011 General Assembly Adopted the recommendation of Task Force II on Proposal 11-211-L.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-211-L.

Proposal Subject:	ISSC Policy Statement on the "Consumption of Raw Oysters"
Specific NSSP Guide Reference:	Section VI. NSSP Policy Setting Documents, ISSC Policy Statement, Paragraph 3
Text of Proposal/ Requested Action:	"Certain medically compromised individuals are at increased risk from common marine bacteria that are unrelated to pollution. Therefore, it may not be possible to address this risk through environmental controls. Although the reported number of illnesses and fatalities from these bacteria in the United States each year is small in comparison with other food borne illnesses, **shellfish that have been processed to reduce the levels of all pathogens of public health concern to safe levels can be eaten by the at-risk population or the at-risk population should eat molluscan shellfish fully cooked or,** total abstinence from raw molluscan shellfish is the best advice for medically compromised."
Public Health Significance:	This new ISSC policy setting language for the consumption of raw oysters will confirm the use of the labeling allowed for PHP shellfish listed in Chapter XVI. This new policy statement language will show the ISSC supports PHPs and that medically compromised individuals can choose safer post harvest processed shellfish rather than consume other raw shellfish that has not undergone a PHP and/or eat shellfish fully cooked.
Cost Information (if available):	None
Action by 2005 Task Force III	Recommended referral of Proposal 05-308 to an appropriate committee as determined by the Conference Chairperson to investigate the possibility of a change to the ISSC Policy Statement on the Consumption of Raw Molluscan Shellfish.
Action by 2005 General Assembly	Adopted recommendation of 2005 Task Force III.
Action by USFDA	Concurred with Conference action.
Action by 2007 Executive Board	Executive Board directed the Executive Director to discuss the ISSC Consumption Policy with the FDA. These discussions were not productive in identifying meaningful language for incorporating changes to the Policy Statement.
Action by 2007 Task Force III	Recommended no action on the proposed changes to the ISSC Policy Statement on the Consumption of Raw Molluscan Shellfish but, Recommended the Executive Board continue to pursue ways to acknowledge Post Harvest Processing in the National Shellfish Sanitation Program.
Action by 2007 General Assembly	Adopted recommendation of 2007 Task Force III.
Action by USFDA	December 20, 2007 Concurred with Conference action.

Action by 2009 ISSC Executive Board	The Executive Board has concluded that it is inappropriate to consider changes to the ISSC Policy Statement on the Consumption of Raw Molluscan Shellfish until the future of PHP is more clearly defined.
Action by 2009 Task Force III	Recommended no action on Proposal 05-308. Rationale: The Conference is in a state of transition regarding PHP approval and it is inappropriate at this time to consider changes to the policy.
Action by 2009 General Assembly	Adopted recommendation of 2009 Task Force III on Proposal 05-308.
Action by USFDA 02/16/2010	Concurred with Conference action on Proposal 05-308.
Action by 2011 Task Force III	The Task Force was advised that the ISSC Executive Board is continuing to discuss consumption policies with the USFDA. No action was required by Task Force III.
Action by 2011 General Assembly	No action was required by the General Assembly on Proposal 05-308.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 05-308.

Proposal Subject: Shellfish Sanitation Plant Element Evaluation Criteria

Specific NSSP Guide Reference: ISSC Constitution, Bylaws, and Procedures
Procedure XV. Procedure for Evaluation of Shellfish Sanitation Elements
Section 6. Subdivision b. Subdivision iv. (NEW)

Text of Proposal/ Requested Action Add new Subdivision iv. as follows:

iv. Shellfish sanitation program element compliance will be based on the following criteria:

(a) All dealers are required to be certified in accordance with the Guide for the Control of Molluscan Shellfish.

(b) 95% of the certified dealers evaluated must have been inspected by the state at the frequency required by the current Guide for the Control of Molluscan Shellfish.

(c) Where compliance schedules are required no more than 10% of the certified dealers evaluated will be without such schedules.

(d) States must demonstrate that they have performed proper follow up for compliance schedules for 90% of dealers evaluated, and if the compliance schedules were not met, that proper administrative action was taken by the state.

(e) All critical deficiencies have been addressed by the state inspector in accordance with the Guide for the Control of Molluscan Shellfish.

Public Health Significance: These criteria will be helpful to both the USFDA and States in the state evaluation process.

Cost Information (if available): No costs associated with this program addition.

Action by 2005 NSSP Evaluation Criteria Committee Recommended adoption of Proposal 05-310 as amended by the NSSP Evaluation Criteria Committee, the submitter.

iv. **Shellfish sanitation program element criteria shall be used to evaluate consecutive full evaluations (not including follow up). If a violation of the same criteria is repeated, the program element is considered out of compliance. This** ~~shellfish sanitation~~ program element compliance will be based on the following criteria:

(a) All dealers are required to be certified in accordance with the Guide for the Control of Molluscan Shellfish.

(b) 95% of the certified dealers evaluated must have been inspected by the state at the frequency required by the current Guide for the Control of Molluscan Shellfish.

(c) Where compliance schedules are required no more than 10% of the certified dealers evaluated will be without such schedules.

(d) States must demonstrate that they have performed proper follow up for compliance schedules for 90% of dealers evaluated, and if the compliance schedules were not met, that proper administrative action was taken by the state.

(e) All critical deficiencies have been addressed by the state inspector in accordance with the Guide for the Control of Molluscan Shellfish.

Action by 2005 Task Force III

Recommended adoption of the recommendations in the NSSP Evaluation Criteria Committee report with an effective date of October 1, 2004.

Action by 2005 General Assembly

Adopted recommendation of 2005 Task Force III.

Action by USFDA

FDA concurs with adoption of the five evaluation criteria for identifying state programs whose plant processing element is seriously out of compliance with the NSSP. FDA Recommended that the ISSC continue with efforts to develop additional criteria that may be used to define when a state program element is sufficiently out of compliance as to pose a public health risk. In particular, criteria are needed that focus on the in-field component of the FDA evaluation process, i.e. criteria to be used during the plant visit component of FDA's evaluation process. The criteria adopted by the 2005 Conference are more specific to the administrative aspects of a state's plant sanitation element. These criteria are examined as part of the central file review of a state program evaluation. Criteria that focus on the in-field component of the evaluation are also needed. New criteria should consider the distinction between sporadic plant deficiencies and those of an egregious and chronic nature that are indicative of systemic plant sanitation and safety problem.

Action by 2007 NSSP Evaluation Criteria Committee

The NSSP Evaluation Criteria Committee recommended that the following criteria be used by the USFDA in evaluating the state Plant Sanitation Element. FDA should provide a report to the ISSC regarding the effectiveness of the criteria.

ISSC Plant Evaluation Guidance

I. Plant Evaluation Criteria

1. Legal Authority – Chapter VIII. @.01 A. (2) (c)
   The plant sanitation element will be deemed in compliance if administrative laws and regulations exist that provide the administrative authority to implement the Dealer Certification requirements listed in Chapter I @.01 and @ 02. [Critical]

2. Initial Certification-Chapter I @ 02 B
   The Plant Sanitation Element will be deemed in compliance with this requirement when all plants are certified in accordance with criteria listed below:
   a. HACCP requirements:
      (i) A HACCP plan accepted by the Authority;
      (ii) No critical deficiencies;
      (iii) Not more than 2 key deficiencies;
      (iv) Not more than 2 other deficiencies
   b. Sanitation and additional Model Ordinance Requirements
      (i) No critical deficiencies;
      (ii) Not more than 2 key deficiencies;
      (iii) Not more than 3 other deficiencies.

3. Inspection frequency - Chapter I @ 02 F and G
   The Plant Sanitation Element will be deemed in compliance with this requirement when no more than one plant inspected doesn't meet the required inspection frequency.

4. Compliance schedules
   The Plant Sanitation Element will be deemed in compliance with this requirement when no more than 10% of the certified dealers evaluated are found to be without schedules.

5. Follow-up
   The Plant Sanitation Element will be deemed in compliance with this requirement when the state demonstrates that they have performed proper follow-up for compliance schedules for 90% of dealers evaluated and if the compliance schedules were not met that administrative action was taken.

6. Deficiency Follow-up
   The Plant Sanitation Element will be deemed in compliance with this requirement when the state demonstrates that all critical deficiencies have been addressed.

