Interstate Shellfish Sanitation Conference

2015 Biennial Meeting

Task Force I Report

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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.
Text of Proposal/ Requested Action	Procedure for Acceptance and Approval of Analytical Methods for the NSSP 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	It is difficult to determine exact costs because many government cost models do not consider capital 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 recommends: 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.
Action by 2013 Laboratory Methods Review and Quality Assurance Committee	Recommended no action on Proposal 05-111 Rationale - Proposal 05-111 is resolved by action on Proposal 13-109.
Action by 2013 Task Force I	Recommended adoption of Laboratory Methods Review and Quality Assurance Committee recommendation on Proposal 05-111.
Action by 2013	Adopted recommendation of 2013 Task Force I on Proposal 05-111.

General Assembly	
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 05-111.
Action by 2015 Laboratory Methods Review Committee	Recommended the following: 1) Change the name of the Jellett Rapid Test to Scotia Rapid Test and the Jellett Rapid Extraction to Scotia Rapid Extraction in the next revision of the NSSP Guide for the Control of Molluscan Shellfish (Section IV. Guidance Documents Chapter II Growing Areas 4. Approved Limited Use Methods for Marine Biotoxin Testing). 2) Refer Proposal 05-111 for PSP to an appropriate committee as determined by the Conference Chair and further recommended to direct the Executive Office to send a letter to the method submitter requesting additional information as detailed by the LMRC. 3) No action on the Scotia Rapid Extraction Method for ASP as there is no data nor did the submitter indicate that data would be submitted for ASP.
Action by 2015 Task Force I	Recommends adoption of the Laboratory Methods Review Committee on Proposal 05-111 with the following amendments: 1) Remove "and ASP" and change "toxins" to "toxin" throughout the proposal and adopt the Laboratory Method Review Committee recommendation 1 2) Refer Proposal 05-111 to appropriate committee as determined by Conference Chair. 3) No action on recommendation 3 as this is covered by the proposal as amended by the Task Force.

Proposal Subject	Re-opening Conditional Areas using Male-specific Coliphage after WTP Malfunction
Specific NSSP Guide Reference	Section II. Model Ordinance Chapter IV. Shellstock Growing Areas
Text of Proposal/ Requested Action	@.03 Growing Area Classification A. (5) (c) (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 105 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	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. To include FDA prepare and provide to the committee data collected using MSC in wastewater treatment plant and to work with 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 Proposal 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.
Action by 2013 Growing Area Classification Committee	Recommended referral of Proposal 11-101 to the appropriate committee as determined by the Conference Chairman. It was additionally recommended that a workgroup be formed to look at current MSC data and the science behind its potential use and applicability for use in the NSSP. The workgroup will organize a summit of outside experts, academia, and scientists to present current information

	and science on MSC. The group will meet at least quarterly and respond back to the Growing Area Classification Committee on its findings and recommendations. Recommended that the ISSC pursue funding to facilitate scheduling a summit to bring together experts to present the current science in the use of MSC.
Action by 2013 Task Force I	Recommended adoption of Growing Area Classification Committee recommendation on Proposal 11-101.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 11-101.
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 11-101.
Action by 2015 Growing Area Classification Committee	Recommended no action on Proposal 11-101. Rationale: This proposal is resolved by Proposal 15-102 and Proposal 15-106.
Action by 2015 Task Force I	Recommends adoption of the Growing Area Classification Committee recommendation on Proposal 11-101.

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	Section II. Model Ordinance Chapter IV. Shellstock Growing Areas
Text of Proposal/ Requested Action	@.03 Growing Area Classification E. (5) (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 105 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	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. To include FDA prepare and provide to the committee data collected using MSC in wastewater treatment plant and to work with 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 Proposal 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.
Action by 2013 Growing Area Classification Committee	Recommended referral of Proposal 11-102 to the appropriate committee as determined by the Conference Chairman. It was additionally recommended that a workgroup be formed to look at current MSC data and the science behind its potential use and applicability for use in the NSSP. The workgroup will organize a summit of outside experts, academia, and scientists to present current information and science on MSC. The group will meet at least quarterly and respond back to the Growing Area Classification Committee on its findings and recommendations. Recommended that the ISSC pursue funding to facilitate scheduling a summit to bring together experts to present the current science in the use of MSC.
Action by 2013 Task Force I	Recommended adoption of Growing Area Classification Committee recommendation on Proposal 11-102.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 11-102.

Action by FDA May 5, 2014	Concurred with Conference action on Proposal 11-102.
Action by 2015 Growing Area Classification Committee	Recommended no action on Proposal 11-102. Rational: This proposal is resolved by Proposal 15-102 and Proposal 15-106.
Action by 2015 Task Force I	Recommends adoption of the Growing Area Classification Committee on Proposal 11-102.

Proposal Subject	Alternative Male-specific Coliphage Meat Standard for Restricted Classification of Growing Areas Impacted by wastewater treatment plant outfall.
Specific NSSP Guide Reference	Section II. Model Ordinance Chapter IV. Shellstock Growing Area @ .02 Bacteriological Standards
Text of Proposal/ Requested Action	G. Standard for the Restricted Classification of Growing Areas Affected by Point Sources and Used as a Shellstock Source for Shellstock Depuration. (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	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	Recommend 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.
Action by 2013 Growing Area	Recommend referral of Proposal 11-103 to the appropriate committee as determined by the Conference Chairman.

Classification Committee	It was additionally recommended that a workgroup be formed to look at current MSC data and the science behind its potential use and applicability for use in the NSSP. The workgroup will organize a summit of outside experts, academia, and scientists to present current information and science on MSC. The group will meet at least quarterly and respond back to the Growing Area Classification Committee on its findings and recommendations. Recommended that the ISSC pursue funding to facilitate scheduling a summit to bring together experts to present the current science in the use of MSC.
Action by 2013 Task Force I	Recommended adoption of Growing Area Classification Committee action on Proposal 11-103.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 11-103.
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 11-103.
Action by 2015 Growing Area Classification Committee	Recommended referral of Proposal 11-103 to appropriate committee as determined by the Conference Chair.
Action by 2015 Task Force I	Recommends adoption of Growing Area Classification Committee recommendation on Proposal 11-103.

Proposal Subject	Update PSP Laboratory Evaluation Checklist
Specific NSSP Guide Reference	Section IV. Guidance Documents Chapter II. Growing Areas .12 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 Checklist 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	None
Action by 2011 Laboratory Methods Review & Quality Assurance Committee	Recommend Proposal 11-109 be referred 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.
Action by 2013 Laboratory Methods Review & Quality Assurance Committee	Recommended Proposal 11-109 be referred to the appropriate committee as determined by the Conference Chairman.
Action by 2013 Task Force I	Recommended adoption of Laboratory Methods Review and Quality Assurance Committee recommendation on Proposal 11-109.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 11-109.
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 11-109.
Action by 2015 Laboratory Methods Review Committee	Recommended that Proposal 11-109 be adopted as amended (attached). Available upon request (14 page document)

Action by 2015 Task Force I	Recommends adoption of Laboratory Methods Review Committee recommendation on Proposal 11-109.

Proposal Subject	Addition to the Requirements for the Authority During a Suspected Shellfish Related Outbreak
Specific NSSP Guide Reference	Section II. Model Ordinance Chapter II. Risk Assessment and Risk Management
Text of Proposal/ Requested Action	@.01 Outbreaks of Shellfish-Related Illness 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.
Public Health Significance	
Cost Information	
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.
Action by 2013 Growing Area Classification Committee	Recommended Proposal 11-115 be referred to the appropriate committee as determined by the Conference Chairman and that a workgroup be formed to further explore available options for PHP methods that could be used for reconditioning recalled product. The workgroup should determine a definition for "validated reconditioned process". The Committee further recommended that the workgroup report back to the Growing Area Classification Committee with its findings.
Action by 2013 Task Force I	Recommended adoption of Growing Area Classification Committee recommendation on Proposal 11-115.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 11-115.
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 11-115.
Action by 2015 Shellfish Reconditioning Committee	Recommended adding a new section as follows: Chapter II. Risk Assessment and Risk Management @ .01 Outbreaks J. Molluscan shellfish products that as a result of illnesses associated with V.v. & V.p. may be reconditioned. Validated reconditioned processes include subjecting products to validated PHPs or placing product into approved, conditionally approved, conditionally restricted, or restricted growing areas for an appropriate period of time, not less than fourteen (14) days, with appropriate controls and documentation to be determined by the State Shellfish Control Authority (SSCA).
Action by 2015 Task Force I	Recommends adoption of Proposal 11-115 as amended. Add a new section as follows: Chapter II. Risk Assessment and Risk Management @ .01 Outbreaks J. Molluscan shellfish product~~s~~ that is recalled as a result of illness~~es~~ outbreak associated with *V.v. & V.p.* may be reconditioned. Validated reconditioned processes include subjecting product~~s~~ to validated PHPs or placing product into approved, conditionally approved, conditionally restricted, or restricted

	growing areas for an appropriate period of time, not less than fourteen (14) days, with appropriate controls and documentation to be determined by the State Shellfish Control Authority (SSCA).

Proposal Subject	Sources of Seed for Aquaculture
Specific NSSP Guide Reference	Section II. Model Ordinance Chapter VI. Shellfish Aquaculture
Text of Proposal/ Requested Action	.03 Seed Shellstock Seed may come from any growing area, or from any growing area in any classification, provided that: A. The source of the seed is sanctioned by the Authority B. Seed from growing areas ~~or growing areas~~ in the restricted or prohibited classification have acceptable levels of poisonous or deleterious substances; and C. Seed from growing areas ~~or growing areas~~ in the prohibited classification are cultured for a minimum of ~~six (6) months~~ one month while average daily water temperatures are above 50 degrees F.
Public Health Significance	Shellfish seed collected or cultured in certain growing areas that are in the prohibited classification have been shown through repeated sampling to be free of deleterious substances (John Mullen RI DOH, unpub. data, Rheault unpubl. data, Rice unpub. data, Leavitt unpub. data). A period of one month is typically adequate to purge viral and bacterial contaminants provided water temperatures are high enough to maintain active metabolic activity (above 60 degrees F or 15 degrees C) (Richards 1988). Once the Authority is satisfied that adequate sampling has demonstrated that the seed have "acceptable levels of deleterious substances", then a 30 day period of culture in open waters should be adequate to allow purging of bacterial and viral contaminants to ensure that public health is protected. The Authority retains the right to deny seed collection and culture in any area, or to require additional testing for deleterious substances, or to require longer periods to purge contaminants as necessary. The original intent of this section was to provide for purging of viral and bacterial contamination prior to harvest for consumption on the assumption that deleterious substances were at acceptable levels prior to moving the seed to grow out areas The six-month requirement was implemented as a short-hand way to ensure that seed were grown for at least one month when water temperatures exceeded 60 degrees F. It makes little sense to require relay times in excess of one month for seed that are typically more than six months from harvest size when shellstock relay times as short as two weeks are common. References Cited: Richards, G. (1988), Microbial Purification of Shellfish: A Review of Depuration and Relaying, J. Food Protection 51(3)218-251. Supporting Information: RI DOH metals data (oyster seed grown in Billington Cove Marina) Unpublished data from Rd. Dale Leavitt (clam seed grown in Warwick Cove Marina)
Cost Information	This change should facilitate record keeping and documentation efforts required to ensure that seed from prohibited waters do not get harvested until bacterial and viral contamination has been purged.
Action by 2013 Task Force I	Recommended referral of Proposal 13-107 to an appropriate committee as determined by the Conference Chairman
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 13-107.

Action by FDA May 5, 2014	Concurred with Conference action on Proposal 13-107.
Action by 2015 Aquaculture Facility Inspection Committee	Recommended the following: (1) Referral of Proposal 13-107 back to Committee as appointed by the Conference Chair. (2) The charge of the Committee be expanded to include updating and revising the Aquaculture Chapter of the Model Ordinance to reflect current practices and methods and submit proposals for the next Annual Meeting.
Action by 2015 Task Force I	Recommends adoption of Aquaculture Facility Inspection Committee recommendations on Proposal 13-107.

Proposal Subject	Expanding the use of the Abraxis Shipboard ELISA for the determination of paralytic shellfish poisoning (PSP) toxins
Specific NSSP Guide Reference	Section IV. Guidance Documents Chapter II. Growing Areas .11 Approved NSSP Laboratory Tests
Text of Proposal/ Requested Action	4. Approved Limited Use Methods for Marine Biotoxin Testing This submission presents the Abraxis Shipboard ELISA for paralytic shellfish poisoning (PSP) toxins as a screening method for consideration as an NSSP Approved Limited Use Method. Currently the Abraxis Shipboard ELISA is approved for limited use in conjunction with the Jellett Rapid Extraction (mixture of rubbing alcohol and vinegar) and specifically for the onboard testing protocol. This proposal presents more data on the Abraxis test using the rapid extraction and also provides new data and comparisons of the test when AOAC extractions (boiling with hydrochloric acid) are performed. The data presented supports expanding the use of the Abraxis Shipboard ELISA to (1) allow for the rapid extraction OR the AOAC extraction method and (2) allow the kit to be used as a screening method beyond the onboard screening protocol
Public Health Significance	Paralytic shellfish poisoning intoxications result from the consumption of seafood (primarily bivalve molluscs) contaminated with neurotoxins known as paralytic shellfish toxins (PSTs). To protect public health, harvesting closures are implemented when toxicity exceeds the guidance level of 80 micrograms saxitoxin equivalents per 100 grams of shellfish tissue. As such, accurate screening and analytical methods are needed to monitor shellfish toxicity for making decisions regarding opening and closing shellfish growing areas accordingly. While the Abraxis Shipboard ELISA is already an NSSP Approved Limited Use Method for PSP toxicity determination, being able to use AOAC extractions with this kit would allow for the same extraction to be used with this method during screening and with the MBA as necessary for confirmation (without requiring a second extraction). Further expanding the use of the method beyond the onboard screening protocol would be beneficial as it would make the Abraxis Shipboard ELISA available for use by monitoring laboratories.
Cost Information	Each 96 well plate costs ~$500.
Action by 2013 Laboratory Method and Quality Assurance Review Committee	Recommended Proposal 13-109 be referred to an appropriate committee as determined by the Conference Chairman.
Action by 2013 Task Force I	Recommended adoption of Laboratory Method and Quality Assurance Review Committee recommendation on Proposal 13-109.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 13-109.
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 13-109.
Action by 2015 Laboratory Methods Review Committee	Recommended that Proposal 13-109 be referred to an appropriate committee as determined by the Conference Chair until data that supports the use of the Abraxis ELISA beyond the use of the onboard procedure is made available.
Action by 2015 Task Force I	Recommends adoption of Laboratory Methods Review Committee recommendation on Proposal 13-109.

Proposal Subject	Immunoassay Method for Detection of Saxitoxin (PSP) from Shellfish
Specific NSSP Guide Reference	Section IV. Guidance Documents Chapter II. Growing Areas .11 Approved NSSP Laboratory Tests
Text of Proposal/ Requested Action	2. Approved Methods for Marine Biotoxin Testing and 4. Approved Limited Use Methods for Marine Biotoxin Testing. Review the validation for Saxitoxin (PSP) Microtiter Plate Test Kit by the Proposal Review Committee. Single Laboratory Validation Protocol for Method Approval attached.
Public Health Significance	Rapid screening method can handle numerous samples and screen out negative samples so that it reduces the size of sample to be confirmed with regulatory methods such as mouse bioassay (MBA) or liquid chromatography with post-column oxidation (PCOX). This results in saving resources of the laboratories, and makes the laboratories able to provide rapid warning. References attached.
Cost Information	Approximate cost for the basic set up of the method is $3600
Action by 2013 Laboratory Methods and Quality Assurance Review Committee	Recommended Proposal 13-110 be referred to an appropriate committee as determined by the Conference Chairman and direct the Executive Office send a letter to the submitter requesting additional information as requested by the Laboratory Methods Review and Quality Assurance Committee.
Action by 2013 Task Force I	Recommended adoption of Laboratory Method Review and Quality Assurance Committee recommendation on Proposal 13-110.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 13-110.
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 13-110.
Action by 2015 Laboratory Methods Review Committee	Recommended that Proposal 13-110 be referred to the appropriate committee as determined by the Conference Chair until additional data are received.
Action by 2015 Task Force I	Recommends adoption of Laboratory Methods Review Committee recommendation on Proposal 13-110.