7. In-Field Plant Criteria
   The in-field Plant Sanitation Element will be deemed in compliance with this requirement when the plant meets the following criteria:

   a. Shucker/packers and repackers
      (i) HACCP requirements:
         (a) A HACCP plan accepted by the Authority; and
         (b) No critical deficiencies; and
         (c) Not more than 4 key deficiencies; or
         (d) Not more than 4 other deficiencies.
      (ii) Sanitation and additional Model Ordinance Requirements
         (a) No critical deficiencies; and
         (b) Not more than 4 key deficiencies; or
         (c) Not more than 6 other deficiencies.
   b. Shellstock shippers and reshippers
      (i) HACCP requirements:
         (a) A HACCP plan accepted by the Authority; and
         (b) No critical deficiencies; and
         (c) Not more than 3 key deficiencies; or
         (d) Not more than 3 other deficiencies.
      (ii) Sanitation and additional Model Ordinance Requirements
         (a) No critical deficiencies; and
         (b) Not more than 3 key deficiencies; or
         (c) Not more than 5 other deficiencies.

II. The following procedures will be implemented when an FDA evaluation identifies deficiencies with the above plant evaluation criteria.

   1. The overall Plant Sanitation Program element will be assigned one of the following designations:

a. Conformance: The program is in compliance with all of the criteria listed above.
b. Conformance with Deficiencies: The program is in compliance with I.1., I.2., I.3., I.4., I.5., I.6 and has less than 25% of plants with deficiencies associated with key or other compliance items in I.7.
c. Non-Conformance: The program is in compliance with I.1., but, does not meet the criteria in I.2., or I.3 or I.4 or I.5 or I.6 has greater than 25% (but less than 51%) of plants with deficiencies associated with key or other compliance items in I.7.
d. Major Non-Conformance: The program has multiple deficiencies. It is noncompliant with I.1, or 2 or more of I.2 or I.3 or I.4 or I.5 or I.6 or 51% or greater of plants with deficiencies associated with I.7.

2. FDA will follow the current compliance program for communication with the state agencies.

Action by 2007 Task Force III

Recommended adoption of the NSSP Evaluation Criteria Committee recommendation on Proposal 05-310. The guidance documents will be incorporated into the NSSP Guide and will be referenced in the ISSC Constitution, By Laws and Procedures. The Task Force recommended these criteria become effective October 1, 2007.

Action by 2007 General Assembly

Approved referral of Proposal 05-310 to the NSSP Evaluation Criteria Committee with the following recommendations:

1. That FDA use the criteria in this proposal as a two-year pilot program beginning October 2007; and
2. That FDA provide in-plant compliance rates for the states evaluated for the past two years and report those compliance rates to the first 2008 ISSC Executive Board meeting.

Action by USFDA

December 20, 2007
Concurred with Conference action with the following comments and recommendations for ISSC consideration.

On December 3, 2007, FDA forwarded correspondence to the ISSC Executive Office regarding the plant sanitation evaluation criteria adopted in Proposal 05-310. That correspondence, which asked for clarification regarding the use of the plant evaluation criteria during FDA's 2008 state program evaluation process, is provided below.

At the 2007 ISSC meeting Task Force II recommended adoption of Proposal 05-310 which set forth criteria developed by the NSSP Evaluation Criteria Committee for evaluating a state's Plant Sanitation Element with an effective date for use by FDA of October 1, 2007. However, the Voting Delegates, at the final General Assembly meeting, voted to refer Proposal 05-310 back to the NSSP Evaluation Criteria Committee with the following recommendations.

1. That FDA use the criteria as a two year pilot program beginning October 1, 2007; and
2. That FDA provide in-plant compliance rates for the states evaluated for the past two years and report those compliance rates to the first 2008 ISSC Executive Board meeting.

There is some confusion regarding the intent o the above ISSC action and how FDA should use the evaluation criteria during the recommended pilot. It was FDA's understanding that during the two year pilot the criteria would be used for purposes of determining the level of compliance of a state's Plant Sanitation Element and for recommending appropriate corrective/regulatory action. However, the State of Florida has indicated that this was not the intent of action taken by the 2007 ISSC. Florida suggests that the intent was for FDA to **not** use the evaluation criteria during the pilot for purposes of determining a state's level of compliance, but rather, for FDA to use the criteria to examine the appropriateness of the criteria by measuring the level of compliance. If this was the intended purpose then FDA does note see the need to conduct both a two year pilot and provide in-plant compliance rates for the states evaluated for the past two years and report those compliance rates to the first 2008 ISSC Executive Board meeting. By implementing #1 only, the ISSC can obtain the data necessary to examine the ability of the criteria and the associated levels of compliance (Conformance, Conformance with Deficiencies, etc.) to accurately reflect how well a state's program conforms with NSSP requirements without expending limited FDA and state resources to conduct a two year retrospective review.

The FDA further suggests that the previous plant evaluation criteria be used until the Conference (Voting Delegates or Executive Board) can give approval to use Proposal 05-310 criteria for compliance purposes.

Action by 2011 NSSP Evaluation Criteria Committee

The NSSP Evaluation Criteria Committee recommended that Section II. (1.)(b.) of the Plant Evaluation Criteria be amended as follows:

b. Conformance with Deficiencies: The program is in compliance with I.1., I.2., I.3., I.4., I.5., I.6 and has ~~less than~~ 25% or less of plants with deficiencies associated with key or other compliance items in I.7.

The NSSP Evaluation Criteria Committee also recommended that Section I. (7.) of the Plant Evaluation Criteria be amended as follows:

7. In-Field Plant Criteria

The in-field Plant Sanitation Element will be deemed in compliance with this requirement when the plant meets each of the following criteria:

a. Shucker/packers and repackers
  (i) HACCP requirements:
    (a) A HACCP plan accepted by the Authority; ~~and~~
    (b) No critical deficiencies; ~~and~~
    (c) Not more than 4 key deficiencies; ~~or~~
    (d) Not more than 4 other deficiencies.
  (ii) Sanitation and additional Model Ordinance Requirements
    (a) No critical deficiencies; ~~and~~
    (b) Not more than 4 key deficiencies; ~~or~~
    (c) Not more than 6 other deficiencies.
b. Shellstock shippers and reshippers
  (i) HACCP requirements:
    (a) A HACCP plan accepted by the Authority; ~~and~~
    (b) No critical deficiencies; ~~and~~

(c) Not more than 3 key deficiencies; ~~or~~
(d) Not more than 3 other deficiencies.
(ii) Sanitation and additional Model Ordinance Requirements
(a) No critical deficiencies; ~~and~~
(b) Not more than 3 key deficiencies; ~~or~~
(c) Not more than 5 other deficiencies.

Action by 2011 Task Force III Recommended adoption of Proposal 05-310 as amended by the NSSP Evaluation Criteria Committee.

Action by 2011 General Assembly Adopted the recommendation of Task Force III on Proposal 05-310.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 05-310.

Proposal Subject: Guidance on Equivalence Criteria for Food

Specific NSSP Guide Reference: N/A

Text of Proposal/ Requested Action: Under Article 4 of the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (SPS) (the SPS Agreement), each member nation of the WTO, including the United States, is obligated to accept as equivalent a food regulatory system of another country if it provides the same level of health protection as is provided to consumers by its own system.

Equivalent regulatory systems need not be identical. Under the concept of equivalence, the "sanitary or phytosanitary measures" used by an exporting country may differ from the measures applied domestically by an importing country as long as these measures "achieve the importing Member's appropriate level of sanitary or phytosanitary protection".

Under the SPS Agreement, the burden of demonstrating that equivalence exist rest with the exporting country. The exporting country has the right to decide for itself whether the regulatory system of the exporting country is equivalent to its own or is inadequate to achieve "the importing Member's appropriate level of sanitary or phytosanitary protection," or that inadequate evidence has been provided to demonstrate equivalence.

One of the roles of the USFDA in the National Sanitation Shellfish Program (NSSP) is the evaluation of foreign programs and the establishment of MOU's with countries that meet the requirements of the NSSP. This responsibility of FDA is outlined in IV. A. 4. of the ISSC/FDA Memorandum of Understanding, March 14, 1984. Article 4 of the WTO Agreement obligates the FDA to accept equivalency in foreign programs. The Agreement requires that the USFDA consider acceptance of foreign shellfish safety programs that, while having a system of sanitary measures that differ from those applied domestically, are recognized as providing an equivalent level of public health protection.

The FDA is seeking input from the ISSC for purposes of incorporating the concept of equivalency into the NSSP. Recognizing that FDA has a clear obligation under the WTO Agreement to take responsibility for equivalency determination, it is important to the Agency that this responsibility be recognized within the NSSP.

Public Health Significance: N/A

Cost Information (if available): N/A

Action by 2007 Task Force III Recommended referral of Proposal 07-303 to Executive Board for developing short term and long term approaches to incorporating equivalency into the NSSP and the ISSC.

Action by 2007 General Assembly Adopted recommendation of 2007 Task Force III.

Action by USFDA December 20, 2007
Concurred with Conference action.

Action by 2009 Executive Board Recommended the Executive Board continue discussions with FDA to address equivalency of food programs.

Action by 2009 Task Force III Recommended adoption of the Executive Board recommendation on Proposal 07-303.

Action by 2009 General Assembly Adopted recommendation of 2009 Task Force III on Proposal 07-303.

Action by USFDA 02/16/2010 Concurred with Conference action on Proposal 07-303.

Action by 2011 Task Force III Recommended adoption of Resolution 11-003 as a substitute for Proposal 07-303.

Resolution No. 11-003

Whereas, the Interstate Shellfish Sanitation Conference, (ISSC), and the Food and Drug Administration, (FDA), agreed to a Memorandum of Understanding, (MOU), on March 14, 1984 which continues to present; and

***Whereas*,** The National Shellfish Sanitation Program (NSSP) and its associated documents, including the FDA/ISSC MOU, do not make provisions for equivalency determinations or recognition of other programs; and

***Whereas*,** under Article 4 of the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (SPS), as a participating member, the U.S. is obligated to consider equivalent food safety measures of a participating country if those measures provide a level of public health protection equal to that provided by the U.S. system - the NSSP; and

***Whereas*,** FDA must address the concept of equivalence and related criteria afforded by non-NSSP shellfish regulatory systems; therefore

***Be it Resolved*,** that the ISSC recognizes that FDA, as a U.S. regulatory agency, is bound by the WTO to consider equivalency if requested by other countries and that the ISSC recognizes and accepts equivalency determinations by FDA; and

***Be it Further Resolved*,** that upon request from FDA, the ISSC will provide input on the criteria and evaluation processes that may be applied by FDA for such determinations.

Action by 2011 General Assembly Adopted the recommendation of Task Force III on Proposal 07-303.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 07-303.

Proposal Subject: Press Releases

Specific NSSP Guide Reference: Section II Model Ordinance Chapter II. Risk Assessment and Risk Management

Text of Proposal/ Requested Action: The US FDA issued press releases associated with outbreaks in the Pacific Northwest in the summer of 2006 and in Texas in March of 2007. These press releases created concern regarding the appropriateness and effectiveness of press releases as a public health measure to address an illness outbreak.
Use of press is to inform consumers.

The ISSC Executive Board discussed the issuance of these press releases and directed the formation of a working group to further investigate and review the use of press by state and federal agencies. The workgroup is to look for ways to coordinate use of press and provide recommendations for discussion at the 2007 Biennial Meeting.

Public Health Significance:

Cost Information (if available):

Action by 2007 Use of Press Committee Recommended that this Committee continue its deliberations and that a meeting be held in January 2008 in conjunction with appropriate FDA officials and report back to the Executive Board in March 2008. In the interim FDA will consult with the involved state regulatory agency on the content and timing of the release of press.

Action by 2007 Task Force III Recommended adoption of the Press Release Committee recommendation on Proposal 07-305.

Action by 2007 General Assembly Adopted recommendation of 2007 Task Force III.

Action by USFDA December 20, 2007
Concurred with Conference action.

Action by 2009 Use of Press Committee The Committee held a conference call on March 13, 2008, and planned a meeting in Washington, DC for April 30, 2008. The plans for this meeting were reported to the Executive Board on April 3, 2008.

On April 30, 2008, several members of the Committee and the ISSC Executive Director met with FDA officials at FDA headquarters and discussed agency procedures regarding use of press. The discussions of this meeting were presented to the Executive Board at the September 11, 2008, Executive Board meeting. The Committee reported that it is working to develop a press protocol for use in addressing press releases associated with outbreaks and product recall

The Committee held a meeting at the 2009 Biennial Conference and is continuing to develop a press protocol. The Committee will continue to fine tune a list of issues to be considered when use of press is contemplated. This list should be incorporated into NSSP Guidance Documents that address outbreaks and product recall.

Action by 2009 Task Force III

Recommended adoption of the Use of Press Committee recommendations on Proposal 07-305. Additionally, the Task Force recommended the Committee address the use of press in situations where significant time lapses have occurred between the last reported illness and the proposed use of press. The protocol should address the rationale for using press in situations where product is not likely to still be available for consumption.

Task Force III further recommended the Use of Press Committee complete the protocol and present the protocol to the Executive Board at the 2010 Spring Meeting. In the interim, as noted in the March 13, 2008, Use of Press Committee report, FDA should be requested to continue to consult with the involved State regulatory agencies on the content and timing of press releases.

Action by 2009 General Assembly

Adopted recommendation of 2009 Task Force III on Proposal 07-305.

Action by USFDA 02/16/2010

Concurred with Conference action on Proposal 07-305.

Action by 2011 Use of Press Committee

Recommended to the Executive Board that the ISSC request that the FDA Core Group coordinate with the ISSC Use of Press Committee concerning use of press protocols.

Criteria should include whether suspect product has been accounted for and the degree of public health risk. The Code of Federal Regulations protocols for use of press should be a guiding document as was the case for recall protocols developed by ISSC and FDA.

The Committee requested that a work group be appointed to craft a decision tree using the work done to date and the CFR guidance.

Members of the Committee that have volunteered for the work group include: Leslie Plamer, Chair; Maryanne Guichard; Don Ulstrom; Bill Dewey; Mike Antee; Tom Mahan; Lori Howell; and Mike Hickey.

Action by 2011 Task Force III

Recommended referral of Proposal 07-305 to an appropriate committee as determined by the Conference Chairman to continue to address the recommendations outlined in the 2011 Use of Press Committee report.

Action by 2011 General Assembly

Adopted the recommendation of Task Force III on Proposal 07-305.

Action by FDA February 26, 2012

Concurred with Conference action on Proposal 07-305.

Proposal Subject: Changes to ISSC State Regions

Specific NSSP Guide Reference: ISSC Constitution, Bylaws, and Procedures, Definitions, (3) ISSC REGION, Page 3

Text of Proposal/ Requested Action: Change Region 4 to include only the States of North Carolina, South Carolina and Georgia and move Florida to Region 5.

(3) ISSC REGION - geographical grouping of shellfish producing states with similar characteristics and interests, established to provide for fairly distributed representation. The ISSC Regions shall be:

Region 1 - Maine, New Hampshire, Massachusetts, Rhode Island
Region 2 - Connecticut, New York, New Jersey
Region 3 - Maryland, Delaware, Virginia
Region 4 - North Carolina, South Carolina, Georgia, ~~Florida~~
Region 5 - Florida, Alabama, Mississippi, Louisiana, Texas
Region 6 - Alaska, Washington, Oregon, California, Hawaii

Public Health Significance: The geographical grouping of shellfish producing states into ISSC Regions should be changed to reflect the environment and geographical area of those states. The current grouping includes Florida with the South Atlantic States of North Carolina, South Carolina and Georgia. Florida issues and interests are and have been Gulf Coast related. Changing this geographical grouping of these shellfish producing states would better align the states with similar characteristics and interests. The South Atlantic States of North Carolina, South Carolina and Georgia should be a separate region from Florida simply due to the geographical differences with the southeast coast.

Cost Information (if available):

Action by 2011 Task Force III Recommended adoption of Proposal 11-300 as submitted.

Action by 2011 General Assembly Voted no action on Proposal 11-300.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-300.

Proposal Subject:	ISSC Board Minutes
Specific NSSP Guide Reference:	ISSC Constitution, Bylaws, and Procedures Article V. Duties of the Board
Text of Proposal/ Requested Action:	Section 11. The Board shall direct the Executive Director to prepare written minutes of all Board meetings and make copies of such minutes for the previous two years available to the ISSC membership ~~at each Biennial Meeting~~ on the ISSC web site at www.issc.org.
Public Health Significance:	N/A
Cost Information (if available):	
Action by 2011 Task Force III	Recommended adoption of Proposal 11-301 as submitted.
Action by 2011 General Assembly	Adopted the recommendation of Task Force III on Proposal 11-301.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 11-301.

Proposal Subject: ISSC State Membership Fees

Specific NSSP Guide Reference: ISSC Constitution, Bylaws, and Procedures
Article III. Registration and Fees

Text of Proposal/ Requested Action: Section 4. There shall be two (2) categories of membership:

Subdivision a. State
Subdivision i. Shellfish producing states
Subdivision ii. Non-producing states

Subdivision b. Individual Member

The fee for each category of membership and the membership period shall be set by the Executive Board. The membership fees may be paid annually or biennially. The state authority membership dues shall include membership for one Voting Delegate. State membership shall be set to provide for forty (40%) per cent and individual membership shall be set to provide for five (5%) per cent of the previous ISSC fiscal year budget. Persons other than Voting Delegates shall be considered members by payment of the individual membership fee. The membership period shall coincide with the calendar year. Applications for membership shall be mailed at least thirty (30) days prior to the beginning of the membership period to the two (2) previous years' membership rolls.