Proposal Subject	DSP PPIA Kit for Determination of Okadaic Acid Toxins Group (OA, DTX1, DTX2) in Molluscan Shellfish
Specific NSSP Guide Reference	Section IV. Guidance Documents Chapter II. Growing Areas .11 Approved NSSP Laboratory Tests: Marine Biotoxin Testing
Text of Proposal/ Requested Action	The DSP PPIA kit be approved as a Marine Biotoxin Laboratory Test Method.
Public Health Significance	Okadaic acid (OA) and its analogues, DTX1, DTX2, together with their ester forms are known as the group of OA-toxins. These toxins, lipophilic and heat stable, are produced by dinoflagellates and can be found in various species of shellfish, mainly in filter feeding bivalve molluscs. The OA-toxins group causes Diarrheic Shellfish Poisoning (DSP), which is characterized by symptoms such as diarrhea, nausea, vomiting and abdominal pain. These symptoms may occur in humans shortly after consumption of contaminated bivalve molluscs such as mussels, clams, scallops or oysters. Inhibition of serine/threonine phosphoprotein phosphatases is assumed to be responsible for these toxic effects. Recently in the Pacific Northwest harvest areas, outbreaks of DSP have occurred.
Cost Information	Refer to Para D.1. of the Checklist
Action by 2013 Laboratory Methods Review and Quality Assurance Committee	Recommended referral of Proposal 13-111 to an appropriate committee as determined by the Conference Chairman and directed the Executive Office send a letter to the submitter requesting additional information as provided by the Laboratory Methods Review and Quality Assurance Committee.
Action by 2013 Task Force I	Recommended adoption of Laboratory Methods Review and Quality Assurance Committee recommendation on Proposal 13-111.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 13-111.
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 13-111.
Action by 2015 Laboratory Methods Review Committee	Recommended that Proposal 13-111 be referred to an appropriate committee as determined by the Conference Chair until additional data are received.
Action by 2015 Task Force I	Recommends adoption of Laboratory Methods Review Committee recommendation on Proposal 13-111.

Proposal Subject	Reveal 2.0 ASP
Specific NSSP Guide Reference	Section IV. Guidance Documents Chapter II. Growing Areas .11 Approved NSSP Laboratory Tests
Text of Proposal/ Requested Action	We request review of the validation study submission for the Reveal 2.0 ASP (domoic acid) test kit and consideration of the method for approval as a screening method for qualitative determination of domoic acid in shellfish. Add Reveal ASP to Section IV. Guidance Documents, Chapter II. Growing Areas, .11 Approved NSSP Laboratory Tests.
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 [1,2]. 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. 1] J. Sobel and J. Painter (2005), Illness caused by Marine Biotoxins. Clin. Infect. Dis. 4, 1290. [2] Van Dolah, Frances M. (2000), Marine algal toxins: origins, health effects, and their increased occurrence. Environmental health perspectives 108. Suppl 1, 133.
Cost Information	Approximately $17.00 per test. Reader based assay – approximate cost of Reader $1995
Action by 2013 Laboratory Method and Quality Assurance Review Committee	Recommended adoption of this method as a Limited Use Method for the purpose of screening and precautionary closure for ASP and direct the Executive Office send a letter to the submitter requesting additional information as provided by the Laboratory Method Review and Quality Assurance Committee.
Action by 2013 Task Force I	Recommended adoption of the Laboratory Method Review and Quality Assurance Committee recommendation on Proposal 13-112 and recommended that the Conference be made aware the submitter of Proposal 13-112 is looking for samples to be used in testing.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 13-112.
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 13-112.
Action by 2015 Laboratory Methods Review Committee	Recommended no action on Proposal 13-112. Rationale: No data has been received and submitter has indicated no plans to submit data at this time.
Action by 2015 Task Force I	Recommends adoption of Laboratory Method Review Committee recommendation on Proposal 13-112.

Proposal Subject	Reveal 2.0 DSP
Specific NSSP Guide Reference	Section IV. Guidance Documents Chapter II. Growing Areas
Text of Proposal/ Requested Action	.11 Approved NSSP Laboratory Tests We request review of the validation study submission for the Reveal 2.0 DSP (okadaic acid group) test kit and consideration of the method for approval as a screening method for qualitative determination of okadaic acid group in shellfish. Add Reveal DSP to Section IV. Guidance Documents, Chapter II. Growing Areas, .11 Approved NSSP Laboratory Tests.
Public Health Significance	Toxins that cause diarrhetic shellfish poisoning (DSP) include the okadaic acid (OA) group of toxins [1, 2] OA is produced by marine dinoflagellates such as Dinophysis, and has structural analogues referred to as the dinophysistoxins (DTXs). The U.S. Food and Drug Administration action limits are 160 ppb OA equivalents (OA, DTX1, DTX2, DTX3) in shellfish. LC-MS/MS methods [3] have been accepted as quantitative reference methods in many parts of the world. Assays facilitating more rapid determination of OA toxins with simplified procedures are needed by the shellfish industry and regulatory authorities. [1] J. Sobel and J. Painter (2005), Illness caused by Marine Biotoxins. Clin. Infect. Dis. 4, 1290. [2] Van Dolah, Frances M. (2000), Marine algal toxins: origins, health effects, and their increased occurrence. Environmental health perspectives 108. Suppl 1, 133. [3]Community Reference Laboratory for Marine biotoxins (CRLMB)., Agencia Española de Seguridad Alimentaria y Nutrición (AESAN). (2009). EU Harmonised Standard Operating Procedure for determination of OA-Group Toxins by LC-MS/MS. Version1. http://www.aesan.msps.es/en/CRLMB/web/procedimientos_crlmb/crlmb_standard_operating_procedures.shtml
Cost Information	Approximately $17.00 per test. Reader based assay – approximate cost of Reader $1995.
Action by 2013 Laboratory Method and Quality Assurance Review Committee	Recommended Proposal 13-113 be referred to an appropriate committee as determined by the Conference Chairman and await data to determine if the method is fit for purpose within the NSSP.
Action by 2013 Task Force I	Recommended adoption of Laboratory Method Review and Quality Assurance Committee recommendation on Proposal 13-113.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 13-113.
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 13-113.
Action by 2015 Laboratory Methods Review Committee	Recommended that Proposal 13-113 be referred to an appropriate committee as determined by the Conference Chair until additional data are received.
Action by 2015 Task Force I	Recommends adoption of Laboratory Methods Review Committee recommendation on Proposal 13-113.

Proposal Subject	Receptor Binding Assay (RBA) for Paralytic Shellfish Poisoning (PSP) Toxicity Determination
Specific NSSP Guide Reference	Section IV. Guidance Documents Chapter II. Growing Areas . 11 Approved NSSP Laboratory Tests
Text of Proposal/ Requested Action	4. Approved Limited Use Methods for Marine Biotoxin Testing This submission presents the 'Receptor Binding Assay (RBA) for Paralytic Shellfish Poisoning (PSP) Toxicity Determination' for consideration as an NSSP Approved Limited Use Method. The RBA is a competition-based assay that employs radiolabeled saxitoxin (3H-STX) to compete with PSP toxins present in standards/samples for binding sites on natural receptors in the assay. Following incubation with the receptors, unbound 3H-STX is removed and the remaining labeled toxin is measured with a scintillation counter. The amount of remaining 3H-STX is inversely proportional to standard/sample toxicity. The RBA offers a high-throughput, sensitive, and quantitative alternative to the mouse bioassay (MBA), which has been the long-standing reference method for PSP toxicity. Further, the RBA eliminates the use of live animals for detection of these toxins. While the RBA still uses receptors prepared from animals, the number of animals required for analysis is significantly reduced. Using native receptors as the analytical recognition elements for the assay allows for a composite measure of overall toxicity, as opposed to toxin concentrations measured by liquid chromatographic methods that require conversion factors of equivalent toxicity to calculate the overall toxicity. The RBA has undergone AOAC single- and multi-laboratory validation and is designated through AOAC as an Official Method of Analysis (OMA 2011.27). Results from those studies, and additional data, are included in this proposal submission for the RBA to be considered for approval as an NSSP Approved Limited Use Method for Marine Biotoxin Testing.
Public Health Significance	Paralytic shellfish poisoning intoxications result from the consumption of seafood (primarily bivalve molluscs) contaminated with neurotoxins known as paralytic shellfish toxins (PSTs). This suite of toxins binds to voltage-gated sodium channels and may result in paralysis if enough toxin is consumed. In extreme cases when respiratory support is not available to the patient, the intoxication may prove fatal. Since the toxins cannot be destroyed during cooking and there is no way to remove the toxins from seafood, the best control strategy is to ensure that contaminated product never reaches the market. To protect public health, harvesting closures are implemented when toxicity exceeds the guidance level of 80 micrograms saxitoxin equivalents per 100 grams of shellfish tissue. As such, accurate analytical methods are needed to monitor shellfish toxicity for making decisions regarding opening and closing shellfish growing areas accordingly. Acceptance of the RBA as an NSSP Approved Limited Use Method for PSP toxicity determination would provide monitoring and management programs with an additional tool that can be used for monitoring toxin levels and making regulatory decisions. Not only does the RBA eliminate the need for live animals for PSP testing, it is also more sensitive than the MBA, thereby providing an early warning system for monitoring programs as toxin levels begin to rise.
Cost Information	The estimated cost for a full 96-well plate assay is ~$95.00. Including standards and samples with triplicate measurements (as well as three dilutions per sample to ensure the unknown samples fall within linear range of assay), the cost per sample for quantitative results would be ~$13.60. If running multiple plates or in screening mode, sample costs would be reduced. Further, the filter plates used in the RBA

	differ from ELISA plates in that all reagents are added to each well as needed rather than already being a component of the plate, making it more practical and cost-effective to analyze samples when there is less than a full plate.
Action by 2013 Laboratory Methods and Quality Assurance Review Committee	1. Recommended approval of this method as an alternative to the mouse bioassay for PSP in mussels. 2. Recommended approval of this method for Limited Use for clams and scallops for the purpose of screening and precautionary closure for PSP. 3. Recommended referral of this proposal to an appropriate committee as determined by the Conference Chairman to address this method in oysters. 4. Recommended Executive Office send a letter to submitter to request a checklist for evaluation of labs using this method with said checklist to be submitted within three (3) months.
Action by 2013 Task Force I	Recommended adoption of Laboratory Method Review and Quality Assurance Committee recommendation on Proposal 13-114.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 13-114.
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 13-114.
Action by 2015 Laboratory Methods Review Committee	Recommended that Proposal 13-114 be referred to an appropriate committee as determined by the Conference Chair until additional data for oyster matrix are received.
Action by 2015 Task Force I	Recommends adoption of Laboratory Methods Review Committee recommendation on Proposal 13-114.

Proposal Subject	Receptor Binding Assay (RBA) for Paralytic Shellfish Poisoning (PSP) Toxicity Determination
Specific NSSP Guide Reference	2011 NSSP Section IV. Guidance Documents Chapter II. Growing Areas .12 Evaluation of Laboratories by State Shellfish Laboratory Evaluation Officers including Laboratory Evaluation Checklist-Laboratory Checklist-PSP
Text of Proposal/ Requested Action	Establish a PSP Laboratory Evaluation Checklist for the HPLC-PCOX method. Please find the HPLC-PCOX checklist attached-word document titled "PSP HPLC PCOX checklist.docx" There is no summary of changes as no previous checklist exists for this procedure
Public Health Significance	The HPLC-PCOX method has been an approved limited use method since 2009, yet no checklist exists to allow evaluation of laboratories who utilize this method. Use of this method provides states much more detailed toxin profiles as well as helping eliminate animal testing. It is important that the checklist items and quality assurance requirements are clear and understandable.
Cost Information	For laboratories that do not already possess a HPLC post column reaction system, the upfront cost can be significant. Once in place, the costs per test are not significantly different than that imposed by the capital cost of the mouse bioassay.
Action by 2013 Laboratory Methods and Quality Assurance Review Committee	Recommended Proposal 13-115 be referred to an appropriate committee as determined by the Conference Chairman.
Action by 2013 Task Force I	Recommended adoption of Laboratory Method Review and Quality Assurance Committee recommendation on Proposal 13-115.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 13-115.
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 13-115.
Action by 2015 Laboratory Methods Review Committee	Recommended adoption of Proposal 13-115 as amended (attached). Available upon request (13 page document).
Action by 2015 Task Force I	Recommends adoption of Laboratory Methods Review Committee recommendation on Proposal 13-115.

Proposal Subject	Shellfish Quarantine Guidance Document
Specific NSSP Guide Reference	Section II. Model Ordinance Chapter IV. Shellstock Growing Areas @.04 Marine Biotoxin Control Section IV. Guidance Documents Chapter II. Growing Areas .02 Guidance for Developing Marine Biotoxin Contingency Plans
Text of Proposal/ Requested Action	Model Ordinance Chapter IV. Shellstock Growing Areas @.04 Marine Biotoxin Control Section A. (4) describes agreements or memoranda of understanding between the Authority and individual shellfish harvesters or individual shellfish dealers, to allow harvesting during marine Biotoxin closures under specific, controlled conditions. The State of Florida has successfully implemented such an agreement to address Neurotoxic Shellfish Poisoning (NSP) for over a decade. This pilot project, developed in consultation with FDA, has resulted in zero cases of NSP in commercially harvested shellfish from Florida waters. NSP may affect any Gulf or South Atlantic state and therefore Florida wishes to provide ISSC member states with a proven quarantine protocol template for incorporation into the Model Ordinance Section IV. Guidance Documents. Guidance Documents Chapter II. Growing Areas .02 Guidance for Developing Marine Biotoxin Contingency Plans. Text of the proposed guidance is as follows: Example Protocol for Quarantine Harvest of Shellfish from Aquaculture Leases During *Karenia brevis* Closures: A. Closure of an entire shellfish growing area due to *Karenia brevis* shall be in accordance with Model Ordinance Chapter IV. @.04 C. (1). B. When a shellfish growing area is closed due to *Karenia brevis*, the Authority may allow harvest of shellfish from selected aquaculture leases within a specific zone by authorized harvesters and subsequent controlled quarantine at a certified shucker packer or shellstock shipper. This option would not be available if any Authority collected water samples in the specific zone exceeded 200,000 cells per liter of *Karenia brevis*. Zone is defined as an Authority delineated geographic area within a Conditionally Approved or Approved classified shellfish growing area. *Controlled quarantine conditions:* The Authority will determine and plot the specific zones. Certified processors possessing a valid shellfish processing plant certification license must have written permission from the Authority to engage in this activity. To be eligible for participation in the quarantine program, the certified processor must: (1) Provide the Authority with written and signed agreements the processor has with shellfish aquaculture leaseholders who would be supplying the shellfish and; (2) Notate on their application letter which FDA-approved marine

Biotoxin laboratory will be used to conduct the approved mouse bioassay and;

(3) Provide the Authority with the cooler capacity, physical address and current certification number of the facility to be used for controlled quarantine of shellfish. All quarantine coolers must be non-mobile, secure from unauthorized access and equipped with warning signs in a language readily understood by all employees.

Participation in each week's quarantine program is only possible for certified processors who:

(1) Have written permission on file with the Authority and are on an Authority-controlled document listing current approved quarantine program processors and;

(2) Possess emailed permission granted by the Authority the day before harvest for that one specific quarantine and;

(3) Propose harvesting a quantity of shellfish that meets the Authority established minimum number but does not exceed the maximum allowed number of shellfish of one specific species for that day.

Under no circumstances may any approved processor participate in any quarantine until they possess written (emailed) documentation sent by the Authority before each specific quarantine event.

- The authorization email sent by the Authority shall explicitly state the permissible species that may be harvested by that approved processor.
- The Authority will notify the appropriate law enforcement entity in charge of patrol of shellfish growing areas with a list of participants in that specific day's harvest.
- Persons harvesting a species not authorized for that day's harvest will be subject to seizure of that harvest by the Authority. In addition, the Authority will immediately seize and destroy product which is improperly tagged, violates any National Shellfish Sanitation Program (NSSP) Model Ordinance regulations, state laws or is from non-authorized participants.
- Co-mingling of species is not allowed to make up an individual lot.

Violation of the terms of this protocol may result in the termination of the participant's future eligibility in the quarantine program, as determined by the Authority.

Prior to being considered for participation in any specific quarantine event, approved processors shall be contacted by the Authority and asked to provide the name of the species they plan to harvest and the quantity they plan on harvesting. Quantities shall be described as approximate total number by species in addition to total number of baskets, containers, bags, etc. with specific weights (if applicable) for those baskets, containers, bags, etc.

Eligible processors should be aware that daily implementation of this program is contingent on marine Biotoxin laboratory availability as well as Authority staffing considerations given staff time necessary to fulfill the

requirements of the program.

Regulatory considerations on behalf of the Authority and staffing considerations on behalf of the marine Biotoxin lab necessitate an Authority developed maximum number of samples that could be potentially tested on any given week.

The Authority may implement a lottery, random rotation or similar procedure to ensure a fair distribution of testing opportunities among the eligible processors. It is suggested that the Authority develop this procedure with industry involvement.

Once specific permission is received from the Authority, the processor:

(2) May receive properly tagged shellfish from eligible aquaculturists only as indicated in the Authority's authorization email;
(3) Must upon receipt of shellfish, separate and maintain the shellfish into specific lots [A Lot is defined as shellfish of one species from no more than one day's harvest from a specific zone within a shellfish growing area];
(4) Must place shellfish under proper controls and quarantine; Proper controls and quarantine are defined by bold, clear, warning signage signaling the properly tagged and segregated shellfish within the processor's cooler are under quarantine and must not be moved until Authority permission is obtained pending outcome of laboratory testing. The signage should be such that it is clear to anyone entering the cooler (including facility employees and/or regulatory inspectors) that the affected shellfish are under quarantine. Wrapping of the entire lot with a single bright red or yellow ribbon or equivalent attached to the bold warning sign will further reinforce the warning message.
(5) Must allow the Authority to take two (2) random samples [minimum of twenty (20) shellfish per each sample] from each lot and deliver to the approved laboratory for approved mouse bioassay;
(6) Must hold all shellfish in quarantine at the approved processor's certified facility until receiving official written test result notice from the Authority via email or fax that the shellfish are cleared for sale;
(7) Must either return shellfish to aquaculture lease(s) in the zone(s) from where harvested if any sample in a lot is 20 Mouse Units / 100 grams or greater or destroy the shellfish, both activities of which must be witnessed and documented by the Authority;
(8) Must cease this activity if any Authority collected red tide cell counts in the specific zone exceeds 200,000 cells per liter of *Karenia brevis*; and
(9) Must document all of the requirements listed above in the approved facility HACCP plan.

C. If cell counts in all water samples fall to 5,000 cells/L or less Karenia brevis in the entire area, the Authority will collect shellfish meat samples for toxicity testing and the entire Shellfish Harvesting Area will be reopened if results of all samples are <20 MU/100g.

I _____ (print name) have received a copy of this quarantine protocol and I agree to abide by all terms and conditions. I understand I

	am bound by the terms of this agreement during the period of time that I am processing shellfish from a shellfish growing area that is currently in the closed status due to Karenia brevis. _____ _____ Signed Date
Public Health Significance	Closures of shellfish growing areas due to Neurotoxic Shellfish Poisoning (NSP) may occur at any time in the Gulf of Mexico and to a lesser degree, the Atlantic coast. Well established procedures for detecting and responding to *Karenia brevis* blooms have safeguarded public health. Clear early warning signs, a cell count action level with a high factor of safety and established sampling networks provide excellent public health protection. A very real impact of *Karenia brevis* blooms is the resulting long-term closures of shellfish growing areas and severe economic impact to commercial shellfish operations. Florida addressed this issue after studying years of water quality samples and mouse bioassay results from shellfish growing areas. Hydrodynamic studies linked to water samples obtained from fixed stations over an extended period of time established clear patterns in distribution of *Karenia brevis*. Working in conjunction with harmful algal bloom researchers, shellfish growing area managers, FDA and industry, Florida developed a NSP quarantine protocol that has resulted in the retention of a shellfish industry in one of the most severely impacted HAB regions of the Gulf while protecting public health as required by the Model Ordinance. An enormous amount of data has been generated and reviewed during the years this protocol has been used. Repeated mouse bioassay testing on shellfish exposed to different levels of *Karenia brevis* has provided Florida with sufficient data to refine the protocol into a powerful management tool. Florida's experience pre-quarantine protocol was unfortunate, as several fledgling businesses failed due to repeated NSP closures. It was this economic damage that spurred the aforementioned collaborative effort between leading edge HAB researchers, shellfish growing area managers, FDA and industry. If adopted, shellfish producing states impacted by *Karenia brevis* could reference this protocol in the Guidance Document and use it to effectively manage NSP closures.
Cost Information	
Action by 2013 Task Force I	Recommended referral of Proposal 13-116 to an appropriate committee as determined by the Conference Chairman.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 13-116.
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 13-116.
Action by 2015 Biotoxin Committee	Recommended adoption of Proposal 13-116 with substitute language as follows: (4) The plan may include agreements or memoranda of understanding, between the Authority and individual shellfish harvesters or individual shellfish dealers, to allow harvesting in designated parts of a state growing area while other parts of the same ~~the~~ growing area are placed in the closed status. Such controlled harvesting shall be conducted with strict assurances of safety. In state growing areas or designated portions of state growing waters that are closed, the authority may allow for harvesting if an end product testing program is developed and~~, such as by batch release of shellfish lots only after~~ samples of each lot are tested and found to be below the action levels specified in Section C. The program must include at a minimum: i. Establishment of appropriate pre-harvest screening levels; ii. Establishment of appropriate screening and end product testing methods; iii. Establishment of appropriate laboratories/analysts to conduct screening

	and end product testing methods; iv. Establishment of representative sampling plan for both i. and ii. above; and v. Other controls as necessary to ensure that shellstock are not released prior to meeting all requirements of the program. Should the above amended proposal be adopted by the conference, then the Biotoxin Committee should develop a Guidance Document that includes guidance for development of end-product testing programs to address biotoxins in closed state waters.
Action by 2015 Task Force I	Recommends adoption of Biotoxin Committee recommendation on Proposal 13-116.

Proposal Subject	Certification of State Shellfish Laboratory Evaluation Officers
Specific NSSP Guide Reference	Section IV. Guidance Documents Chapter II. Growing Areas
Text of Proposal/ Requested Action	.12 Evaluation of Laboratories By State Shellfish Laboratory Evaluation Officers Including Laboratory Evaluation Checklists Laboratory results from the ~~bacteriological~~ microbiological and marine Biotoxin testing of shellfish and shellfish growing waters ~~and meats~~ are widely used in the National Shellfish Sanitation Program (NSSP) to aid in determining the safety of shellfish for human consumption. Experience with the ~~bacteriological~~ microbiological and marine Biotoxin analyses of shellfish and shellfish growing waters have indicated that minor differences in laboratory procedures or techniques might cause wide variations in the results. ~~Improper handling of the sample may also cause variations in results during collection or transportation to the laboratory.~~ To ensure uniformity ~~nationwide~~ NSSP wide in the application of standards for shellfish and shellfish growing waters, a comprehensive, effective laboratory quality assurance (QA) program is necessary to ~~substantiate~~demonstrate the validity of analytical results. ~~A~~ Thee laboratory ~~quality assurance~~QA program is the systematic application of the practices essential to remove or minimize errors that may occur in any laboratory operation caused by personnel, ~~apparatus,~~ equipment, media, reagents, ~~sampling procedures,~~ and analytical methodology. ~~(APHA, 1985).~~ Integral to laboratory quality assurance is a strong program for the external assessment or evaluation of laboratory performance. The laboratory evaluation process has evolved over the years to accommodate changes in microbiology and marine Biotoxin procedures brought about by NSSP Workshops and more recently by the Interstate Shellfish Sanitation Conference (ISSC). In 1985, FDA issued an interpretation entitled "Evaluation of Laboratories by State Shellfish Laboratory Evaluation Officers" (SS#35). This Interpretation allowed NSSP laboratories which had been previously evaluated by FDA Shellfish Laboratory Evaluation Officers to be subsequently evaluated by qualified state personnel as certified State Shellfish Laboratory Evaluation Officers. This guidance describes the procedure for the certification of these individuals as State Shellfish Laboratory Evaluation Officers. ~~Requirements for evaluating laboratories that analyze samples under the NSSP have increased significantly since the 1970's. The number of laboratories participating in the shellfish program has also increased. Several states now have multiple laboratories that provide these analyses. Some states have officially designated city, county or private laboratories to conduct analyses supporting their shellfish sanitation programs. Some states are also authorizing the use of private laboratories to monitor depuration operations. More states are maintaining a marine biotoxin analytical capability in their laboratories; and more foreign laboratories are involved in the NSSP. Historically, FDA has evaluated all these laboratories. Reduction in FDA staffing has made it difficult to evaluate the many state, county, municipal, and foreign shellfish laboratories operating in support of the NSSP. If states with multiple laboratory support would exercise their option to accept responsibility for evaluating their laboratories by employing a State Shellfish Laboratory Evaluation Officer (State Shellfish LEO), FDA would be able to better meet its NSSP responsibilities.~~ General Provisions 1. If the State Shellfish Control Authority (Authority) uses the analytical services of private/commercial/fee for services laboratories to support

the NSSP, then he/she should select a qualified individual to become certified as a State Shellfish Laboratory Evaluation Officer (State Shellfish LEO).

2. If the Authority uses the analytical services of multiple public laboratories (state, county, parish town, etc.) to support the NSSP, then he/she may select a qualified individual to become a State Shellfish LEO.
3. If the Authority chooses not to participate in the certification process, FDA can evaluate the state's public laboratories. FDA, however, does not normally evaluate private/commercial/fee for services laboratories. FDA may, under certain circumstances as resources permit, evaluate these laboratories on a case-by-case basis at the request of the Authority. This request must be in writing and made through the FDA Regional Shellfish Specialist.
4. State Shellfish LEOs will perform official NSSP evaluations of laboratories which have been previously evaluated by FDA and been found to fully conform to NSSP laboratory requirements.
5. State Shellfish LEOs may evaluate laboratories in a different state under a memorandum of understanding between the states involved and FDA consistent with NSSP requirements.
6. State Shellfish LEOs may not evaluate laboratories in which they are employed or which they supervise or laboratories within the same supervisory chain of command to ensure complete objectivity in the evaluation process and avoid the appearance of a conflict of interest.
7. To qualify for certification, the prospective State Shellfish LEO should be:
   a. A state employee;
   b. Have shellfish laboratory experience or a laboratory background;
   c. Preferably have laboratory evaluation experience; and,
   d. Be free from any commercial, financial or other pressures or conflicts of interest that might cause or appear to cause the prospective State Shellfish LEO to act in other than an impartial or non-discriminatory manner.
8. If the prospective or current State Shellfish LEO is employed by the laboratory supporting the NSSP, that laboratory must be fully conforming to NSSP requirements or the individual will not be certified and if currently certified, certification will be revoked.

Responsibilities of the State Shellfish Control Authority

1. The Authority must ensure that appropriate written documentation is provided to FDA to demonstrate that a prospective State Shellfish LEO is adequately qualified to assume the responsibilities of a State Shellfish LEO as described above.
2. The Authority must provide or ensure that adequate time, resources and support are made available to the State Shellfish LEO to fully participate in the certification process and to fulfill his/her obligation as a State Shellfish LEO.

FDA's Responsibilities

1. FDA is responsible for the certification/recertification of State

Shellfish LEOs.

2. As a result FDA must:
   a. Select qualified individuals to receive training based upon the documentation supplied by the Authority;
   b. Develop and provide training that will enable prospective and current State Shellfish LEOs to consistently and uniformly apply evaluation criteria in determining the competence of laboratories to support or continue to support the NSSP;
   c. Certify prospective State Shellfish LEOs that successfully complete the certification process;
   d. Maintain communication with State Shellfish LEOs as needed to provide guidance and updates relevant to the NSSP laboratory evaluation program;
   e. Recertify current State Shellfish LEOs pursuant to the criteria established for satisfactory performance below;
   f. Monitor the performance of State Shellfish LEOs to ensure that the evaluation process is being performed consistent with NSSP requirements as described in the current NSSP Guide for the Control of Molluscan Shellfish and this guidance;
   g. Maintain communication as needed with the Authority and other pertinent state officials, prospective and current State Shellfish LEOs and FDA Regional Shellfish Specialists relevant to the certification/recertification process;
   h. Revoke certification of State Shellfish LEOs for cause; and,
   i. Void certification when the need for a State Shellfish LEO no longer exists within the state shellfish sanitation program or when the State Shellfish LEO is no longer employed by the state.

~~Selection of State Shellfish LEOs should be based on the following criteria:~~

~~1.The individual must be administratively attached to a state central shellfish sanitation laboratory that has been found by the FDA to be in full conformance with NSSP requirements. To avoid the appearance of impropriety and maintain objectivity in the evaluation process, individuals certified as State Shellfish LEOs will not be allowed to evaluate their own laboratories. FDA will maintain the responsibility for evaluating these laboratories.~~

~~2.The individual must be an experienced analyst and should have laboratory supervision experience. To maintain the integrity of the evaluation process, this individual should not, however, have overall supervisory responsibilities for the laboratory or laboratories to be evaluated. If deemed necessary by an FDA Laboratory Evaluation Officer, the individual must conduct several laboratory evaluations jointly with the FDA Laboratory Evaluation Officer.~~

~~3.During the joint on-site laboratory evaluation with an FDA Laboratory Evaluation Officer, the individual must demonstrate competence in evaluating the laboratory's capability to support the NSSP. The evaluation will be performed and documented using the most current version of the applicable FDA Shellfish Laboratory Evaluation Checklist.~~

~~4 The individual must submit a written narrative report of the joint on-site evaluation to the FDA co-evaluator for review and comment. The report should consist of the completed FDA Shellfish Laboratory Evaluation Checklist and a narrative discussion that accurately and concisely describes the overall operation of the laboratory. All nonconformities noted should be described in this evaluation write-up; and, where relevant an explanation~~

~~provided relating the potential impact of the deficiency on the analytical results. Recommendations for corrective action or, if applicable, suggestions to enhance laboratory operations must be included in this write-up.~~

~~The FDA will issue a letter certifying each individual who successfully completes the certification process and will clear the evaluation report(s) for distribution to the laboratories evaluated with copies to the appropriate Shellfish Specialist.~~

~~Certification is normally effective for a period of three (3) years. Once certified, the individual is then expected to assume the following responsibilities:~~

State Shellfish Laboratory Evaluation Officer's Responsibilities

1. Conduct onsite laboratory evaluations at least every three (3) years. However, more frequent evaluations are strongly encouraged and may be ~~required~~necessary with marginally performing laboratories, or when major changes in workloads or priorities have occurred or when there has been a substantial turnover of personnel, or, at the specific request of the Authority. ~~State Shellfish Control Authorities:~~
2. Provide appropriate post-evaluation follow-up for each laboratory evaluated;
3. Prepare timely narrative evaluation reports for all laboratories evaluated. The report should consist of the completed FDA Shellfish Laboratory Evaluation Checklist for the component(s) evaluated and a narrative discussion that accurately and concisely describes the overall operation of the laboratory. All nonconformities noted should be described in this narrative; and, where relevant, an explanation provided relating the potential impact of the deficiency on the analytical results. Recommendations for corrective action or, if applicable, suggestions to enhance laboratory operations should also be included in the narrative report. ~~Incorporating the requirements specified in 4 above;~~
4. Distribute completed evaluation reports with checklists with checklists to FDA and to FDA and to the appropriate FDA Regional Shellfish Specialist.~~:~~
5. Inform ~~the appropriate~~ FDA Shellfish Laboratory Evaluation Officers when a laboratory has been found to be in nonconforming status.~~;~~
6. Coordinate proficiency testing at least yearly for all laboratories in the state supporting the microbiology component of the NSSP.
7. Prepare ~~at least~~ annually (in December) a summary list of ~~qualified analysts for each~~ all laboratories and qualified analysts within each laboratory by NSSP laboratory component supported ~~laboratory supporting the NSSP in the state~~ and transmit it to the ~~appropriate~~ FDA Shellfish Laboratory Evaluation Officers.

Certification Process

Certification is designed to be accomplished through individualized training and field standardization. Individuals are certified for evaluating either the microbiological and/or post-harvest processing (PHP) and/or marine Biotoxin components of the NSSP depending on their qualifications and the needs of the state shellfish sanitation program and at the discretion of FDA.

Field Standardization

1. Field standardization is designed to evaluate the prospective State Shellfish LEO's ability to determine the competence of the laboratory to meet NSSP laboratory requirements; recognize laboratory practices inconsistent with NSSP requirements when they occur; make appropriate recommendations for corrective action; and, provide the necessary follow-up activity to bring the laboratory into conformity with the NSSP.
2. Field standardization consists of one or several joint but independent onsite evaluations with an FDA Shellfish Laboratory Evaluation Officer and preparation of the corresponding narrative evaluation reports. The report(s) should consist of the completed FDA Shellfish Laboratory Evaluation Checklist(s) and a narrative discussion that accurately and concisely describes the overall operation of the laboratory. All nonconformities noted should be described in the narrative; and where relevant an explanation provided relating the potential impact of the deficiency on the analytical results. Recommendations for corrective action or, if applicable, suggestions to enhance laboratory operations should be included in this narrative report(s).
3. Field standardization should be performed in NSSP laboratories within the prospective State Shellfish LEO's home state to provide realistic evaluation scenarios. The narrative evaluation report detailing the evaluation findings must be prepared. The draft narrative report(s) with accompanying checklist(s) must be submitted to the certifying FDA Shellfish Laboratory Evaluation Officer within 60 days of the evaluation(s). All documents submitted will be reviewed for appropriate content, accuracy and uniformity of approach by the certifying FDA Shellfish Laboratory Evaluation Officer.
4. Field standardization is based on a pass fail system.

Certification

1. Certification is dependent upon the perspective State Shellfish LEO satisfying all the following performance criteria.
   a. Demonstration of good familiarity with evaluation requirements.
   b. Demonstration of a thorough knowledge of the evaluation methods and documents.
   c. Demonstration of the technical knowledge/familiarity with the analytical procedures being used.
   d. Ability to communicate effectively both orally and in writing.
   e. Successful completion of both training and field standardization.
2. Upon successful completion of the certification process, a letter of certification will be issued by the FDA Shellfish Laboratory Evaluation Officer and a copy will be sent to both the requesting Authority and the FDA Regional Shellfish Specialist.
3. Certification is normally valid for up to five (5) years unless revoked or voided.

Failure to be Certified

1. If a prospective State Shellfish LEO fails to satisfy any of the performance criteria listed above, he/she will not be certified.
2. As resources permit and at the discretion of FDA, the prospective State Shellfish LEO may receive additional training to better prepare him/her to be certified.
3. The requesting Authority may withdraw the prospective State Shellfish LEO from consideration.