Public Health Significance:

Cost Information (if available):

Action by 2011 Task Force III Recommended referral of Proposal 11-302 to the appropriate committee as determined by the Conference Chairman.

Action by 2011 General Assembly Adopted the recommendation of Task Force III on Proposal 11-302.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-302.

Proposal Subject: ISSC Task Force Consultants

Specific NSSP Guide Reference: ISSC Constitution, Bylaws, and Procedures
Article II. Task Force Consultant

Text of Proposal/ Requested Action:

Section 1. The Board Chairperson shall appoint a consultant for each Task Force from the Board.

Section 2. FDA and NMFS may provide a consultant for each Task Force. EPA may provide a consultant to Task Force I and Task Force III.

Section 3. Consultants will have no voting rights in Task Force action but will attend Task Force deliberations to offer advice as needed.

Public Health Significance: N/A

Cost Information (if available):

Action by 2011 Task Force III Recommended adoption of Proposal 11-303 as amended.

Section 1. The Board Chairperson shall appoint a consultant for each Task Force from the Board.

Section 2. FDA, EPA. and NMFS may provide a consultant for each Task Force. ~~EPA may provide a consultant to Task Force I and Task Force III.~~

Section 3. Consultants will have no voting rights in Task Force action but will attend Task Force deliberations to offer advice as needed.

Action by 2011 General Assembly Adopted the recommendation of Task Force III on Proposal 11-303.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-303.

Proposal Subject: Procedure for Handling and Disseminating Interpretations of the Manual by FDA

Specific NSSP Guide Reference: ISSC Constitution, Bylaws, and Procedures
Procedure XII.

Text of Proposal/ Requested Action: PROCEDURE XII. PROCEDURE FOR HANDLING AND DISSEMINATING

INTERPRETATIONS OF THE NSSP GUIDE FOR THE CONTROL OF

MOLLUSCAN SHELLFISH~~MANUAL~~ BY FDA.

Section 1. A request for Interpretation must be submitted to FDA Headquarters (Office of Food Safety~~Field Programs~~) through either an FDA Regional Office or the ISSC Executive Director according to the following routes:

Subdivision a. The interpretation request is submitted to the Office of Food Safety~~Field Programs~~ following the administrative chain of communication from industry to the State and, to the FDA Regional Office~~, to FDA Headquarters~~; or

Subdivision b. The interpretation request is submitted to the ISSC Executive Director by industry, a State, or the general public. The ISSC forwards the interpretation request to Office of Food Safety~~Field Programs~~ for a response.

Section 2. The interpretation request submitted to Office of Food Safety~~Field Programs~~ must be written and include the following:

Subdivision a. The question to be interpreted. Clearly state what the issue(s) is and include the NSSP Guide for the Control of Molluscan Shellfish reference(s) that is unclear and requires interpretation. Include any NSSP Guide for the Control of Molluscan Shellfish references related to the question.

Subdivision b. Who is requesting the interpretation? Give the name, state, area of interest (i.e., an industry person who operates an oyster shucker/packer operation, a State Shellfish Standardization Officer, etc.) and his/her address and phone number.

Subdivision c. The background surrounding the interpretation request. It is very important to understand the circumstances, motivation, and purpose for an interpretation to put it into context.

Subdivision d. An opinion on resolving the problem. Include

ideas on what the Interpretation should be. This includes what the NSSP Guide for the Control of Molluscan Shellfish means, the intent of the NSSP Guide for the Control of Molluscan Shellfish, how appropriate reference (CFR, EPA Guidance Document, etc.) should be interpreted.

Section 3. Within seven (7) days, the Office of Food Safety~~Field Programs~~ will acknowledge receipt of the letter to the requestor and FDA's Division of Federal and State Relations (DFSR)~~ and report which branch in FDA is responsible for developing the interpretation~~.

Section 4. All requests for interpretations must be sent to the Office of Food Safety~~Field Programs~~. ~~The Office of Field Programs will decide if the request is a technical or policy issue. The Office of Field Programs will develop technical interpretations and the FDA Office of Seafood will develop policy interpretations. Therefore, in the following subdivisions, if the request is a policy issue, substitute FDA Office of Seafood for Office of Field Programs.~~

Subdivision a. Within sixty (60) days of acknowledgment of the letter, the Office of Food Safety~~Field Programs~~ will provide a draft proposal to the FDA Regional Offices, the ISSC Executive Director, and DFSR for comment. The ISSC Executive Director shall distribute the draft proposal to the requestor and ISSC members from states, industry, and the general public.

Subdivision b. An additional thirty (30) days may be permitted for draft development if circumstances warrant. The requestor must be notified of the additional development time.

Section 5. Comments on the Draft Interpretation.

Subdivision a. The FDA ~~Office of Seafood, the~~ Regional Offices, ISSC Executive Director, and DFSR~~A~~ have thirty (30) days from receipt to comment on the draft proposal to the Office of Food Safety~~Field Programs~~. The ISSC Executive Director is responsible for receiving, consolidating, and forwarding to the Office of Food Safety~~Field Programs~~ comments from ISSC members from states, industry, and the general public.

Subdivision b. The FDA ~~Office of Seafood, the FDA~~ Regional Offices, ISSC Executive Director, and DFSR may request, in writing to the Office of Food Safety~~Field Programs~~, an additional thirty (30) days to comment on the draft proposal.

Section 6. Action on Draft Interpretation Comments.

Subdivision a. The Office of Food Safety~~Field Programs~~ has thirty (30) days from receipt of comments to complete the final interpretation by:

Subdivision i. Incorporating the comments and issuing a final interpretation; or

Subdivision ii. Issuing the final interpretation without revision.

Subdivision b. FDA may request an additional thirty (30) days for issuance of the final interpretation if circumstances warrant. The requestor and ISSC Executive Director must be notified of the additional development time.

Section 7. The Office of Food Safety~~Field Programs~~ shall disseminate final interpretations to the ISSC and DFSR for dissemination as follows:

Subdivision a. Upon receipt of the final interpretation, the ISSC Executive Director shall distribute it to the requestor and ISSC members from states, industry, and the general public.

Subdivision b. Upon receipt of the final interpretation, DFSR shall distribute it to the FDA Regional Offices and the Office of Food Safety~~Field Programs~~.

Subdivision c. Final interpretation shall be incorporated into the NSSP Guide for the Control of Molluscan Shellfish.

Public Health Significance:

Cost Information (if available):

Action by 2011 Task Force III Recommended adoption of Proposal 11-304 as submitted.

Action by 2011 General Assembly Adopted the recommendation of Task Force III on Proposal 11-304.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-304.

Proposal Subject: ISSC Constitutional Cost-Benefit Requirement for New Proposals that have a Significant Financial Impact on the States and Shellfish Industry

Specific NSSP Guide Reference:

Text of Proposal/ Requested Action: Article XIII. Procedure for the Submission of Proposals

Section 1. The Executive Director shall provide each registrant of the preceding Conference meeting at least one hundred sixty-five (165) days prior to the next Conference meeting with forms on which proposal for problems are to be submitted to the Executive Director for assignment to the appropriate Task Force.

Section 2. All proposals must be submitted to the Executive Office no later than one hundred twenty (120) days prior to the Conference meeting.

Section 3. Proposals submitted by any Conference participants requiring Conference action are to be referred to the Executive Director for assignment to the appropriate Task Force.

Section 4. Proposals submitted by any Conference participant that may have a significant cost to implement by either the SSCA or the shellfish industry must include an independent cost benefit analysis and an economic impact study.

Section 5~~4~~. The Executive Director shall review and assign all problems or proposals received for Task Force and Conference deliberation. Problem or proposal assignment shall be made according to subject matter and in accordance with Article XIII. Section 4., Section 5., Section 6., and Section 7. of the Constitution of the Conference.

Section 6~~5~~. Task Force I - Growing Areas: all proposals submitted to the Conference dealing with the classification or patrol of shellfish growing waters, relaying, training and research, or similar items concerning growing areas shall be assigned to Task Force I by the Executive Director.

Section 7~~6~~. Task Force II – Harvesting, Handling, and Distribution: all proposals submitted to the Conference dealing with the sanitation of harvesting, depuration, processing, labeling, transporting, storage, fill or content, training and research, or similar items concerning processing and distribution shall be assigned to Task Force II by the Executive Director.

Section 8~~7~~. Task Force III - Administration: all proposals submitted to the Conference dealing with Conference agreements, memorandums of understanding, complaints and challenges of reciprocity and program evaluations, or similar items, or items not specifically relating to Task Force I or II shall be assigned to Task Force III by the Executive Director.