Recertification

1. Recertification normally occurs every five (5) years and is contingent upon the continuing need in the state shellfish sanitation program for the services of a State Shellfish LEO.
2. Recertification is based on the State Shellfish LEO satisfactorily meeting the following employment and performance criteria.
   a. The individual must continue to be employed by the state and be free of any commercial, financial or other pressures or conflicts of interest real or perceived that may cause the State Shellfish LEO to act in other than an impartial and non-discriminatory manner.
   b. The individual must demonstrate continued competence in the evaluation of NSSP laboratories by performing one to several joint evaluations with an FDA Shellfish Laboratory Evaluation Officer and providing an appropriate narrative evaluation report to the FDA co-evaluator for review and comment for each of the laboratories jointly evaluated.
   c. The individual must have performed laboratory evaluations at the minimum frequency prescribed in the current edition of the Guide for the Control of Molluscan Shellfish and have all Narrative evaluation reports up to date.
3. State Shellfish LEOs who successfully complete recertification will be issued a letter of recertification by FDA and be cleared to distribute the completed report(s) to the appropriate Regional Shellfish Specialist. A copy of this letter will be sent to the State Shellfish Control Authority and appropriate Regional Shellfish Specialist.
4. If FDA is unable to conduct a recertification visit by the expiration of the individual's certification, his/her certification may be extended until such time as recertification can be completed. If requested, a letter extending the certification can be provided as appropriate.

Revocation of Certification

1. State Shellfish LEO's who fail to meet any of the certification/recertification, employment or performance criteria listed above will have their certification revoked.
2. Certification may be voided when state shellfish sanitation programs no longer have a need for the services of a State Shellfish LEO.
3. Voided certifications may be reactivated at the discretion of FDA if the need for the analytical services of additional laboratories by the state shellfish sanitation program recurs.
4. Revoked certifications will not normally be restored.

~~Recertification of State Shellfish LEOs will normally occur triennially and will be based on satisfactorily meeting the following criteria:~~

	~~1. The individual must continue to be administratively attached to a central state shellfish laboratory which is in full conformance with NSSP requirements;~~ ~~2. The individual is not the supervisor of any of the laboratories to be evaluated;~~ ~~3. The individual must demonstrate continued competence in evaluating the capability of laboratories to support the NSSP. If considered necessary, the individual will be required to performance to several joint evaluations with FDA Laboratory Evaluation Officer.~~ ~~4. The individual must submit a written narrative report of the joint evaluation(s) to the FDA co-evaluator for review and comment. The report should consist of the completed FDA Shellfish Laboratory Evaluation Checklist and the narrative portion should be prepared as above;~~ ~~5. The individual must have all state laboratory evaluations, split sample(proficiency) test examinations, and reports current;~~ ~~6. The individual should receive training as necessary, in laboratory evaluations and analytical procedures to remain proficient.~~ ~~State Shellfish LEOs who successfully complete this process will be issued a Letter of recertification by FDA and be cleared to distribute the evaluation reports to the laboratories evaluated with a copy to the appropriate Regional Shellfish Specialist. Normally recertification is effective for a period of three (3) years. Individuals who fail to meet the requirements for recertification will lose their certification until it is demonstrated that all requirements including adequate training are met.~~
Public Health Significance	This guidance document is virtually unchanged since the inception of the program for utilizing State Shellfish Laboratory Evaluation Officers (State Shellfish LEOS) in the NSSP. This revised guidance updates and clarifies the process for selection, certification and recertification of State Shellfish LEOs
Cost Information	N/A
Action by 2013 Task Force I	Recommended referral of Proposal 13-117 to an appropriate committee as determined by the Conference Chairman.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 13-117.
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 13-117.
Action by 2015 Laboratory Methods Review Committee	Recommended adoption of Proposal 13-117 as amended. .12 Evaluation of Laboratories By State Shellfish Laboratory Evaluation Officers Including Laboratory Evaluation Checklists Laboratory results from the ~~bacteriological~~ microbiological and marine Biotoxin testing of shellfish and shellfish growing waters ~~and meats~~ are widely used in the National Shellfish Sanitation Program (NSSP) to aid in determining the safety of shellfish for human consumption. Experience with the ~~bacteriological~~ microbiological and marine Biotoxin analyses of shellfish and shellfish growing waters have indicated that minor differences in laboratory procedures or techniques might cause wide variations in the results. ~~Improper handling of the sample may also cause variations in results during collection or transportation to the laboratory.~~ To ensure uniformity ~~nationwide~~ NSSP wide in the application of standards for shellfish and shellfish growing waters, a comprehensive, effective laboratory quality assurance (QA) program is necessary to ~~substantiate~~demonstrate the validity of analytical results. ~~A~~ Thee laboratory ~~quality assurance~~QA program is the systematic application

of the practices essential to remove or minimize errors that may occur in any laboratory operation caused by personnel, ~~apparatus,~~ equipment, media, reagents, ~~sampling procedures,~~ and analytical methodology. ~~(APHA, 1985).~~ Integral to laboratory quality assurance is a strong program for the external assessment or evaluation of laboratory performance.

The laboratory evaluation process has evolved over the years to accommodate changes in microbiology and marine Biotoxin procedures brought about by NSSP Workshops and more recently by the Interstate Shellfish Sanitation Conference (ISSC). In 1985, FDA issued an interpretation entitled "Evaluation of Laboratories by State Shellfish Laboratory Evaluation Officers" (SS#35). This Interpretation allowed NSSP laboratories which had been previously evaluated by FDA Shellfish Laboratory Evaluation Officers to be subsequently evaluated by qualified state personnel as certified State Shellfish Laboratory Evaluation Officers. This guidance describes the procedure for the certification of these individuals as State Shellfish Laboratory Evaluation Officers.

~~Requirements for evaluating laboratories that analyze samples under the NSSP have increased significantly since the 1970's. The number of laboratories participating in the shellfish program has also increased. Several states now have multiple laboratories that provide these analyses. Some states have officially designated city, county or private laboratories to conduct analyses supporting their shellfish sanitation programs. Some states are also authorizing the use of private laboratories to monitor depuration operations. More states are maintaining a marine biotoxin analytical capability in their laboratories; and more foreign laboratories are involved in the NSSP. Historically, FDA has evaluated all these laboratories. Reduction in FDA staffing has made it difficult to evaluate the many state, county, municipal, and foreign shellfish laboratories operating in support of the NSSP. If states with multiple laboratory support would exercise their option to accept responsibility for evaluating their laboratories by employing a State Shellfish Laboratory Evaluation Officer (State Shellfish LEO), FDA would be able to better meet its NSSP responsibilities.~~

General Provisions

1. If the State Shellfish Control Authority (Authority) uses the analytical services of private/commercial/fee for services laboratories to support the NSSP, then he/she should select a qualified individual to become certified as a State Shellfish Laboratory Evaluation Officer (State Shellfish LEO).
2. If the Authority uses the analytical services of multiple public laboratories (state, county, parish town, etc.) to support the NSSP, then he/she may select a qualified individual to become a State Shellfish LEO.
3. If the Authority chooses not to participate in the certification process, FDA can evaluate the state's public laboratories. FDA, however, does not normally evaluate private/commercial/fee for services laboratories. FDA may, under certain circumstances as resources permit, evaluate these laboratories on a case-by-case basis at the request of the Authority. This request must be in writing and made through the FDA Regional Shellfish Specialist.
4. State Shellfish LEOs will perform official NSSP evaluations of laboratories which have been previously evaluated by FDA and been found to fully conform to NSSP laboratory requirements.
5. State Shellfish LEOs may evaluate laboratories in a different state

under a memorandum of understanding between the states involved and FDA consistent with NSSP requirements.

6. State Shellfish LEOs may not evaluate laboratories in which they are employed or which they supervise or laboratories within the same supervisory chain of command to ensure complete objectivity in the evaluation process and avoid the appearance of a conflict of interest.
7. To qualify for certification, the prospective State Shellfish LEO should be:
   a. A state employee;
   b. Have shellfish laboratory experience or a laboratory background;
   c. Preferably have laboratory evaluation experience; and,
   d. Be free from any commercial, financial or other pressures or conflicts of interest that might cause or appear to cause the prospective State Shellfish LEO to act in other than an impartial or non-discriminatory manner.
8. If the prospective or current State Shellfish LEO is employed by the laboratory supporting the NSSP, that laboratory must be fully conforming to NSSP requirements or the individual will not be certified and if currently certified, certification will be revoked.

Responsibilities of the State Shellfish Control Authority

1. The Authority must ensure that appropriate written documentation is provided to FDA to demonstrate that a prospective State Shellfish LEO is adequately qualified to assume the responsibilities of a State Shellfish LEO as described above.
2. The Authority must provide or ensure that adequate time, resources and support are made available to the State Shellfish LEO to fully participate in the certification process and to fulfill his/her obligation as a State Shellfish LEO.

FDA's Responsibilities

1. FDA is responsible for the certification/recertification of State Shellfish LEOs.
2. As a result FDA must:
   a. Select qualified individuals to receive training based upon the documentation supplied by the Authority;
   b. Develop and provide training that will enable prospective and current State Shellfish LEOs to consistently and uniformly apply evaluation criteria in determining the competence of laboratories to support or continue to support the NSSP;
   c. Certify prospective State Shellfish LEOs that successfully complete the certification process;
   d. Maintain communication with State Shellfish LEOs as needed to provide guidance and updates relevant to the NSSP laboratory evaluation program;
   e. Recertify current State Shellfish LEOs pursuant to the criteria established for satisfactory performance below;
   f. Monitor the performance of State Shellfish LEOs to ensure that the evaluation process is being performed consistent with NSSP requirements as described in the current NSSP Guide for the Control of Molluscan Shellfish and this guidance;

g. Maintain communication as needed with the Authority and other pertinent state officials, prospective and current State Shellfish LEOs and FDA Regional Shellfish Specialists relevant to the certification/recertification process;

h. Revoke certification of State Shellfish LEOs for cause; and,

i. Void certification when the need for a State Shellfish LEO no longer exists within the state shellfish sanitation program or when the State Shellfish LEO is no longer employed by the state.

~~Selection of State Shellfish LEOs should be based on the following criteria:~~

~~1.The individual must be administratively attached to a state central shellfish sanitation laboratory that has been found by the FDA to be in full conformance with NSSP requirements. To avoid the appearance of impropriety and maintain objectivity in the evaluation process, individuals certified as State Shellfish LEOs will not be allowed to evaluate their own laboratories. FDA will maintain the responsibility for evaluating these laboratories.~~

~~2.The individual must be an experienced analyst and should have laboratory supervision experience. To maintain the integrity of the evaluation process, this individual should not, however, have overall supervisory responsibilities for the laboratory or laboratories to be evaluated. If deemed necessary by an FDA Laboratory Evaluation Officer, the individual must conduct several laboratory evaluations jointly with the FDA Laboratory Evaluation Officer.~~

~~3.During the joint on-site laboratory evaluation with an FDA Laboratory Evaluation Officer, the individual must demonstrate competence in evaluating the laboratory's capability to support the NSSP. The evaluation will be performed and documented using the most current version of the applicable FDA Shellfish Laboratory Evaluation Checklist.~~

~~4 The individual must submit a written narrative report of the joint on-site evaluation to the FDA co-evaluator for review and comment. The report should consist of the completed FDA Shellfish Laboratory Evaluation Checklist and a narrative discussion that accurately and concisely describes the overall operation of the laboratory. All nonconformities noted should be described in this evaluation write-up; and, where relevant an explanation provided relating the potential impact of the deficiency on the analytical results. Recommendations for corrective action or, if applicable, suggestions to enhance laboratory operations must be included in this write-up.~~

~~The FDA will issue a letter certifying each individual who successfully completes the certification process and will clear the evaluation report(s) for distribution to the laboratories evaluated with copies to the appropriate Shellfish Specialist.~~

~~Certification is normally effective for a period of three (3) years. Once certified, the individual is then expected to assume the following responsibilities:~~

State Shellfish Laboratory Evaluation Officer's Responsibilities

1. Conduct onsite laboratory evaluations at least every three (3) years. However, more frequent evaluations are strongly encouraged and may be ~~required~~necessary with marginally performing laboratories, or when major changes in workloads or priorities have occurred or when there has been a substantial turnover of personnel, or, at the specific

request of the Authority. ~~State Shellfish Control Authorities:~~

2. Provide appropriate post-evaluation follow-up for each laboratory evaluated;
3. Prepare timely narrative evaluation reports for all laboratories evaluated. The report should consist of the completed FDA Shellfish Laboratory Evaluation Checklist for the component(s) evaluated and a narrative discussion that accurately and concisely describes the overall operation of the laboratory. All nonconformities noted should be described in this narrative; and, where relevant, an explanation provided relating the potential impact of the deficiency on the analytical results. Recommendations for corrective action or, if applicable, suggestions to enhance laboratory operations should also be included in the narrative report. ~~Incorporating the requirements specified in 4 above;~~
4. Distribute completed evaluation reports with checklists with checklists to FDA and to FDA and to the appropriate FDA Regional Shellfish Specialist.~~:~~
5. Inform ~~the appropriate~~ FDA Shellfish Laboratory Evaluation Officers when a laboratory has been found to be in nonconforming status.~~;~~
6. Coordinate proficiency testing at least yearly for all laboratories in the state supporting the microbiology component of the NSSP.
7. Prepare ~~at least~~ annually (in December) a summary list of ~~qualified analysts for each~~ all laboratories and qualified analysts within each laboratory by NSSP laboratory component supported ~~laboratory supporting the NSSP in the state~~ and transmit it to the ~~appropriate~~ FDA Shellfish Laboratory Evaluation Officers.

Certification Process

Certification is designed to be accomplished through individualized training and field standardization. Individuals are certified for evaluating either the microbiological and/or post-harvest processing (PHP) and/or marine Biotoxin components of the NSSP depending on their qualifications and the needs of the state shellfish sanitation program and at the discretion of FDA.

Field Standardization

1. Field standardization is designed to evaluate the prospective State Shellfish LEO's ability to determine the competence of the laboratory to meet NSSP laboratory requirements; recognize laboratory practices inconsistent with NSSP requirements when they occur; make appropriate recommendations for corrective action; and, provide the necessary follow-up activity to bring the laboratory into conformity with the NSSP.
2. Field standardization consists of one or several joint but independent onsite evaluations with an FDA Shellfish Laboratory Evaluation Officer and preparation of the corresponding narrative evaluation reports. The report(s) should consist of the completed FDA Shellfish Laboratory Evaluation Checklist(s) and a narrative discussion that accurately and concisely describes the overall operation of the laboratory. All nonconformities noted should be described in the narrative; and where relevant an explanation provided relating the potential impact of the deficiency on the analytical results. Recommendations for corrective action or, if applicable, suggestions

to enhance laboratory operations should be included in this narrative report(s).

3. Field standardization should be performed in NSSP laboratories within the prospective State Shellfish LEO's home state to provide realistic evaluation scenarios. The narrative evaluation report detailing the evaluation findings must be prepared. The draft narrative report(s) with accompanying checklist(s) must be submitted to the certifying FDA Shellfish Laboratory Evaluation Officer within 60 days of the evaluation(s). All documents submitted will be reviewed for appropriate content, accuracy and uniformity of approach by the certifying FDA Shellfish Laboratory Evaluation Officer.
4. Field standardization is based on a pass fail system.

Certification

1. Certification is dependent upon the perspective State Shellfish LEO satisfying all the following performance criteria.
   a. Demonstration of good familiarity with evaluation requirements.
   b. Demonstration of a thorough knowledge of the evaluation methods and documents.
   c. Demonstration of the technical knowledge/familiarity with the analytical procedures being used.
   d. Ability to communicate effectively both orally and in writing.
   e. Successful completion of both training and field standardization.
2. Upon successful completion of the certification process, a letter of certification will be issued by the FDA Shellfish Laboratory Evaluation Officer and a copy will be sent to both the requesting Authority and the FDA Regional Shellfish Specialist.
3. Certification is normally valid for up to five (5) years unless revoked or voided.

Failure to be Certified

1. If a prospective State Shellfish LEO fails to satisfy any of the performance criteria listed above, he/she will not be certified.
2. As resources permit and at the discretion of FDA, the prospective State Shellfish LEO may receive additional training to better prepare him/her to be certified.
3. The requesting Authority may withdraw the prospective State Shellfish LEO from consideration.

Recertification

1. Recertification normally occurs every five (5) years and is contingent upon the continuing need in the state shellfish sanitation program for the services of a State Shellfish LEO.
2. Recertification is based on the State Shellfish LEO satisfactorily meeting the following employment and performance criteria.
   a. The individual must continue to be employed by the state and be free of any commercial, financial or other pressures or conflicts of interest real or perceived that may cause the State Shellfish LEO to act in other than an impartial and non-

discriminatory manner.

b. The individual must demonstrate continued competence in the evaluation of NSSP laboratories by performing one to several joint evaluations with an FDA Shellfish Laboratory Evaluation Officer and providing an appropriate narrative evaluation report to the FDA co-evaluator for review and comment for each of the laboratories jointly evaluated.

c. The individual must have performed laboratory evaluations at the minimum frequency prescribed in the current edition of the Guide for the Control of Molluscan Shellfish and have all Narrative evaluation reports up to date.

3. State Shellfish LEOs who successfully complete recertification will be issued a letter of recertification by FDA and be cleared to distribute the completed report(s) to the appropriate Regional Shellfish Specialist. A copy of this letter will be sent to the State Shellfish Control Authority and appropriate Regional Shellfish Specialist.

4. If FDA is unable to conduct a recertification visit by the expiration of the individual's certification, his/her certification may be extended until such time as recertification can be completed. If requested, a letter extending the certification can be provided as appropriate.

Revocation of Certification

1. State Shellfish LEO's who fail to meet any of the certification/recertification, employment or performance criteria listed above will have their certification revoked.
2. Certification may be voided when state shellfish sanitation programs no longer have a need for the services of a State Shellfish LEO.
3. Voided certifications may be reactivated at the discretion of FDA if the need for the analytical services of additional laboratories by the state shellfish sanitation program recurs.
4. Revoked certifications will not normally be restored.

~~Recertification of State Shellfish LEOs will normally occur triennially and will be based on satisfactorily meeting the following criteria:~~

~~1. The individual must continue to be administratively attached to a central state shellfish laboratory which is in full conformance with NSSP requirements;~~

~~2. The individual is not the supervisor of any of the laboratories to be evaluated;~~

~~3. The individual must demonstrate continued competence in evaluating the capability of laboratories to support the NSSP. If considered necessary, the individual will be required to performance to several joint evaluations with FDA Laboratory Evaluation Officer.~~

~~4. The individual must submit a written narrative report of the joint evaluation(s) to the FDA co-evaluator for review and comment. The report should consist of the completed FDA Shellfish Laboratory Evaluation Checklist and the narrative portion should be prepared as above;~~

~~5. The individual must have all state laboratory evaluations, split sample(proficiency) test examinations, and reports current;~~

~~6. The individual should receive training as necessary, in laboratory evaluations and analytical procedures to remain proficient.~~

~~State Shellfish LEOs who successfully complete this process will be issued a~~

	~~Letter of recertification by FDA and be cleared to distribute the evaluation reports to the laboratories evaluated with a copy to the appropriate Regional Shellfish Specialist. Normally recertification is effective for a period of three (3) years. Individuals who fail to meet the requirements for recertification will lose their certification until it is demonstrated that all requirements including adequate training are met.~~
Action by 2015 Task Force I	Recommends adoption of Laboratory Method Review Committee recommendations on Proposal 13-117.

Proposal Subject	Dilution Guidance for Prohibited Zones Associated with Wastewater Discharges
Specific NSSP Guide Reference	NSSP Guide Section IV. Guidance Documents Chapter II. Growing Areas
Text of Proposal/ Requested Action	Refer to 2015 Proposal Package
Public Health Significance	The public health purpose of this guidance is to provide the scientific basis and recommendations for determining appropriately sized Prohibited Areas (closure zones) around waste water treatment plants (WWTP). Section II, Chapter IV. @.03 (5) currently mandates that a prohibited zone be established, but there is no specific guidance information on how to calculate the size of the prohibited zone to ensure that microbiological pathogens (particularly viruses) from WWTP do not adversely impact the growing area at the time of harvest. It is expected that this guidance will provide all ISSC stakeholders with better information on which to make informed, scientifically based decisions
Cost Information	
Action by 2013 Task Force I	Recommended referral of Proposal 13-118 to an appropriate committee as determined by the Conference Chairman with additional instructions to the ISSC Executive Office to create a workgroup to meet quarterly and report back to the Conference at the next ISSC meeting.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 13-118.
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 13-118.
Action by 2015 Growing Area Classification Committee	Recommended adoption of Proposal 13-118 with substitute language as follows: Determining Appropriately Sized Prohibited Areas Associated with Wastewater Treatment Plants <u>Introduction</u> The original National Shellfish Sanitation Program (NSSP) principles have proved effective in controlling bacterial illness associated with shellfish harvested from polluted waters. These principles, namely a robust sanitary survey, regular water and shellfish monitoring using bacterial indicators, controlled harvest times and labelling the origin of shell stock remain applicable as the primary preventative food safety control measures for growing areas. However, there is now ample scientific evidence to show that the current bacterial indicators are inadequate to predict the risk of viral illness for the following reasons: (1) Enteric viruses are resistant to treatment and disinfection processes in a wastewater treatment plant (WWTP) and are frequently detected in the WWTP's final effluent under normal operating conditions (Baggi et al. 2001; Burkhardt et al. 2005, Pouillot et al. 2015). (2) Shellfish can bioaccumulate enteric viruses up to 100-fold from surrounding water (Seraichekas et al. 1968; Maalouf et al. 2011). (3) Certain enteric viruses are retained by molluscan shellfish to a greater extent and for longer than the indicator bacteria currently used to classify shellfish growing areas (Sobsey et al. 1987; Dore & Lees 1995; Love et al. 2010). It has been well documented that enteric virus detection is not indexed by levels of conventional indicator bacteria.

For several decades now viral illnesses, in particular norovirus (NoV) and Hepatitis A (HAV), have been the most common food safety problem associated with bivalve molluscan shellfish (Woods 2010; Iwamoto et al 2010; Scallan et al. 2011; Batz et al. 2012; Hall et al 2012). NoV genogroups I, II and IV and HAV are typically associated with ill-individuals and transferred by the fecal-oral route. Because WWTPs do not completely remove infectious enteric viruses emphasis should be placed on the importance of ensuring there is adequate dilution between a sewage source and a shellfish growing area.

In addition to the risk of enteric viruses WWTP effluents may also contain other chemicals and deleterious substances including pharmaceuticals, nanoparticles, and other contaminants of emerging concern. Establishment of a prohibitive area in proximity to WWTP discharges is an effective strategy to reduce the risk posed by both enteric viruses and other contaminants found in WWTP effluents. This guide provides information on the recommended dilution rates with respect to enteric viruses to ensure WWTP effluent does not cause a significant viral food safety risk within shellfish growing areas. The guide also considers the factors that should be used to assess a WWTP.

Delineation of the Prohibited Zone around a Wastewater Treatment Plant

The NSSP Model Ordinance Section II, Chapter IV. @.03 (2) (b) and @.03 E(5) states that all growing areas which have a sewage treatment plant outfall or other point source outfall of public health significance within or adjacent to the shellfish growing area must have a prohibited classification established adjacent to the outfall taking account of the following factors:

(1) The volume flow rate, location of discharge, performance of the wastewater treatment plant and the microbiological quality of the effluent;

(2) The decay rate of the contaminants of public health significance in the wastewater discharged;

(3) The wastewater's dispersion and dilution and the time of waste transport to the area where shellstock may be harvested; and

(4) The location of the shellfish resources, classification of adjacent waters and identifiable landmarks or boundaries.

There are several important considerations for the shellfish authority to consider when establishing the size of each prohibited zone:

(1) The area to ensure that there is adequate dilution when the WWTP is operating as normal. “Normal” means that the WWTP is operating fully within the plant’s design specifications, including design flows; treatment stages; disinfection; as well as compliance with all permit conditions that relate to the WWTPs effectiveness in reducing enteric viruses in sewage.

Below is not an exhaustive list but serves as examples of situations that could occur and are critical for Shellfish Control Authorities (SCAs) on evaluating each WWTP when developing Conditional Area Management Plan (CAMP):

Bypassing stage of treatment

A plant may be considered operating outside of normal operation if a treatment stage such as primary or secondary treatment is bypassed which

may result in an increased load of solids in the disinfection step and reduce the effectiveness of disinfection. An additional example would be when a WWTP experiences a loss in disinfection and thus the ability to effectively treat the final effluent. SCAs should determine the significance of these types of events and make appropriate provisions in the CAMP.

Operating outside design specifications/other types of failures or events

It is not uncommon for a WWTP to periodically experience mechanical failures of equipment that could alter the treatment of sewage. Additionally, a WWTP may also need to periodically perform routine maintenance to the various stages of treatment and may need to temporarily take a portion of a treatment stage off-line for cleaning. Other unexpected maintenance may need to occur for example bio-fouling of filters or membranes used in treatment. SCAs should be informed by WWTP operators of these events to determine if any additional temporary action is needed if not addressed in the CAMP.

Operating above design flow

Some WWTPs may operate above its design flow and not necessarily bypass any particular stage of treatment. During these events it is typical for WWTP operators to adjust the operation of the WWTP which may include reducing the treatment time in the aeration stage and/or solids separation/settling stage of treatment. Under some circumstances this could lead to a significant reduction in the effectiveness of disinfection. SCAs may consider assessing the efficiency of WWTPs to determine the significance of these type of events and if additional provisions should be made in the CAMP.

WWTP permit violations

If a WWTP is exceeding the permitted bacterial indicator levels in the final effluent this indicates that effectiveness of the disinfection step has been reduced. Other measured parameters in the effluent (e.g. TSS, BOD) may also indicate a reduction in treatment efficiency as occurred. SCAs may consider assessing the efficiency of WWTPs to determine the significance of these type of events and if additional provisions should be made in the CAMP.

Situations where compliance with permit but risk to shellfish growing area

There could be situations in which a particular WWTP could be in compliance with a permit, and could still pose a risk to the shellfish harvest area. For example, a WWTP may have permit conditions to allow for flow blending during high flow periods where a portion of the sewage may receive full treatment but a portion of the sewage may only be partially treated and "blended" in the final disinfection step. Although this may be an acceptable practice under a permit it could result in conditions in which the efficiency of the WWTP to remove enteric viruses is considerably reduced. SCAs may consider assessing the efficiency of WWTPs to determine the significance of these type of events and if additional provisions should be made in the CAMP.

(2) That the collection system has no malfunctions, bypasses or other factors that would lead to significant leakages of untreated sewage to the marine environment.

(3) That there is adequate detection and response time when any malfunction occurs to ensure that all harvesting ceases and closures are enforced, so that contaminated product does not reach the market.

Additional considerations

It is critical for SCAs to communicate with WWTP operators and ensure that there is no confusion over how SCAs define "outside of normal operation" in a Conditional Area Management Plan (CAMP) which may differ from how "malfunctions" or "violations" are defined in a permit. The SCAs also need to ensure that the WWTP operators understand the CAMP and that shellfish growing areas may close based on conditions of the CAMP even though the WWTP is operating in compliance within permitted conditions. Thus, it is important to communicate with WWTP operators to ensure that when shellfish closures occur and are reported that SCAs are using terminology that is understood by both parties.

Guidelines for Dilution, Dispersion, and Time of Travel of Effluent

Dilution refers to the dilution of effluent that occurs when the effluent is subjected to a number of physical processes in the receiving waters including turbulent mixing of the effluent in the vicinity of the outfall and at further distances primarily through tidal action, wind, and density stratification. Dispersion refers to the spread, location, and shape of the effluent discharge plume with time as it leaves the WWTP outfall. Time of travel refers to the time it takes effluent to reach the shellfish harvest site starting from the point of discharge.

It is essential to recognize that water samples collected near discharge outfalls are not useful for determining the size of prohibited zones because normal operating conditions in WWTPs can effectively reduce or even eliminate the fecal and total coliforms which are the current indicator microorganisms used to assess treatment efficiency. In contrast, many human enteric viruses are not inactivated by functioning WWTP treatment and disinfection systems, hence the need for an adequate dilution zone between the outfall and the shellfish resource.

It is important to consider not only the WWTP discharge, but also overflow points on the collection system such as those from pumping stations. While a malfunctioning WWTP may provide partial treatment, the discharge from a collection system is untreated and may be a more common failure point in the overall system.

When determining if a WWTP or collection system discharge within the watershed or catchment area draining to a shellfish estuary potentially impacts a shellfish growing area, in the absence of a performance history of the treatment and collection system, and a database of influent and effluent quality, the NSSP recommends that a worst case raw sewage discharge be assumed. In this circumstance, if a level of 1.4×10^6 FC/100ml is assumed for a raw sewage release, a 100,000:1 dilution would be required to dilute the sewage sufficient to meet the approved area standard of 14 FC/100ml. If dilution analysis determines that the location of the discharge is such that the dilution of effluent would be greater than 100,000:1 then the WWTP could be considered located outside the zone of influence to the shellfish growing area. Different dilution ratios may be applied depending on the known concentration of sewage, provided that the water

quality objective of the downstream harvest area is met.

In areas where the required WWTP discharge dilution is less than 100,000:1 and/or a raw sewage release results in FC levels in the growing area of >14 FC/100 ml a conditional management may be considered. However, conditional management is only recommended for, highly efficient WWTPs that are well monitored to detect malfunctions and changes in effluent quality and when the shellfish authority has the resources to effectively administrate and patrol the conditions of the growing area management plan.

In all cases the FDA recommends the minimum of a 1000:1 dilution around a WWTP outfall to mitigate the impact of viruses on shellfish growing areas.

A dye study can be used to measure the dilution and dispersion of the effluent during specific discharge conditions. Computer modeling programs can also be used to estimate the dispersion and dilution of the effluent plume from WWTPs and collection system overflows.

Scientific Rationale for 1000:1 Dilution Guidance

In 1995 the FDA determined the 1000:1 dilution was necessary using the most relevant the scientific literature available at that time (Kohn, et al. 1995; Havelaar et al. 1993; Kapikian et al. 1990; Liu et al. 1966). In 2008 FDA performed an investigation in the upper portion of Mobile Bay, Alabama, the results of which were published in the Journal of Shellfish Research (Goblick, et al., 2011). The article describes how FDA used technical advances to assess the 1995 1000:1 dilution recommendation. The Mobile Bay study confirmed that this level of dilution was appropriate to mitigate the risk of viruses discharged in treated wastewater effluent.

Since the 2008 Mobile Bay study there have been major advances in the detection and enumeration of NoV in wastewater and shellfish and fluorometer technologies have enabled more sophisticated hydrographic dye study methods. Using these advances, FDA has now conducted numerous dye studies supplemented with the testing of shellfish sentinels for enteric viruses and their surrogates. The findings from these studies demonstrate that achieving a steady-state 1000:1 dilution level in the requisite Prohibited area appears to be adequate for mitigating the impacts of viruses on shellfish when WWTPs have typical treatment and disinfection practices, such as secondary treatment and chlorination, and when operating under normal conditions.

While evaluating the 1000:1 dilution level Male Specific Coliphage (MSC) results in shellfish from the 2008-2015 studies were evaluated. These collaborative studies with State Shellfish Control Authorities and Industry were conducted in the Gulf, Mid-Alantic, East and West Coast, and under varying hydrographic and meteorological conditions. Various additional factors were considered such as type of wastewater treatment and disinfection technology, seasonal conditions, and shellfish species etc. and are represented in the data collected. In some cases, data was collected during a period of which the WWTP was considered to be operating outside of “normal” operating conditions. In other cases, the WWTP was considered not suitable for conditional area management due to design/poor performance even during routine/normal operation. Focus was given to the MSC threshold of 50 PFU/100 grams of shellfish tissue which is the level used for re-opening harvest areas after an emergency closure due to raw untreated sewage discharged from a large community sewage collection system or a WWTP (Model Ordinance (Section II, Chapter IV,

@.03 A(5)(C)(ii))). From the 2008-2015 studies, a total 216 samples were assessed including conditions when the WWTPs were considered operating normally as well as under a bypass or degraded operation conditions. In summary, 216 samples were analyzed for MSC of which 176 samples (81%) were positive for MSC; 118 samples (67%) contained MSC levels > than 50 PFU/100 grams; and 43 samples (20%) had MSC levels > 50 PFU/100 grams and wastewater effluent dilution was greater than 1000:1. These results are shown in Figure 1 and Table 1 below.