Section 9~~8~~. The Executive Director shall provide the appropriate shellfish control authorities in each state and all members, at least ninety (90) days prior to each Conference meeting, with the proposals to be discussed under the heading of Unfinished Business.

Section 10~~9~~. Proposals submitted after the deadline, established in Article XIII Section 2 of the Constitution, will be reviewed and may be accepted by the Executive Board for Task Force Consideration. The Executive Board will use the following criteria in accepting late proposals.

Subdivision a. Why is the proposal being submitted after the deadline?

Subdivision b. Was the information available prior to the deadline?

Subdivision c. What is the criticality of the proposal to the safety of molluscan shellfish or the future of the ISSC?

Subdivision d. Does the proposal involve an NSSP Guide for the Control of Molluscan Shellfish change or an ISSC administrative change?

Section 11~~10~~. The Executive Director will consult with the Proposal Review Committee before declaring any problem or proposal invalid.

Section 12~~11~~. The Proposal Review Committee will review and prioritize proposals for Task Force consideration. The Committee will also provide consultation as needed to the Executive Director in assigning proposals to Task Forces.

Public Health Significance: Cost-Benefit Analyses and Economic Impact Studies are required by Federal and State Agencies prior to imposing new regulations. For too many years the ISSC through amendments made to the NSSP without any regards to the costs imposed on the SSCA and Shellfish Industry to implement the new guidelines.

Cost Information (if available): The cost to conduct cost-benefit analyses and economic impact studies will be much less on the SSCA'S and Shellfish Industry than the cost to implement by the SSCA's or by the shellfish industry.

Action by 2011 Task Force III Recommended referral of Proposal 11-305 to the appropriate committee as determined by the Conference Chairman. The committee is instructed to identify ways to better utilize the cost information portion of the proposal submission form.

Action by 2011 General Assembly Adopted the recommendation of Task Force III on Proposal 11-305.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-305.

Proposal Subject: Determining Effectiveness of NSSP Changes

Specific NSSP Guide Reference: ISSC Constitution, Bylaws, and Procedures
Article I. Task Forces
Procedure X. Procedure for Handling ISSC Summary of Actions

Text of Proposal/ Requested Action:

Article I. Task Forces

Section 6. Each Task Force shall deliberate all proposals during the times specified at the Conference meeting. Each Task Force Chairperson shall report the actions recommended by his/her respective Task Force to the voting delegates at the Conference under the heading of New Business for final Conference consideration. Any "No Action" recommended by a Task Force shall contain the reasons for the "No Action" recommendation. The Task Force will designate each proposal with a determination of cost of implementation. The designation will be all of the following:

Subdivision a. Significant costs to industry.
Subdivision b. Significant costs to State Shellfish Control Authority.
Subdivision c. Insignificant costs.

Procedure X. Procedure for Handling ISSC Summary of Actions

Section 5. All NSSP changes that have significant costs will be reviewed and assessed for effectiveness. This assessment will occur as part of the Conference meeting held in the fourth calendar year following adoption of the change. Those changes that are determined to be ineffective will be deleted.

Public Health Significance: N/A

Cost Information (if available):

Action by 2011 Task Force III Recommended referral of Proposal 11-306 to the appropriate committee as determined by the Conference Chairman.

Action by 2011 General Assembly Adopted the recommendation of Task Force III on Proposal 11-306.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-306.

Proposal Subject: Procedure for the Approval of Analytical Methods for the NSSP

Specific NSSP Guide Reference: ISSC Constitution, Bylaws, and Procedures
Procedure XVI. Procedure for the Approval of Analytical Methods for the NSSP

Text of Proposal/ Requested Action: PROCEDURE XVI. Procedure for the Approval of Analytical Methods for the NSSP

Section 1. Prior to NSSP adoption, all laboratory methods shall be ~~Systematic~~ evaluat~~ion~~ed by the ISSC ~~of the analytical method~~ using the validation criteria developed ~~by the ISSC~~ as detailed in the Single Laboratory Validation Protocol;

Section 2. All methods shall be submitted ~~Proposal~~ to the ISSC in proposal form requesting approval of the analytical method for use in the NSSP~~;~~

Subdivision a. Proposals shall include a ~~Submission of the Proposal~~ completed Single Laboratory Validation Method Application and Checklist.~~;~~

Subdivision b. The ISSC ~~Proposal presented to~~ Executive Director shall submit the proposal to the Laboratory Methods Review ~~for~~ Committee for review and development of recommendations to Task Force I~~consideration for acceptance~~.

Section 3. Review by Laboratory Methods Review Committee;

Subdivision a. The Laboratory Methods Review Committee shall conduct an ~~review and~~ evaluation of the data ~~submitted~~ which describe~~sing~~ the performance characteristics of the method;

Subdivision i. These performance characteristics include:

Subdivision (a) Accuracy (Trueness);
Subdivision (b) Measurement uncertainty;
Subdivision (c) Precision;
Subdivision (d) Recovery;
Subdivision (e) Specificity;
Subdivision (f) Linear range;
Subdivision (g) Limit of detection;
Subdivision (h) Limit of quantitation (sensitivity);
Subdivision (i) Ruggedness;
Subdivision (j) Comparability if applicable (comparison of the performance of the new/modified method to the accepted method.

Subdivision ii. Method documentation including:
Subdivision (a) Method title, scope and references;
Subdivision (b) Equipment and reagents required;
Subdivision (c) Sample collection,

preservation and storage requirements;
Subdivision (d) Safety requirements;
Subdivision (e) Step by step procedure;
Subdivision (f) Specific quality control measures associated with the method;
Subdivision (g) Cost of the method;
Subdivision (h) Sample turnaround time.

Subdivision iii. Specific application(s);

Subdivision b. Review of need for the method;

Subdivision i. Method meets an immediate or continuing need;

Subdivision ii. Improves analytical capability under the NSSP as an alternative to an accepted method(s);

Subdivision iii. Replaces other approved or accepted method(s).

Section 4. ~~Possible Actions by t~~The Laboratory Methods Review Committee shall submit one of the following recommendations to Task Force I:~~;~~

Subdivision a. Non-acceptance pending further information as defined by the Committee;

Subdivision b. Accept as an Approved NSSP ~~Type IV~~ Method;

Subdivision c. Accept as an Approved Limited Use NSSP ~~Type III~~ Method;

Subdivision d. Accept as an Emergency Use NSSP ~~Type III~~ Method~~, and recommend adoption as Type II or Type I Method;~~

~~Subdivision e. Rescind acceptance for cause (the need no longer exists, poor performance, equipment or reagents no longer available, etc.)~~

Section 5. Requests for ISSC recantation of an approved method shall be submitted using the ISSC proposal form. The request for recantation must include reason for the request, i.e. the need no longer exists, poor performance, equipment or reagents no longer available, etc.~~Task Force recommendation (or non-recommendation) for adoption by ISSC;~~

Section 6. ~~Section 6. Adoption (or non-adoption) by ISSC General Assembly;~~ Types of NSSP analytical methods.

Subdivision a. Approved NSSP Methods.
Approved NSSP methods are those accepted for use as permanent methods and cited in the NSSP Guide for the Control of Molluscan Shellfish, Guidance Documents Chapter II. Growing Areas .10 Approved National Shellfish Sanitation

Program Laboratory Tests. These methods have been long used in the NSSP or have completed the Single Laboratory Validation Method Protocol to show that the method is fit for purpose in the NSSP. Approved NSSP Methods have been:

Subdivision i. Described in a scientific or other peer-reviewed professional publication;

Subdivision ii. Used successfully to detect or quantify;

Subdivision iii. Evaluated and the performance characteristics for specific applications have been determined and found fit for purpose;

Subdivision iv. Collaboratively studied and/or collaboratively tested.

Subdivision b. Approved Limited Use Methods.

Approved Limited Use Methods are methods accepted for use in NSSP and listed in the NSSP Guide for the Control of Molluscan Shellfish, Guidance Documents Chapter II. Growing Areas .10 Approved National Shellfish Sanitation Program Laboratory Tests. These methods are alternative methods within the NSSP that can meet an immediate need of the NSSP, improve turnaround time, cost effectiveness, and / or increase analytical capacity. Approved Limited Use Methods can include screening, provisional, or methods with limitations as defined by the LMRC evaluation of the method.

Subdivision c.Emergency Use Methods.