Figure 1: Comparison of dilution in receiving water and MSC levels in shellfish – all conditions

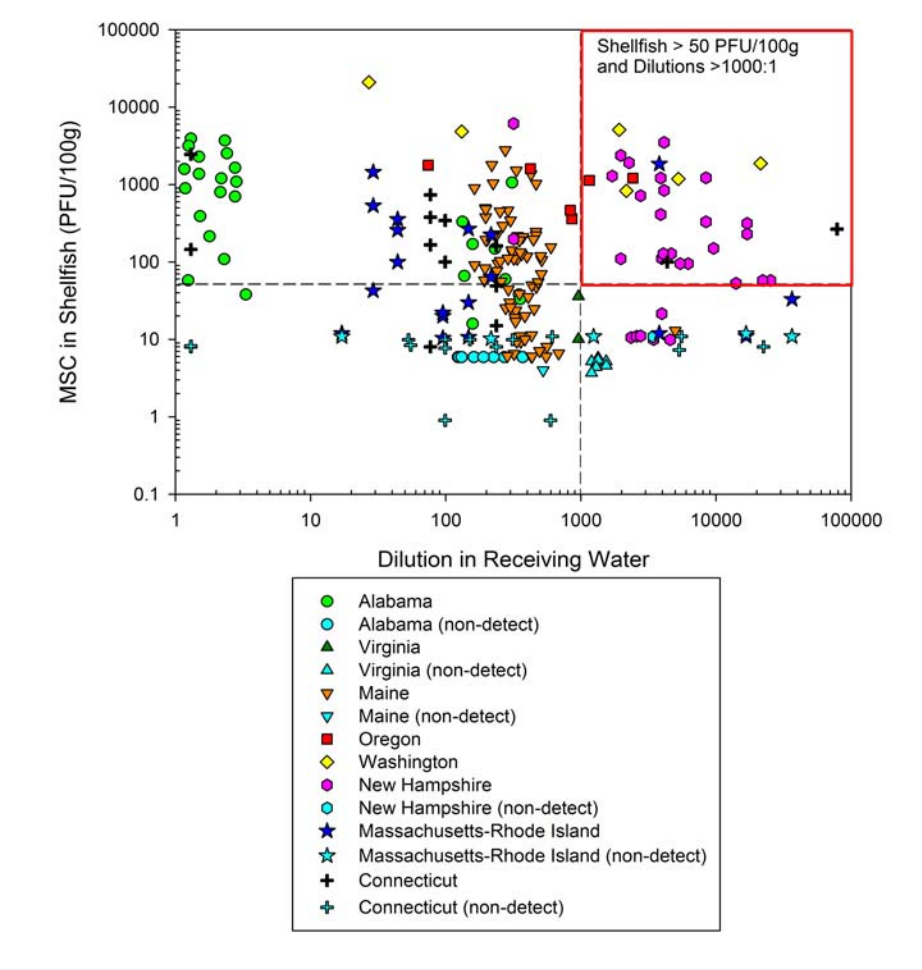


Table 1: MSC in shellfish operating under "normal" and outside of normal operation

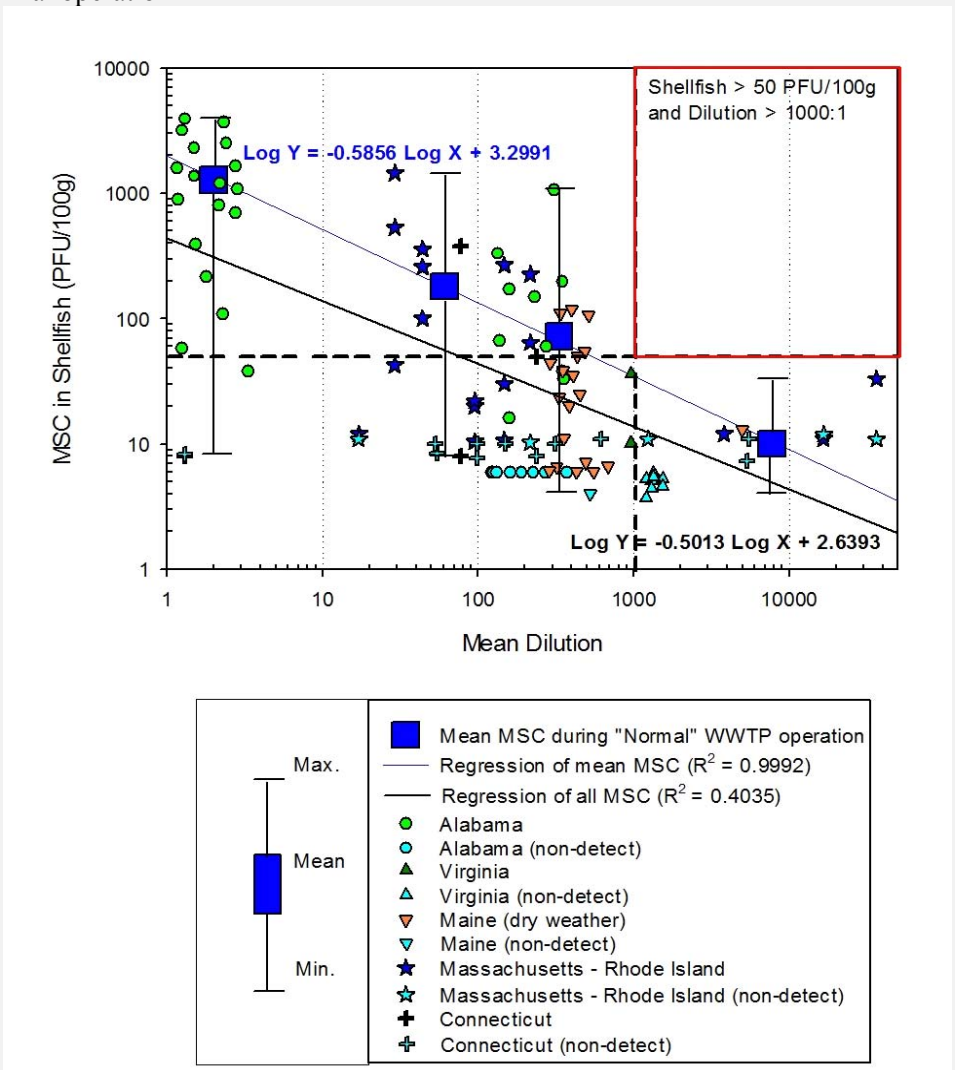
MSC Results	All Conditions (n=216)	Normal Operating Conditions (n=129)
MSC detectable	81% (176)	62% (80)
MSC levels >50 pfu/100g	67% (118)	36% (46)
MSC levels >50 pfu/100g and Dilution in Growing Area >1000:1	20% (43)	0% (0)

In separating the data attributed to "normal" operation from other conditions, 129 of the 216 total samples were considered to be attributed to "normal" WWTP operation, also shown on Table 1. Eighty seven (87) samples were removed as they were attributed to conditions of WWTP malfunction or situations considered not suitable

for conditional area management. From the 87 samples, 80 were associated with degraded WWTP performance or malfunction of which 6 were associated with a primary bypass, 13 were associated within a period of a WWTP upgrade during which the WWTP reportedly was operating an extended period (weeks) without disinfection, 31 were associated with degraded treatment quality because of rainfall/flows exceeding the WWTP design capacity, and 30 were attributed to a WWTP with no secondary treatment and operated frequently with flows exceeding the design capacity. Of the remaining 7 samples, 6 were associated with a WWTP utilizing unconventional disinfection technology (membrane filtration) and demonstrated poor performance in removing viruses compared to other conventional technologies during normal operating conditions, and 1 sample was attributed to a potential point source sewage discharge other than the WWTP.

When considering the remaining 129 samples attributed to "normal" WWTP operating conditions there were no samples that were above 50 PFU/100 grams when dilution was greater than 1000:1. In comparison, of the 87 samples attributed to malfunction or unsuitable conditions, 43 samples exceeded 50 PFU/100 grams when dilution was greater than 1000:1. These results are shown in Figure 2 below.

Figure 2: Comparison of dilution in receiving water and MSC levels in shellfish under normal operation



Comparing MSC with NoV sample results, out of the 216 samples analyzed for MSC, 161 samples were also analyzed for NoV. Of the 161 samples tested for NoV, 66 were positive (41% of total) were positive for NoV. Out of the 66 NoV positive samples, 62 (94% of total) were also positive for MSC and 53 (85% of total) had levels greater than 50 PFU/100 grams. There were only 4 cases where NoV was positive but MSC was not detected. However, in these cases, 3 of the sample results were near the Limit of Detection (LOD) for NoV enumeration. In one case it is suspected that both MSC and NoV may have been present but not likely viable as the WWTP utilized UV disinfection and was operating under normal conditions. These results are shown in Figure 3 and Table 2 below:

Figure 3: Comparison of MSC and NoV results

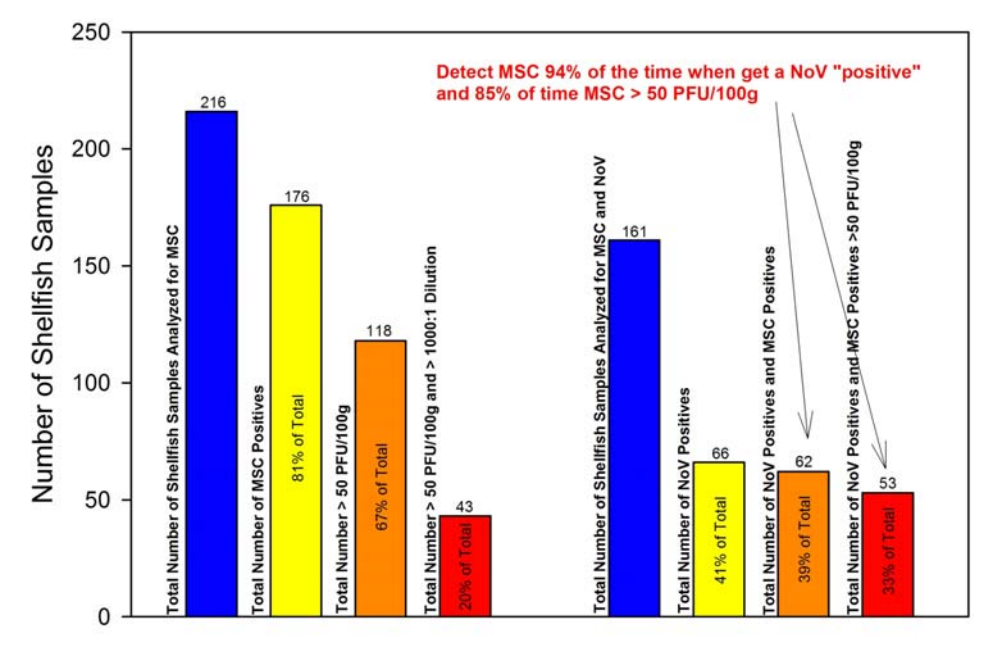


Table 2: Comparison of MSC and NoV Results in shellfish

MSC and NoV Results	
NoV detected in shellfish	41% (66 of 161)
MSC detectable	39% (62 of 161)
MSC negative when NoV detected (MSC<10 pfu/100g)	7% (4 of 66)*
MSC present when NoV detected (MSC>10 pfu/100g)	94% (62 of 66)
MSC present when NoV detected (MSC>50 pfu/100g)	85% (53 of 66)

*NoV detected at LOD of Assay

The overall results of FDA's field studies demonstrate a strong relationship between increased levels of enteric viruses and MSC and decreased levels of dilution. This trend was observed in all of the studies conducted by FDA at conventional WWTPs. These results also emphasize the critical need for sufficient notification time, meaning travel time from the WWTP discharge in the prohibited area is long enough to close the shellfish growing area in the event of a malfunction. This preventative measure may necessitate the Prohibited Area be larger than the zone necessary to achieve 1000:1 dilution. Furthermore, this analysis demonstrates the need to individually assess each WWTP, to assess their performance to remove enteric viruses.

In addition to the FDA field studies, as part of a Joint United States-Canada Norovirus in Bivalve Molluscan Shellfish Risk Assessment, a Meta-Analysis of the

Reduction of NoV and MSC Concentrations by Wastewater Treatment was conducted (Pouillot, 2015). The meta-analysis included previously unpublished surveillance data from the United States and Canada and relevant data reported in the literature (2,943 measurements in total).

For WWTPs with mechanical systems and chlorine disinfection, mean log10 reductions were 2.4 log10 gc/liter, for NoV GI, 2.7 log10 gc/liter, for NoV GII, and 2.9 log10 PFU per liter for MSCs. Comparable values for WWTPs with lagoon systems and chlorine disinfection were 1.4 log10 gc/liter for NoV GI, 1.7 log10 gc/liter for NoV GII, and 3.6 log10 PFU per liter for MSCs. WWTPs with ultra-violet (UV) disinfection demonstrated slightly higher mean log10 reductions with 3.0 log10 gc/liter, for NoV GI, 3.3 log10 gc/liter, for NoV GII, and 4.3 log10 PFU per liter for MSCs. The results of the reduction of NoV and MSC are shown in Table 3 below:

Table 3: Log reduction in NoV and MSC in treated wastewater with disinfection

Wastewater Treatment and Disinfection	Log_{10} NoV GI Reduction	Log_{10} NoV GII Reduction	Log_{10} MSC Reduction
Mechanical with Chlorine Disinfection	2.4	2.7	2.9
Lagoon with Chlorine Disinfection	1.4	1.7	3.6
Mechanical with UV Disinfection	3.0	3.3	4.3

This meta-analysis also demonstrated that Chlorine Disinfection had little effect on the mean reductions of the NoV and MSC. The mean log10 reduction that occur due to mechanical and biological treatment of the facility (prior to disinfection) were 2.2 log10 gc/liter, for NoV GI, 2.5 log10 gc/liter, for NoV GII, and 2.4 log10 PFU per liter for MSCs which varied little from mean log reduction after disinfection. In addition, a strong correlation, 0.8, existed between the reductions of NoV GII and MSC that occurred following treatment at the same WWTP indicating that MSCs could be useful in evaluating the efficiency of a WWTP.

Alternate Options

The FDA studies also suggested that certain factors, such as the quality of sewage treatment or the time of year, may exert influences on the levels of viruses discharged. However, at this time FDA does not have reliable data to justify specific dilution levels associated with environmental variables. It is recognized that such criteria could be determined by SCAs on a case by case basis, where factors of WWTP performance, disinfection method, tidal flushing, shellfish species and seasonal impacts may vary.

For example, in consideration of a raw sewage discharge, a lower dilution level than a 100,000:1 could be justified provided that specific data to that particular WWTP demonstrates that a lower bacteriological level associated with a potential raw sewage discharge is supported. Additional or other site specific information also can be used to justify alternative approaches that take into account other factors (such as no prior history of raw sewage discharges or containment structures sufficiently sized to accommodate a raw sewage event preventing a discharge).

Alternative options for calculating the size of the prohibited zone to mitigate the virological effects of WWTP discharges at the shellfish growing area may be used provided that they are based on sound scientific principles that can be verified. For example, it is reasonable to expect a potentially higher reduction in viral load from a properly maintained wastewater treatment system employing ultraviolet (UV)

disinfection, tertiary treatment and operating under optimum design flow conditions. Regardless of the technology employed any proposed alternative minimum level of dilution for conditional management other than 1000:1 would need validation. MSC could potentially be used on a case-by-case basis as the validation process (for example to validate treatment efficiency) if demonstrated it is a successful/feasible strategy for the given location/situation. However, when there is insufficient information available for a growing area to support the use of a lower level of dilution, the 1000:1 dilution should be employed. If MSC is selected as an alternative option for calculating the size of the prohibited zone of a WWTP discharge, the authority should select an MSC criteria that adequately protects shellfish growing areas from virological effects and should be based on the most recent data and regional studies.

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Action by 2015 Task Force I	Recommends adoption of Growing Area Classification Committee recommendation on Proposal 13-118.

Proposal Subject	Definition of Laboratory Method Types
Specific NSSP Guide Reference	Section I. Definitions
Text of Proposal/ Requested Action	Add the following new definitions in Section I. Definitions: **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 .11 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 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 .11 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. **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 criteria in Procedure XVI. of the ISSC Constitution, Bylaws, and Procedures are provided.
Public Health Significance	These terms are used in Chapter III. and in the ISSC Constitution, Bylaws, and Procedures and should be defined.
Cost Information	
Action by 2015 Task Force I	Recommends adoption of the following substitute language to be included in both Section I. Definitions and Section 9, Subdivisions a and b of Procedure XVI of the ISSC Constitution Bylaws and Procedures. **Approved NSSP Methods.** Approved NSSP Methods are the primary/core methods used in the NSSP~~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 .11 Approved National Shellfish Sanitation Program Laboratory Tests. These methods have been described in scientific or other peer-reviewed professional publications; have been used historically or are used throughout the NSSP and elsewhere to effectively detect or quantify and have been extensively evaluated and the performance characteristics for specific applications in the NSSP determined as ~~long used in the NSSP or have completed the Single Laboratory Validation Method Protocol to show that the method is~~ fit for purpose through long use in the NSSP and/or Single Laboratory Validation (SLV) testing and/or collaborative study.. **Approved Limited Use Methods.** Approved Limited Use Methods are permanent methods accepted for use in NSSP and listed in the NSSP Guide for the Control of Molluscan Shellfish, Guidance Documents Chapter II. Growing Areas .11 Approved National Shellfish Sanitation Program Laboratory Tests. These methods include new methods, alternative methods or screening 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. These methods have been evaluated and the performance characteristics for specific applications in the NSSP have been

	determined through the Single Laboratory Validation Method Protocol (SLV) to be fit for purpose within the NSSP. These methods are referred to as being of limited use within the NSSP either because of their status as newly adopted methods with little corroborating data beyod the SLV or because the application for which the method can be or is used within the NSSP is limited in scope with little laboratory participation within the NSSP and little to no subsequent corroborating data or because of the nature of the test method itself and/or restrictions that have been placed on its use that limit its usefulness within the NSSP. ~~Approved Limited Use Methods can include screening, provisional, or methods with limitations as defined by the LMRC evaluation of the method.~~ **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 criteria in Procedure XVI. of the ISSC Constitution, Bylaws, and Procedures are provided.

Proposal Subject	Monthly Laboratory Grade Water Testing
Specific NSSP Guide Reference	Section II. Model Ordinance Chapter III. Laboratory
Text of Proposal/ Requested Action	@.02 Methods. A. 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 .11 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. Methods for the analysis 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 .11 Approved National Shellfish Sanitation Program Laboratory Tests. (2) Results shall be expressed for chemical and physical measurements in standard units and not instrument readings. (3) 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. C. Biotoxin. Methods for the analyses of shellfish and shellfish 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 .11 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. D. Emergency Use Methods. (1) When there is an immediate or critical need and no Approved NSSP Method exists, an unapproved or non-validated method may be used for a specific purpose provided that: (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 Shellfish Control Authority submitter. (h) Developer/submitter contact information. (3) Within two (2) years of Executive Board interim approval of the Emergency Use Method, the entire Single Lab Validation Protocol should be submitted. The Laboratory Methods Review Committee will report to the Executive Board on the status of the Single Lab Validation Protocol data submission. E. Laboratory Grade Water, AKA Reagent Water Microbiologically Suitable Water, Type 1 Water. For the required monthly testing of the laboratory's reagent grade water for microbiological contamination, the following may be used: (1) An AOAC, BAM, or EPA approved method; (2) Heterotrophic plate count equivalent methods as described in *Standard Methods for the Examination of Water and Wastewater or Compendium of Methods for the Microbiological Examination of Foods.*
Public Health Significance	Although this is a monthly requirement, there are currently no approved NSSP methods that specifically address reagent water. For labs that support multiple Federal programs with this requirement, adding this would provide clearer guidance while allowing each lab to choose the method that best conforms to the analysis they routinely perform. The savings of time and money allows resources to be used to protect public health more wisely.
Cost Information	Cost will be determined by each lab dependent on method used.
Action by 2015 Laboratory Methods Review Committee	Recommended no action on Proposal 15-101. Rationale: This test is for internal laboratory use so the method of analysis used is at the discretion of the laboratory. The only requirement is that the test method chosen be recognized as fit for purpose.
Action by 2015 Task Force I	Recommends adoption of the 2015 Laboratory Method Reviews Committee recommendation on Proposal 15-101.

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	Section II. Model Ordinance Chapter IV. Shellstock Growing Areas
Text of Proposal/ Requested Action	@.01 Sanitary Survey. A. General. (1) The sanitary survey is the written evaluation report of all environmental factors, including actual and potential pollution sources, which have a bearing on water quality in a shellfish growing area. The sanitary survey shall include the data and results of: (a) A shoreline survey; (b) A survey of the ~~bacteriological~~ microbiological quality of the water and in growing areas adjacent to wastewater system discharges the State Shellfish Control Authority may utilize MSC results from analysis of shellfish meat samples and the analysis of the data will be included in the sanitary survey report; (c) An evaluation of the effect of any meteorological, hydrodynamic, and geographic characteristics on the growing area; (d) An analysis of the data from the shoreline survey, the bacteriological and the hydrodynamic, meteorological and geographic evaluations; (e) A determination of the appropriate growing area classification. B. Sanitary Survey Required… C. Sanitary Survey Performance. (5) On an annual basis, the sanitary survey shall be updated to reflect changes in the conditions in the growing area. The annual reevaluation shall include: (a) A field observation of the pollution sources which may include: (i) A drive-through survey; (ii) Observations made during sample collection; and (iii) Information from other sources. (b) Review, at a minimum, of the past year's water quality sample results by adding the year's sample results to the data base collected in accordance with the requirements for the bacteriological standards and sample collection required in Section .02; (c) Review of available inspection reports and effluent samples collected from pollution sources; (d) Review of available performance standards for various types of discharges that impact the growing area; ~~and~~ (e) A brief report which documents the findings of the annual reevaluation; and (f) The SSCA may use MSC meat sampling data and/or MSC waste water sampling data in the annual reevaluation of (5)(b), (c), and (d) above to evaluate the viral contributions of the performance standards of waste water system discharge (WWSD) impacts on shellfish growing areas.

(g) If MSC meat and/or water data is being used, the SSCA shall conduct annual sample collection and analysis in determining performance standards.

D. Shoreline Survey Requirements…

@.02 ~~Bacteriological~~ Microbiological Standards.

Note: The NSSP allows for a growing area to be classified using either a total or fecal coliform standard. The NSSP further allows the application of either standard to different water bodies within the state. The NSSP also allows for two (2) sample collection strategies for the application of the total or fecal coliform standard: adverse pollution condition and systematic random sampling. The 1992 Task Force II recommended that this portion of the Ordinance be codified in two (2) ways: a total coliform strategy and a fecal coliform strategy so that the state may choose sampling plans on a growing area basis. Within each strategy, provisions would appear for use of both systematic and adverse pollution condition sample collection. The Ordinance has been recodified in this manner. For maximum flexibility, a state may wish to adopt the use of both standards and both sampling strategies for each standard. This codification represents the fecal coliform standards. Additionally, states may choose to use MSC sample data in conjunction with total or fecal coliform data to evaluate areas impacted by waste water system discharges.

A. General. Either the total coliform or fecal coliform standard shall be applied to a growing area. The SSCA may utilize MSC data in conjunction with bacteriological data to evaluate waste water system discharge (WWSD) impacts on shellfish growing areas.
B. Water Sample Stations…
C. Exceptions…
D. Standards for the Approved Classification of Growing Areas in the Remote Status…
E. Standard for the Approved Classification of Growing Areas Affected by Point Sources…
F. Standard for the Approved Classification of Growing Areas Affected by Nonpoint Sources…
G. Standard for the Restricted Classification of Growing Areas Affected by Point Sources and Used as a Shellstock Source for Shellstock Depuration…
H. Standard for the Restricted Classification of Growing Areas Affected by Nonpoint Sources and Used as a Shellstock Source for Shellstock Depuration…

@.03 Growing Area Classification.

A. General…
    (1) Emergency Conditions…
    (2) Classification of All Growing Areas…
    (3) Boundaries…
    (4) Revision of Classifications…
    (5) Status of Growing Areas…
        (a) Open Status…
        (b) Closed Status...
        (c) Reopened Status. A growing area temporarily placed in the closed status as provided in (b) above, shall be returned to the open status only when:

(i) The emergency situation or condition has returned to normal and sufficient time has elapsed to allow the shellstock to reduce pathogens or poisonous or deleterious substances that may be present in the shellstock to acceptable levels. Studies establishing sufficient elapsed time shall document the interval necessary for reduction of contaminant levels in the shellstock to pre-closure levels. In addressing pathogen concerns, the study may establish criteria for reopening based on coliform levels in the water; or

(ii) For emergency closures ~~(not applicable for conditional closures)~~ of harvest areas caused by the occurrence of raw untreated 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 fifty (50) male-specific coliphage per 100 grams from shellfish samples collected no sooner than seven (7) days after contamination has ceased and from representative locations in each growing area potentially impacted; or

(iii) The requirements for Biotoxins or conditional area management plans as established in Section .04 and Section .03, respectively, are met; and

(iv) Supporting information is documented by a written record in the central file.

(d) Inactive Status…

(e) Remote Status…

(f) Seasonally Remote/Approved Status…

B. Approved Classification…

C. Conditional Classifications. Growing areas may be classified as conditional when the following criteria are met:

(1) Survey Required. The sanitary survey meets the following criteria:

(a) The area will be in the open status of the conditional classification for a reasonable period of time. The factors determining this period are known, are predictable, and are not so complex as to preclude a reasonable management approach;

(b) Each potential source of pollution that may adversely affect the growing area is evaluated;

(c) ~~Bacteriological~~ Microbiological water quality correlates with environmental conditions or other factors affecting the distribution of pollutants into the growing area;~~.~~ and

(d) For SSCAs utilizing MSC meat sample data, this data correlates with environmental conditions or other factors affecting the distribution and persistence of viral contaminants into the growing area.

(2) Management Plan Required. For each growing area, a written management plan shall be developed and shall include:

(a) For management plans based on wastewater treatment plant function, performance standards that include:

(i) Peak effluent flow, average flow, and infiltration flow;

(ii) Microbiological quality of the effluent;
(iii) Physical and chemical quality of the effluent;
(iv) Conditions which cause plant failure;
(v) Plant or collection system bypasses;
(vi) Design, construction, and maintenance to minimize mechanical failure, or overloading;
(vii) Provisions for monitoring and inspecting the waste water treatment plant; and
(viii) Establishment of an area in the prohibited classification adjacent to a wastewater treatment plant outfall in accordance with Section E. Prohibited Classification;

(b) For management plans based on pollution sources other than waste water treatment plants:
(i) Performance standards that reliably predict when criteria for conditional classification are met; and
(ii) Discussion and data supporting the performance standards.

(c) For management plans based on waste water system discharge ~~treatment plant~~ function or pollution sources other than waste water system discharge ~~treatment plants~~, criteria that reliably predict when an area that was placed in the closed status because of failure to comply with its conditional management plan can be returned to the open status. The minimum criteria are:
(i) Performance standards of the plan are fully met;
(ii) Sufficient time has elapsed to allow the water quality in the growing area to return to acceptable levels;
(iii) Sufficient time has elapsed to allow the shellstock to reduce pathogens that might be present to acceptable levels. Studies establishing sufficient elapsed time shall document the interval necessary for reduction of coliform levels in the shellstock to pre-closure levels. The study may establish criteria for reopening based on coliform levels in the water; ~~and~~
(iv) For Conditional Management Plans based on waste water system discharge performance and for SSCAs utilizing MSC, sufficient time has elapsed to allow the shellstock to reduce pathogens that might be present to acceptable levels. Studies establishing sufficient elapsed time shall document the interval necessary for reduction of viral levels in the shellstock. Analytical sample results shall not exceed background levels or a level of 50 MSC per 100 grams. The study may establish criteria for reopening based on viral levels in the shellfish meats or the area must be in the closed status until the event is over and twenty-one (21) days have passed; and
(v) Shellstock feeding activity is sufficient to achieve ~~coliform~~ microbial reduction.

	(d) For management plans based on a risk assessment made in accordance with Chapter II. Risk Assessment and Risk Management, criteria that reliably determine when the growing area may be placed in the open status and shellfish may be harvested; (e) For management systems based on marine Biotoxins, the procedures and criteria that reliably determine when the growing area may be placed in the open status; (f) Procedures for immediate notification to the Authority when performance standards or criteria are not met; (g) Provisions for patrol to prevent illegal harvest; and (h) Procedures to immediately place the growing area in the closed status in 24 hours or less when the criteria established in the management plan are not met. (3) Reevaluation of Conditional Classification… (4) Understanding of and Agreement With the Purpose of the Conditional Classification and Conditions of Its Management Plan by All Parties Involved… (5) Conditional Area Types… (6) Conditionally Approved Classification… (7) Conditionally Restricted Classification... D. Restricted Classification… E. Prohibited Classification. (1) Exception… (2) General… (3) Sanitary Survey… (4) Risk Assessment… (5) Wastewater Discharges. (a) An area classified as prohibited shall be established adjacent to each sewage treatment plant outfall or any other point source outfall of public health significance. (b) The determination of the size of the area to be classified as prohibited adjacent to each outfall shall include the following minimum criteria: (i) The volume flow rate, location of discharge, performance of the wastewater treatment plant and the microbiological quality of the effluent; The SSCA may utilize MSC wastewater sample data in the determination of the performance of the sewage treatment plant; (ii) The decay rate of the contaminants of public health significance in the wastewater discharged; (iii) The wastewater's dispersion and dilution, and the time of waste transport to the area where shellstock may be harvested; and (iv) The location of the shellfish resources, classification of adjacent waters and identifiable landmarks or boundaries. NOTE: All references in Section II. Model Ordinance Chapter IV. Shellstock Growing Areas will be changed to Waste Water System Discharge (WWSD).
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 105 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. A better assessment of the risk of viral contamination at a particular location in an adjacent growing area can be ascertained directly using MSC assays of the shellstock. Performing and evaluating dye studies on waste water treatment plant outfall discharges, although effective, 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. There are advantages of using this specialty viral indicator to assess the overall impact of a municipal wastewater treatment system on a particular growing area. The ISSC held an MSC meeting in Charlotte on August 18-19, 2014 to discuss the available MSC science and knowledge. A panel of MSC experts provided MSC information and consensus regarding usage of MSC in the NSSP. (Click here to view, download, or print the MSC meeting report)
Cost Information	The use of MSC is not a requirement; rather, it is an option for States to use, so there would be no cost to States who do not choose to use it. For States that do choose to use MSC, the cost is discussed in the ISSC MSC Meeting Report, August 18-19, 2014, where it states: The MSC assay for shellfish is relatively easy to perform and the cost is roughly equivalent to that of performing fecal coliform testing. The initial cost to prepare laboratory to perform analysis, depends on the lab, and may be approximately $8000 to $10,000, if additional equipment is needed. There may also be cost associated with sample collection.
Action by 2015 Task Force I	Recommends adoption of Proposal 15-102 as amended. @.01 Sanitary Survey. A. General. (1) The sanitary survey is the written evaluation report of all environmental factors, including actual and potential pollution sources, which have a bearing on water quality in a shellfish growing area. The sanitary survey shall include the data and results of: (a) A shoreline survey; (b) A survey of the microbiological quality of the water and in growing areas adjacent to wastewater system discharges the State Shellfish Control Authority may utilize MSC results from analysis of shellfish meat samples and the analysis of the data will be included in the sanitary survey report; (c) An evaluation of the effect of any meteorological, hydrodynamic, and geographic characteristics on the growing area; (d) An analysis of the data from the shoreline survey, the bacteriological and the hydrodynamic, meteorological and geographic evaluations; (e) A determination of the appropriate growing area classification. B. Sanitary Survey Required…

C. Sanitary Survey Performance.

(5) On an annual basis, the sanitary survey shall be updated to reflect changes in the conditions in the growing area. The annual reevaluation shall include:

(a) A field observation of the pollution sources which may include:

(i) A drive-through survey;

(ii) Observations made during sample collection; and

(iii) Information from other sources.

(b) Review, at a minimum, of the past year's water quality sample results by adding the year's sample results to the data base collected in accordance with the requirements for the bacteriological standards and sample collection required in Section .02;

(c) Review of available inspection reports and effluent samples collected from pollution sources;

(d) Review of available performance standards for various types of discharges that impact the growing area;

(e) A brief report which documents the findings of the annual reevaluation; and

(f) The SSCA may use MSC meat sampling data and/or MSC waste water sampling data in the annual reevaluation of (5) (b), (c), and (d) above to evaluate the viral contributions of the performance standards of waste water system discharge (WWSD) impacts on shellfish growing areas.

(g) If MSC meat and/or water data is being used, the SSCA shall conduct annual sample collection and analysis in determining performance standards.

D. Shoreline Survey Requirements…

@.02 Microbiological Standards.

Note: The NSSP allows for a growing area to be classified using either a total or fecal coliform standard. The NSSP further allows the application of either standard to different water bodies within the state. The NSSP also allows for two (2) sample collection strategies for the application of the total or fecal coliform standard: adverse pollution condition and systematic random sampling. The 1992 Task Force II recommended that this portion of the Ordinance be codified in two (2) ways: a total coliform strategy and a fecal coliform strategy so that the state may choose sampling plans on a growing area basis. Within each strategy, provisions would appear for use of both systematic and adverse pollution condition sample collection. The Ordinance has been recodified in this manner. For maximum flexibility, a state may wish to adopt the use of both standards and both sampling strategies for each standard. This codification represents the fecal coliform standards. Additionally, states may choose to use MSC sample data in conjunction with total or fecal coliform data to evaluate areas impacted by waste water system discharges.

A. General. Either the total coliform or fecal coliform standard shall be applied to a growing area. The SSCA may utilize MSC data in conjunction with bacteriological data to evaluate waste water system discharge (WWSD) impacts on shellfish growing areas.

B. Water Sample Stations…

C. Exceptions…

D. Standards for the Approved Classification of Growing Areas in the Remote Status…
E. Standard for the Approved Classification of Growing Areas Affected by Point Sources…
F. Standard for the Approved Classification of Growing Areas Affected by Nonpoint Sources…
G. Standard for the Restricted Classification of Growing Areas Affected by Point Sources and Used as a Shellstock Source for Shellstock Depuration…
H. Standard for the Restricted Classification of Growing Areas Affected by Nonpoint Sources and Used as a Shellstock Source for Shellstock Depuration…

@.03 Growing Area Classification.

A. General…
(1) Emergency Conditions…
(2) Classification of All Growing Areas…
(3) Boundaries…
(4) Revision of Classifications…
(5) Status of Growing Areas…
(a) Open Status…
(b) Closed Status...
(c) Reopened Status. A growing area temporarily placed in the closed status as provided in (b) above, shall be returned to the open status only when:
(i) The emergency situation or condition has returned to normal and sufficient time has elapsed to allow the shellstock to reduce pathogens or poisonous or deleterious substances that may be present in the shellstock to acceptable levels. Studies establishing sufficient elapsed time shall document the interval necessary for reduction of contaminant levels in the shellstock to pre-closure levels. In addressing pathogen concerns, the study may establish criteria for reopening based on coliform levels in the water; or
(ii) For emergency closures of harvest areas caused by the occurrence of raw untreated 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 fifty (50) male-specific coliphage per 100 grams or pre-determined levels established by the Authority bssed on studies conductd on regional species under regional conditions from shellfish samples collected no sooner than seven (7) days after contamination has ceased and from representative locations in each growing area potentially impacted~~;~~ or until the event is over and 21 day have passed; or
(iii) The requirements for Biotoxins or conditional area management plans as established in Section .04 and Section .03, respectively, are met; and
(iv) Supporting information is documented by a written record in the central file.