Emergency Use Methods are methods used to meet an immediate or ongoing critical need for a method of analysis and no NSSP approved method exists. Emergency Use Methods may be given interim approval by the ISSC Executive Board provided the following criteria are provided:

Subdivision i. Name of Method;

Sudivision ii. Date of Submission;

Subdivision iii. Specific purpose or intent of the method for use in the NSSP;

Subdivision iv. Step by step procedure including equipment, reagents and safety requirements necessary to run the method;

Subdivision v. Data generated in support of the efficacy of the method if available;

Subdivision vi. Any peer reviewed articles detailing the method and its efficacy;

Subdivision vii. Name of the developer or SSCA submitter;

Subdivision viii. Developer or submitter contact information.

~~Section 7. Review and Acceptance by FDA Office of Food Safety in the Summary of Actions;~~

~~Section 8. Addition to/removal from the NSSP *Guide for the Control of Molluscan Shellfish*, Guidance Documents Chapter II. Growing Areas .10 Approved National Shellfish Sanitation Program Laboratory Tests: Microbiological and Biotoxin Analytical Methods.~~

~~Section 9. Types of NSSP analytical methods.~~

~~Subdivision a. Type I Methods. Type I methods are methods accepted for use in the NSSP and cited in the NSSP *Guide for the Control of Molluscan Shellfish*, Guidance Documents Chapter II. Growing Areas .10 Approved National Shellfish Sanitation Program Laboratory Tests: Microbiological and Biotoxin Analytical Methods that have been:~~

~~Subdivision i. Described in a scientific or other peer-reviewed professional publication;~~

~~Subdivision ii. Used successfully to detect or quantify;~~

~~Subdivision iii. Evaluated, and the performance characteristics for specific applications have been determined and found fit for purpose;~~

~~Subdivision iv. Collaboratively studied and/or collaboratively tested; and/or,~~

~~Subdivision v. Long used as an accepted method in the NSSP. Examples: APHA MPNs for total and fecal coliforms, Modified A-1 MPN (MA-1), and the mouse bioassay for saxitoxins (PSP).~~

~~Subdivision b. Type II Methods. Type II methods are methods accepted for use in the NSSP and cited in the NSSP *Guide for the Control of Molluscan Shellfish*, Guidance Documents Chapter II. Growing Areas .10 Approved National Shellfish Sanitation Program Laboratory Tests: Microbiological and Biotoxin Analytical Methods that have been:~~

~~Subdivision i. Described in a scientific or other peer-reviewed professional publication;~~

~~Subdivision ii. Used successfully to detect or quantify;~~

~~Subdivision iii. Evaluated, and the performance characteristics for specific applications have been determined and found fit for purpose;~~

~~Subdivision iv. Long used and accepted for use in the NSSP. Examples: Elevated temperature coliform pour plate method (ETCP) and the mouse bioassay for brevetoxins (NSP).~~

~~Subdivision c. Type III Methods. Type III methods include those methods accepted by unanimous vote of the Laboratory Methods Review Committee for use on an interim basis and cited in the NSSP *Guide for the Control of Molluscan Shellfish*,~~

~~Guidance Documents, Chapter II, Growing Areas .10 Approved National Shellfish Sanitation Program Laboratory Tests: Microbiological and Biotoxin Analytical Methods that have been:~~

~~Subdivision i. Described in a scientific or other peer-reviewed professional publication;~~

~~Subdivision ii. Used successfully to detect or quantify;~~

~~Subdivision iii. Evaluated, and the performance characteristics for specific applications have been determined and found fit for purpose;~~

~~Subdivision iv. Selected to fulfill a continuing need;~~

~~Subdivision v. Designated for review and assessment by the Laboratory Methods Review Committee for continued use, re-designation or deletion.~~

~~Subdivision d. Type IV Methods. Type IV methods include those methods accepted by majority vote of the Laboratory Methods Review Committee for use on an interim basis and cited in the NSSP *Guide for the Control of Molluscan Shellfish*, Guidance Documents, Chapter II, Growing Areas .10 Approved National Shellfish Sanitation Program Laboratory Tests: Microbiological and Biotoxin Analytical Methods that have been:~~

~~Subdivision i. Described in a scientific or other peer-reviewed professional publication;~~

~~Subdivision ii. Used successfully to detect or quantify;~~

~~Subdivision iii. Evaluated, and the performance characteristics for specific applications have been determined and found fit for purpose;~~

~~Subdivision iv. Selected to fulfill an immediate need;~~

~~Subdivision v. Designated for review and assessment by the Laboratory Methods Review Committee for continued use, re-designation or deletion.~~

Public Health Significance:

Cost Information (if available):

Action by 2011 Task Force III Recommended adoption of Proposal 11-307 as submitted.

Action by 2011 General Assembly Adopted the recommendation of Task Force III on Proposal 11-307.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-307.

Proposal Subject: Revision of Procedure XVI of the ISSC's Constitution, Bylaws and Procedures

Specific NSSP Guide Reference: ISSC Constitution, Bylaws and Procedures Procedure XVI. Procedure for Acceptance and Approval of Analytical Methods for the NSSP (Section 3, subdivision b, b i, b ii, b iii, Section 4, subdivision a, subdivision b, subdivision c, subdivision d, subdivision e, subdivision f and subdivision g, Section 5, Section 6, Section 7, Section 8, Section 9, subdivision a, a ii, a iii, a iv, Subdivision b, b ii, b iii, b iv, Subdivision c, c ii, c iii, c iv, c v, Subdivision d, d ii, d iii, d iv, d v.)

Text of Proposal/ Requested Action Revise Procedure XVI ISSC Constitution, Bylaws and Procedures as follows.

Section 3 Review by Laboratory Methods Review Committee;

Subdivision a. Committee review ……

Subdivision i. These performance ……..

Subdivision (a) Accuracy (Trueness);
Subdivision (b) Measurement uncertainty;
Subdivision (c) Precision;
Subdivision (d) Recovery;
Subdivision (e) Specificity;
Subdivision (f) Linear range;
Subdivision (g) Limit of detection;
Subdivision (h) Limit of quantitation (sensitivity);
Subdivision (i) Ruggedness;
Subdivision (j) Comparability if applicable……..

Subdivision ii. Method documentation including: ……

Subdivision (a) Method title…….
Subdivision (b) Equipment and…….
Subdivision (c) Sample collection ……
Subdivision (d) Safety requirements;
Subdivision (e) Step by step procedure;
Subdivision (f) Specific quality…….
Subdivision (g) Cost of the method;
Subdivision (h) Sample turnaround time.

Subdivision iii. Specific application(s);

Subdivision b. Review of the need for the method;

Subdivision i. ~~Method~~ Meets ~~an immediate or continuing~~ a critical need for a method of analysis where there is no existing NSSP method available;

Subdivision ii. Improves turnaround time, cost effectiveness or develops analytical capacity beyond existing NSSP methods; ~~capability under the NSSP as an alternative to an accepted~~

~~method(s);~~

Subdivision iii. Replaces an obsolete NSSP method of analysis. ~~other approved or accepted method(s).~~

Section 4. Possible Actions by the Laboratory Methods Review Committee;

Subdivision a. Recommend non-adoption as the proposed method does not meet a critical need; does not replace an obsolete NSSP method or improve turnaround time, cost-effectiveness or develop analytical capacity beyond existing NSSP methods;

Subdivision b. Recommend non-adoption ~~Non-acceptance~~ pending further information as ~~defined~~ required by the Committee;

Subdivision c. Recommend adoption ~~Accept~~ as a Type IV Method;

Subdivision d. Recommend adoption ~~Accept~~ as a Type III Method;

Subdivision e. ~~Accept as a Type III Method and~~ Recommend adoption as a Type II ~~or Type I~~ Method;

Subdivision f Recommend adoption as a Type I Method;

Subdivision g. ~~Rescind acceptance~~ Recommend rescinding the method for cause (the need no longer exists, poor performance, equipment or reagents no longer available, little or unused etc.).

Subdivision h. Recommend no action as there has been no response to the Committee's request for further information or additional data.

Section 5. Task force Recommendation (or non-recommendation) for adoption by the ISSC;

Section 6. Adoption (or non-adoption) by the ISSC General Assembly;

Section 7. Review and concurrence (or non-concurrence) ~~Acceptance~~ by FDA Office of Food Safety in the Summary of Actions;

Section 8. If the Task Force's action is adopted by the Conference and concurred with by FDA, the method is added ~~Addition~~ to/~~removal~~ removed from the Table of Approved National Shellfish Sanitation Program Laboratory Tests in the NSSP *Guide for the Control of Molluscan Shellfish*, Guidance Documents Chapter II. Growing Areas .10. ~~Approved National Shellfish Sanitation Program Laboratory Tests: Microbiological and Biotoxin Analytical Methods.~~

Section 9. Types of NSSP analytical methods.

Subdivision a. Type I Methods. Core Methods. ~~Type 1~~ Core methods are methods accepted for use in the NSSP and ~~cited~~ listed in the NSSP *Guide for the Control of Molluscan Shellfish*, Guidance Documents Chapter II. Growing Areas .10 Approved National

Shellfish Sanitation Program Laboratory Tests~~:~~ ~~Microbiological and Biotoxin Analytical Methods~~ that have been:

Subdivision i. Described in a scientific or other peer-reviewed professional publication;

Subdivision ii. Used successfully throughout the NSSP to detect or quantify;

Subdivision iii. Evaluated, and the performance characteristics for specific applications in the NSSP have been determined and found fit for purpose;

Subdivision iv. Collaboratively studied and/or collaboratively tested ; and/or,

Subdivision v. Long used as ~~an accepted method~~ the "gold standard" throughout ~~in~~ the NSSP to meet established Program requirements. Examples of Type I methods the: the ~~APHA~~ MPN~~s~~ methods for both total and fecal coliforms, ~~Modified A-1 MPN~~ ~~(MA-1)~~ and the mouse bioassays for ~~saxitoxin~~Paralytic shellfish toxins (PSP) and brevetoxins (NSP).

Subdivision b. Type II Methods. Permanent Methods. ~~Type II~~ Permanent methods are methods accepted for use in the NSSP and ~~cited~~ listed in the NSSP *Guide for the Control of Molluscan Shellfish*, Guidance Documents Chapter II Growing Areas .10 Approved National Shellfish Sanitation Program Laboratory Tests~~: Microbiological and Biotoxin Analytical Methods~~ that have been:

Subdivision i. Described in a scientific or other peer-reviewed professional publication;

Subdivision ii. Used successfully within the NSSP to detect or quantify;

Subdivision iii. ~~Evaluated~~ NSSP validated and the performance characteristics for specific applications within the NSSP have been determined and found fit for purpose;

Subdivision iv. ~~Long~~ Widely used ~~and accepted for use~~ within the NSSP as alternative methods to improve turnaround time, cost effectiveness or to develop analytical capacity beyond what is achieved by the core methods. Examples of

Type II methods: the Elevated temperature coliform ~~pour~~ plate method (ETCP) for fecal coliform analysis and the ~~mouse bioassay for brevetoxins (NSP)~~ mTEC membrane filtration method for fecal coliforms.

Subdivision c. Type III Methods.

Interim Methods. ~~Type III~~ Interim methods are ~~include those~~ methods accepted ~~by unanimous vote of the Laboratory Methods Review Committee~~ for use in the NSSP ~~on an interim basis~~ and listed ~~cited~~ in the NSSP *Guide for the Control of Molluscan Shellfish*, Guidance Documents , Chapter II, Growing Areas .10 Approved National Shellfish Sanitation Program Laboratory Tests~~: Microbiological and Biotoxin Analytical Methods~~ that have been:

Subdivision i. Described in a scientific or other peer-reviewed professional publication;

Subdivision ii. Used within the NSSP to detect or quantify;

Subdivision iii. ~~Evaluated~~ NSSP validated and the Performance characteristics for specific applications within the NSSP have been determined and found fit for purpose;

Subdivision iv. Selected to fill ~~fulfill~~ an ongoing NSSP Program ~~continuing~~ need;

Subdivision v. Used effectively outside the laboratory in which the method was developed and/or validated;

Subdivision vi. Designated for periodic review and assessment by the Laboratory Methods Review Committee as to the feasibility for continued use, re-designation or deletion of the method. Examples of Type III methods: the Jellett Rapid Test (JRT) for PSP and the mEndo-LES membrane filtration method for UV treated process water.

Subdivision d. Type IV Methods. Provisional Methods. ~~Type iv~~ Provisional methods are ~~include those~~ methods accepted ~~by majority vote of the Laboratory Methods Review Committee~~ for use in the NSSP ~~on an interim basis~~ and ~~cited~~ listed in the NSSP *Guide for the Control of Molluscan Shellfish* , Guidance Documents, Chapter II Growing Areas .10 Approved National Shellfish Sanitation Program Laboratory Tests~~: Microbiological and Biotoxin Analytical Methods~~ that: ~~have been:~~

Subdivision i. Have been described in a scientific or other

peer-reviewed professional publication;

Subdivision ii. Can be used ~~successfully~~ within the NSSP to detect or quantify;

Subdivision iii. ~~Evaluated,~~ Have been NSSP validated and the performance characteristics for specific applications within the NSSP have been determined and found fit for purpose;

Subdivision iv. Have been selected to ~~fulfill~~ fill an ongoing NSSP Program ~~Immediate~~ need;

Subdivision v. Have been Newly accepted for use and/or not yet used for Program support outside the laboratory in which the method was developed and/or validated;

Subdivision vi. Designated for periodic review and assessment by the Laboratory Methods Review Committee as to the feasibility for ~~continued~~ use, redesignation or deletion of the method. Examples of Type IV methods: the HPLC post column oxidation (PCOX) method for paralytic shellfish toxins and the Male Specific Coliphage (MSC) method for soft shell clams and American Oysters.

Public Health Significance: The revision of Procedure XVI is meant to clarify the overall process of analytical method acceptance into the NSSP and ensure that only proven methods of analysis are available for use to support NSSP Program requirements.

Cost Information (if available): None

Action by 2011 Task Force III Recommended no action on Proposal 11-308.

Rationale: Proposal 11-308 was resolved by Task Force action on Proposal 11-307.

Action by 2011 General Assembly Adopted the recommendation of Task Force III on Proposal 11-308.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-308.

Proposal Subject:	ISSC Procedure for the Submission of Proposals
Specific NSSP Guide Reference:	ISSC Constitution, Bylaws, and Procedures Article XIII. Procedure for the Submission of Proposals
Text of Proposal/ Requested Action:	Section 8. The Executive Director shall provide the appropriate shellfish control authorities in each state and all members, at least ninety (90) days prior to each Conference meeting, with the proposals to be discussed under the heading of Unfinished Business or New Business. ~~Section 11. The Proposal Review Committee will review and prioritize proposals for Task Force consideration. The Committee will also provide consultation as needed to the Executive Director in assigning proposals to Task Forces.~~
Public Health Significance:	N/A
Cost Information (if available):	
Action by 2011 Task Force III	Recommended adoption of Proposal 11-309 as submitted.
Action by 2011 General Assembly	Adopted the recommendation of Task Force III on Proposal 11-309.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 11-309.

Proposal Subject: Internal Authority Self-Assessment Using a National Program Standards Manual

Specific NSSP Guide Reference: Section II Model Ordinance
Chapter I Shellfish Sanitation Program Requirements for the Authority
@.01 Administration

Text of Proposal/ Requested Action

@.01 Administration

A. Scope…
B. State Law and Regulations…
C. Records…
D. Shared Responsibilities…
E. Administrative Procedures…
F. Epidemiologically Implicated Outbreaks of Shellfish-Related Illness…
G. Commingling…
H. Program Evaluation. The Authority shall conduct a self-assessment using the National Program Standards Manual and report annually to the U.S. Food and Drug Administration the results of the assessment.

Public Health Significance: The purpose of this proposal is to begin discussions on how a self-assessment can be used by Authorities to conduct a comprehensive evaluation of their ability to promote the protection of public health. An assessment conducted by an Authority may encourage continuous improvement and innovation and can assure that individual program activities provide comparability among other domestic and international shellfish programs. The evaluation can be used to assist both the FDA and shellfish Authorities in fulfilling regulatory obligations and ensuring the implementation of the requirements set forth in the NSSP Model Ordinance.

Cost Information (if available)

Action by 2011 Task Force III Recommended referral of Proposal 11-310 to the appropriate committee as determined by the Conference Chairman.

Action by 2011 General Assembly Adopted the recommendation of Task Force III on Proposal 11-310.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-310.

Resolution Subject: Memorial Resolution Justin Taylor

Text of Resolution: ***Whereas***, Justin Taylor was born in Shelton, Mason County, on February 16, 1921, and died 90 years later on February 22, 2011. Mr. Taylor helped build his family business, Taylor Shellfish, into a family dynasty now in the fifth generation, and the largest shellfish-farming business in the country. A Navy veteran, Mr. Taylor served on the USS Texas during World War II, enduring enemy fire at Normandy, Iwo Jima and Okinawa, then went back to serve his country again on a Navy oil tanker during the Korean War.

Whereas, Justin grew up alongside Squaxin Island tribal members, with whom his family formed close friendships. His family gifted to the tribe the land on which its museum and cultural center stands, and his relationships with tribal members stretch back to childhood.

Whereas, as a young man Justin logged by hand with a crosscut saw, later using a chain saw in the redwoods of Northern California. He gave up working in the woods when he married, considering the work too dangerous for a family man, and returned in 1956 to the shellfish beds of Puget Sound, slowly buying up property and building the business. Known for his modesty, he was the type to park his rig a long way from the headquarters office or processing plant, to leave the good spots for other people — even though he was nearly 90.

Whereas, Justin was married 55 years to Carol Hunter Taylor and raised three children, and lived to see the business founded by his father not only grow, but pass on successfully to the fifth generation. The company has about 600 employees, 9,000 acres of shellfish beds in production, customers all over the world and operations in the U.S., Canada, Hong Kong and Fiji.

Whereas, Justin had a high-school education, and the mind of a Renaissance man, stoked by a lifelong curiosity, reading everything he could get his hands on, and still coming into the office at Taylor Shellfish in Shelton nearly every day to share a clipping he had just read, and go out for lunch with his sons or visit with employees.

Whereas, Puget Sound was Justin's home, and he fought for its cleanup and conservation long before it was a popular cause, filing one of the first environmental lawsuits ever in Washington against the pulp-mill industry. He kept at it. A letter written in his firm hand in 2009 to Gov. Chris Gregoire reads: "I am writing this because of my concern for Puget Sound. I am 88 years old and still get out on the tide flats most daylight tides. I am appalled at the ever-increasing decline of water quality."

Whereas, Justin was a humble giant, and a real friend of the water. He had a real bond with the Sound, and the waterman's life.

Be It Therefore Resolved that the Interstate Shellfish Sanitation Conference extends its gratitude for Justin's leadership and lasting contributions to the organization; and

Be It Further Resolved, that the Interstate Shellfish Sanitation Conference acknowledge his contributions by a letter to that effect to his family.

Action by 2011 Resolutions Committee	Recommended adoption of Resolution 11-001 as submitted.
Action by 2011 Executive Board	Recommended adoption of Resolutions Committee recommendation on Resolution 11-001.
Action by 2011 General Assembly	Adopted Resolution 11-001.
Action by FDA February 26, 2012	Concurred with Conference action on Resolution 11-001.

Resolution Subject: Memorial Resolution Peter Maistrellis

Text of Resolution: ***Whereas,*** Peter A. Maistrellis was born March 2, 1942, in Peabody, Massachusetts and passed away at the age of 68 on November 23, 2009, after a long, courageous battle with his illness.

Whereas, Peter was the second of three sons. He was an alumnus of Peabody High School and attended the United States Merchant Marine Academy at King's Point, N.Y. In 1963, he received his Bachelor of Science degree in Marine Engineering and was commissioned as an Ensign in the U.S. Naval Reserve. While attending King's Point, Peter was captain of the basketball team and captain of the Color Guard. Proudly serving in the Merchant Marines from 1962 through 1965. Peter traveled throughout the world and forged friendships that would last his lifetime.

Whereas, Peter was also an honored Associate Member of the Society of Naval Architects and Engineers. He loved hard work. After working along side his father-in-law, George Pappas, Peter went on to become a well-respected founding partner in Ipswich Maritime Products. Known for his common sense and business acumen, Peter served for many years as a member of the First National Bank of Ipswich Board of Directors and sat on the Finance Committee of Beverly Hospital.

Whereas, Peter was also an accomplished athlete and an avid tennis player. Peter was a supportive son, a loving father, a loyal friend and brother, and a devoted husband. He could be relied upon to offer his experience and lend a hand to many young friends who sought his guidance throughout his life. Peter approached the world with grace and dignity and treated those around him with respect and kindness. We will remember his quick wit and his compassion. He will be missed by all who knew him.

Whereas, Peter leaves his beloved wife of 48 years, Estelle Sally; his son, Christopher, daughter-in-law, Michelle, and his granddaughter, Sophia Christine. Peter is also survived by his brother, Nicholas and family in Annapolis, Mass.; and his brother, Dimitri and family in Andover. Peter was predeceased by his son, Philip.

Be It Therefore Resolved that the Interstate Shellfish Sanitation Conference extends its gratitude for Peter's leadership and lasting contributions to the organization; and

Be It Further Resolved**,** that the Interstate Shellfish Sanitation Conference acknowledge his contributions by a letter to that effect to his family.

Action by 2011 Resolutions Committee Recommended adoption of Resolution 11-002 as submitted.

Action by 2011 Executive Board Recommended adoption of Resolutions Committee recommendation on Resolution 11-002.

Action by 2011 General Assembly Adopted Resolution 11-002.

Action by FDA February 26, 2012 Concurred with Conference action on Resolution 11-002.

Resolution Subject: Equivalence Criteria for Food

Text of Resolution: ***Whereas***, the Interstate Shellfish Sanitation Conference, (ISSC), and the Food and Drug Administration, (FDA), agreed to a Memorandum of Understanding, (MOU), on March 14, 1984 which continues to present; and

***Whereas*,** The National Shellfish Sanitation Program (NSSP) and its associated documents, including the FDA/ISSC MOU, do not make provisions for equivalency determinations or recognition of other programs; and

***Whereas*,** under Article 4 of the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (SPS), as a participating member, the U.S. is obligated to consider equivalent food safety measures of a participating country if those measures provide a level of public health protection equal to that provided by the U.S. system - the NSSP; and

***Whereas*,** FDA must address the concept of equivalence and related criteria afforded by non-NSSP shellfish regulatory systems; therefore

***Be it Resolved*,** that the ISSC recognizes that FDA, as a U.S. regulatory agency, is bound by the WTO to consider equivalency if requested by other countries and that the ISSC recognizes and accepts equivalency determinations by FDA; and

***Be it Further Resolved*,** that upon request from FDA, the ISSC will provide input on the criteria and evaluation processes that may be applied by FDA for such determinations.

Action by 2011 Resolutions Committee Recommended adoption of Resolution 11-003 as submitted.

Action by 2011 Task Force III Recommended adoption of Resolution 11-003 as a substitute for Proposal 07-303.

Action by 2011 General Assembly Adopted Resolution 11-003 as a substitute for Proposal 07-303.

Action by FDA February 26, 2012 Concurred with Conference action on Resolution 11-003.

Resolution Subject: Resolution of Appreciation

Text of Resolution: ***Whereas***, the twenty-fourth meeting of the Interstate Shellfish Sanitation Conference convened October 1 – 7, 2011, at The Renaissance Seattle Hotel, Seattle, Washington and

Whereas, several individuals and organizations were instrumental in contributing to the outstanding success of this meeting.

Be It Therefore Resolved that the Interstate Shellfish Sanitation Conference goes on record expressing appreciation to:

The staff of the Renaissance Seattle Hotel particularly,

Rene Neidhart, General Manager
Kay Washington, Director of Operations
Nancy Helms, Director of Sales and Marketing
Pieter Koomen, Director of Catering and Events
Heather Moreno, Director of Housekeeping
Ernie Timog, Banquet Manger
Richard Frame, Assistant Banquet Manager
Mark Talbert, Assistant Banquet Manager
Kris Forrest, Swank Audio Visuals
Todd Keller, Swank Audio Visuals
Julie Nolasco, Credit Manager
Karyn Tanaka, Event Manager
Carrie Zimmerman, Senior Sales Manager
Kyle Asher, Rooms Manager
Erick Gonzalez, Executive Chef

The Volunteer ISSC Staff,

Office Manger, William Eisele, Retired

Be It Further Resolved, that the Interstate Shellfish Sanitation Conference directs the Executive Director to write a letter of appreciation to each of the above-mentioned individuals and organizations.

Action by 2011 General Assembly Adopted Resolution 11-004.

Action by FDA February 26, 2012 Concurred with Conference action on Resolution 11-004.

Resolution Subject: Resolution of Appreciation

Text of Resolution: ***Whereas***, the twenty-fourth meeting of the Interstate Shellfish Sanitation Conference convened October 1 – 7, 2011, at The Renaissance Seattle Hotel, Seattle, Washington and

Whereas, the following industry sponsors, companies, and individuals were instrumental in contributing to the outstanding success of the Interstate Shellfish Sanitation Conference Chairman's Reception.

Be It Therefore Resolved that the Interstate Shellfish Sanitation Conference goes on record expressing appreciation to:

Taylor Shellfish Farms - Shelton, Washington
Bill Taylor
Bill Dewey
Marco Pinchot

Chelsea Farms - Olympia, Washington
John Lentz
Linda Lentz

Seattle Shellfish - Olympia, Washington
Jim Gibbons

Be It Further Resolved, that the Interstate Shellfish Sanitation Conference directs the Executive Director to write a letter of appreciation to each of the above-mentioned individuals and organizations.

Action by 2011 General Assembly Adopted Resolution 11-005.

Action by FDA February 26, 2012 Concurred with Conference action on Resolution 11-005.