(d) Inactive Status…
(e) Remote Status…
(f) Seasonally Remote/Approved Status…

B. Approved Classification…

C. Conditional Classifications. Growing areas may be classified as conditional when the following criteria are met:

(1) Survey Required. The sanitary survey meets the following criteria:

(a) The area will be in the open status of the conditional classification for a reasonable period of time. The factors determining this period are known, are predictable, and are not so complex as to preclude a reasonable management approach;

(b) Each potential source of pollution that may adversely affect the growing area is evaluated;

(c) Microbiological water quality correlates with environmental conditions or other factors affecting the distribution of pollutants into the growing area; and

(d) For SSCAs utilizing MSC meat sample data, this data correlates with environmental conditions or other factors affecting the distribution and persistence of viral contaminants into the growing area.

(2) Management Plan Required. For each growing area, a written management plan shall be developed and shall include:

(a) For management plans based on wastewater treatment plant function, performance standards that include:

(i) Peak effluent flow, average flow, and infiltration flow;
(ii) Microbiological quality of the effluent;
(iii) Physical and chemical quality of the effluent;
(iv) Conditions which cause plant failure;
(v) Plant or collection system bypasses;
(vi) Design, construction, and maintenance to minimize mechanical failure, or overloading;
(vii) Provisions for monitoring and inspecting the waste water treatment plant; and
(viii) Establishment of an area in the prohibited classification adjacent to a wastewater treatment plant outfall in accordance with Section E. Prohibited Classification;

(b) For management plans based on pollution sources other than waste water treatment plants:

(i) Performance standards that reliably predict when criteria for conditional classification are met; and
(ii) Discussion and data supporting the performance standards.

(c) For management plans based on waste water system discharge function or pollution sources other than waste water system discharge, criteria that reliably predict when an area that was placed in the closed status because of failure to comply with its conditional management plan can be returned to the open status. The minimum criteria are:

(i) Performance standards of the plan are fully met;
(ii) Sufficient time has elapsed to allow the water

quality in the growing area to return to acceptable levels;

(iii) Sufficient time has elapsed to allow the shellstock to reduce pathogens that might be present to acceptable levels. Studies establishing sufficient elapsed time shall document the interval necessary for reduction of coliform levels in the shellstock to pre-closure levels. The study may establish criteria for reopening based on coliform levels in the water;

(iv) For Conditional Management Plans based on waste water system discharge performance and for SSCAs utilizing MSC, sufficient time has elapsed to allow the shellstock to reduce pathogens that might be present to acceptable levels. Studies establishing sufficient elapsed time shall document the interval necessary for reduction of viral levels in the shellstock. Analytical sample results shall not exceed ~~background levels or~~ a level of 50 MSC per 100 grams or pre-determined levels established by the Authority based on studies conducted on regional species under regional conditions. These studies~~y~~ may establish criteria for reopening based on viral levels in the shellfish meats or the area must be in the closed status until the event is over and twenty-one (21) days have passed; and

(v) Shellstock feeding activity is sufficient to achieve microbial reduction.

(d) For management plans based on a risk assessment made in accordance with Chapter II. Risk Assessment and Risk Management, criteria that reliably determine when the growing area may be placed in the open status and shellfish may be harvested;

(e) For management systems based on marine Biotoxins, the procedures and criteria that reliably determine when the growing area may be placed in the open status;

(f) Procedures for immediate notification to the Authority when performance standards or criteria are not met;

(g) Provisions for patrol to prevent illegal harvest; and

(h) Procedures to immediately place the growing area in the closed status in 24 hours or less when the criteria established in the management plan are not met.

(3) Reevaluation of Conditional Classification…

(4) Understanding of and Agreement With the Purpose of the Conditional Classification and Conditions of Its Management Plan by All Parties Involved…

(5) Conditional Area Types…

(6) Conditionally Approved Classification…

(7) Conditionally Restricted Classification...

D. Restricted Classification…

E. Prohibited Classification.

(1) Exception…

(2) General…

(3) Sanitary Survey…

	(4) Risk Assessment… (5) Wastewater Discharges. (a) An area classified as prohibited shall be established adjacent to each sewage treatment plant outfall or any other point source outfall of public health significance. (b) The determination of the size of the area to be classified as prohibited adjacent to each outfall shall include the following minimum criteria: (i) The volume flow rate, location of discharge, performance of the wastewater treatment plant and the microbiological quality of the effluent; The SSCA may utilize MSC wastewater sample data in the determination of the performance of the sewage treatment plant; (ii) The decay rate of the contaminants of public health significance in the wastewater discharged; (iii) The wastewater's dispersion and dilution, and the time of waste transport to the area where shellstock may be harvested; and (iv) The location of the shellfish resources, classification of adjacent waters and identifiable landmarks or boundaries. NOTE: All references in Section II. Model Ordinance Chapter IV. Shellstock Growing Areas will be changed toWaste Water System Discharge (WWSD).

Proposal Subject	Ineffective Model Ordinance Requirements
Specific NSSP Guide Reference	Section II. Model Ordinance Chapter IV. Shellstock Growing Areas
Text of Proposal/ Requested Action	@.01 Sanitary Survey. A. General. (1) The sanitary survey is the written evaluation report of all environmental factors, including actual and potential pollution sources, which have a bearing on water quality in a shellfish growing area. The sanitary survey shall include the data and results of: (a) A shoreline survey; (b) A survey of the bacteriological quality of the water; (c) An evaluation of the effect of any meteorological, hydrodynamic, and geographic characteristics on the growing area; and ~~(d) An analysis of the data from the shoreline survey, the bacteriological and the hydrodynamic, meteorological and geographic evaluations; and~~ (~~e~~d) A determination of the appropriate growing area classification. (2) The sanitary survey shall be periodically updated through the triennial reevaluation and the annual review in accordance with Section C. to assure that data is current and that conditions are unchanged. (3) The documentation supporting each sanitary survey shall be maintained by the Authority. For each growing area, the central file shall include all data, results, and analyses from: (a) The sanitary survey; (b) The triennial reevaluation; and (c) The annual review. (4) Wherever possible, the Authority shall provide the necessary information to Federal, State, or local agencies which have the responsibility to minimize or eliminate pollution sources identified in the sanitary survey. (5) The Authority shall maintain a current comprehensive, itemized list of all growing areas, including maps showing the boundaries and classification of each shellstock growing area.
Public Health Significance	This section is redundant and confusing. It does not add anything. Whatever would be included here should be addressed by analyses conducted during efforts to meet the Chapter IV. @.01 A. (1) (a) requirement for shoreline survey to be conducted according to the instructions provided in Chapter IV. @.01 D., Chapter IV. A. (1) (c) requirement for evaluating the effects of various factors impacting the area, and the Chapter IV. @.01 A. (1) (d) requirement for determining the appropriate growing area classification.
Cost Information	
Action by 2015 Task Force I	Recommends adoption of Proposal 15-103 as submitted.

Proposal Subject	Sanitary Survey Report Format
Specific NSSP Guide Reference	Section II. Model Ordinance Chapter IV. Shellstock Growing Areas @01. Sanitary Survey and Section IV. Guidance Documents Chapter II. Growing Areas .04 Sanitary Survey and the Classification of Growing Waters.
Text of Proposal/ Requested Action	Model Ordinance Chapter IV. Shellstock Growing Areas @.01 Sanitary Survey (C) Sanitary Survey Performance (1) A sanitary survey of each growing area shall be performed at least once every twelve (12) years and shall include the components in Section A. (1.) in the following outline: A. Executive Summary B. Description of Growing Area (1) Location map or chart showing growing area (2) Description of area and its boundaries (3) History of growing area classification (i) Date of last sanitary survey (ii) Previous classification(s) map(s) C. Pollution Source Survey (1) Summary of Sources and Location (i) Information gathered under the shoreline survey requirements outlined in (D). (ii) Map or chart showing the location of major sources of actual or potential pollution in the survey area including a table of sources of pollution cross-referenced to the survey area map. (2) Detailed description, identification, evaluation, and determination of impact of all actual and potential pollution sources identified during the shoreline survey on water quality throughout the growing area. D. Hydrographic and Meteorological Characteristics (1) Tides (type and amplitude), and currents (velocity and direction) (2) Rainfall and/or snowmelt (i) Amount (ii) When (e.g. time of year) (iii) Frequency of significant rainfalls (iv) Winds (Seasonality and effects on pollution dispersion) (3) River discharges (volume and seasonality) (4) Discussion concerning effects of pollution distribution and hydrographic factors (dilution, dispersion, and time of travel) on water quality throughout the growing area (i) Salinity, depth, and stratification characteristics (ii) Computer model verification if used for classification. E. Water Quality Studies (1) Map of sampling stations (2) Sampling plan and justification (i) Adverse condition sampling; and/or (ii) Random sampling (3) Sample Data Analysis and Presentation: Tables containing the basic NSSP statistics (number of samples, median or

geometric mean, and the respective variability factors)
(i) Station by station monitoring data array collected under the adverse condition or systematic random sampling monitoring strategy
(ii) Daily sampling results and number of samples collected for survey
(iii) Overall compliance with NSSP criteria
(iv) Sorting of data by environmental pollution, seasonal, and/or meteorological condition
(v) Classification assigned to each station

F. Interpretation of Data in Determining Classification to Be Assigned to Growing Area: A discussion of how actual or potential pollution sources, wind, tide, rainfall, etc. affect or may affect water quality, that will address the following:
(1) Effects of meteorological and hydrographic conditions on bacterial loading
(2) Variability in the bacteriological data and causes

G. Conclusions
(1) Map or chart showing classification assigned to growing area(s) (closure lines, boundary lines separating various classifications)
(2) Legal description of growing area boundaries
(3) Management plan for growing area if in the conditionally approved or conditionally restricted classification meeting the requirements in (C.)
(4) Recommendations for sanitary survey improvement
(i) Changes in monitoring schedules, addition of sampling stations or station relocation, etc.

H. Comments

Guidance Documents Chapter II. Growing Areas
.04 Sanitary Survey and the Classification of Growing Waters

~~**Minimum Requirements of the Sanitary Survey Report**~~

~~The following outline contains the minimum requirements for the written growing area sanitary survey report required in the NSSP Model Ordinance.~~

~~A. Executive Summary~~
~~B. Description of Growing Area~~
~~(1) Location map or chart showing growing area~~
~~(2) Description of area and its boundaries~~
~~(3) History of growing area classification~~
~~* Date of last sanitary survey~~
~~* Previous classification(s) map(s) C. Pollution Source Survey~~
~~(1) Summary of Sources and Location~~
~~* Information gathered under the shoreline survey procedures outlined above.~~
~~* Map or chart showing the location of major sources of actual or potential pollution in the survey area.~~
~~* Table of sources of pollution cross-referenced to the survey area map.~~
~~(2) Identification and evaluation of pollution sources~~
~~* Domestic wastes (discussion and maps)~~

	~~* Storm water~~ ~~* Agricultural waste (farms, feedlots, & slaughterhouse operations)~~ ~~* Wildlife areas~~ ~~* Industrial wastes~~ ~~D. Hydrographic and Meteorological Characteristics~~ ~~(1) Tides (type and amplitude), and currents (velocity and direction)~~ ~~(2) Rainfall~~ ~~* Amount~~ ~~* When (e.g. time of year)~~ ~~* Frequency of significant rainfalls~~ ~~* Winds (Seasonality and effects on pollution dispersion)~~ ~~(3) River discharges (volume and seasonality)~~ ~~(4) Discussion concerning effects of pollution distribution and hydrographic factors (dilution, dispersion, and time of travel) on water quality throughout the growing area~~ ~~* Salinity, depth, and stratification characteristics~~ ~~* Computer model verification if used for classification.~~ ~~E. Water Quality Studies~~ ~~(1) Map of sampling stations~~ ~~(2) Sampling plan and justification~~ ~~* Adverse condition sampling~~ ~~* Random sampling~~ ~~(3) Sample Data Analysis and Presentation: Tables containing the basic NSSP statistics (number of samples, median or geometric mean, and the respective variability factors)~~ ~~* Station by station monitoring data array collected under the adverse condition or systematic random sampling monitoring strategy~~ ~~* Daily sampling results and number of samples collected for survey~~ ~~* Overall compliance with NSSP criteria~~ ~~* Sorting of data by environmental pollution condition~~ ~~* Classification assigned to each station~~ ~~F. Interpretation of Data in Determining Classification to Be Assigned to Growing Area: A discussion of how actual or potential pollution sources, wind, tide, rainfall, etc. affect or may affect water quality, that will address the following:~~ ~~(1) Effects of meteorological and hydrographic conditions on bacterial loading~~ ~~(2) Variability in the bacteriological data and causes~~ ~~G. Conclusions~~ ~~(1) Map or chart showing classification assigned to growing area(s) (closure lines, boundary lines separating various classifications)~~ ~~(2) Legal description of growing area boundaries~~ ~~(3) Management plan for growing area if in the conditionally approved or conditionally restricted classification~~ ~~(4) Recommendations for sanitary survey improvement~~ ~~* Changes in monitoring schedules, addition of sampling stations or station relocation, etc.~~ ~~* Comments~~
Public Health Significance	The Model Ordinance Guidance Documents contain the outline of the minimum requirements for the written sanitary survey report based on the requirements of the Model Ordinance. The guidance represents the ISSC's (state, federal, and industry) current thinking on the requirements for a sanitary survey, other reports, and the classification of growing areas. An alternative approach may be used if such approach

	satisfies the requirements of the applicable statute, regulations, and the Guide for the Control of Molluscan Shellfish. The requirement should not be in Guidance, but in the compliance language portion of the Model Ordinance. The primary responsibility of the State Shellfish Control Authority is to ensure the public health safety of the shellfish growing areas through compliance with the NSSP Model Ordinance. The Authority must perform a sanitary survey that collects and evaluates information concerning actual and potential pollution sources that may adversely affect the water quality in each growing area. Based on the sanitary survey information, the authority determines what use can be made of the shellstock from the growing area and assigns the growing area classification. Experience has shown that the minimum sanitary survey components required in this guidance are necessary for a reliable sanitary survey and since the State Shellfish Control Authorities are evaluated for conformance with the minimum requirements, the language should be moved to the satisfactory compliance section.
Cost Information	N/A
Action by 2015 Task Force I	Recommends no action on Proposal 15-104. Rationale: This is already adequately addressed in the Guidance Documents.

Proposal Subject	Opening Growing Areas Closed to Biotoxins
Specific NSSP Guide Reference	Section II. Model Ordinance Chapter IV. Shellstock Growing Areas
Text of Proposal/ Requested Action	@.04 Marine Biotoxin Control C. Closed Status of Growing Areas (4) The closed status shall remain in effect until the Authority has data to show that the toxin content of the shellfish in the growing area is below the level established for closing the area. A minimum of two (2) consecutive shellfish samples must be collected at least three (3) days apart and the toxin levels must be below the regulatory limit(s) to reopen an area. At the discretion of the Authority, an additional sample may be required before the area is reopened if the toxin levels are just below the regulatory limit.
Public Health Significance	There is growing evidence that toxic algal blooms have been increasing over the last 20 years and not only are becoming more frequent, but more intense, occurring in new places and with longer durations. See, e.g., R.M. Kudela et al. 2015. Harmful Algal Blooms: A Scientific Summary for Policy Makers IOC/UNESCO, Paris (IOC/INF-1320). Because Biotoxins from algae bioaccumulate in shellfish, human and animal consumers of shellfish are at risk from Biotoxin poisoning. Human illnesses caused by consumption of contaminated shellfish include paralytic shellfish poisoning ("PSP"), diarrheic shellfish poisoning and amnesic shellfish poisoning. These illnesses manifest in human victims via symptoms including gastrointestinal disorders and neurologic and muscular problem, including paralysis of the chest and abdominal muscles possibly leading to death (PSP). See Raymond RaLonde (1996), Paralytic Shellfish Poisoning: The Alaska Problem, Alaska's Marine Resources Vol. 8, No. 2. There are no antidotes available to counteract Biotoxin poisoning and victims need immediate medical support. The only reliable means of protecting against the harvest and consumption of Biotoxin-contaminated shellfish is frequent sampling of harvest areas followed by qualified laboratory analysis and quick regulatory action. The presence of Biotoxins in shellfish at harmful or fatal levels cannot be detected by simple observation; affected shellfish do not differ in odor or appearance from shellfish that are safe to consume. Thus in States such as Alaska, where subsistence and recreational harvest of shellfish from unregulated beaches is common; there is a high incidence of PSP illness and even death. Between 1993 and 2014, there were 117 reported cases of PSP poisoning in Alaska, with fatalities occurring in three of those years (1994, 1997 and 2010). Further, because Biotoxin sampling results can vary significantly between lethal and safe levels in just a matter of days, it is unsafe to base a re-opening decision on a single sampling event. For example, geoduck clams sampled in Alaska's Steamboat harvest area on March 9, 2014 returned a paralytic shellfish toxin ("PST") level of 206 ug/100 grams while geoduck sampled from the same area on March 16, 2014 returned a PST level of 57 um/100 grams. With the March 16 sample showing levels below the 80 ug/100 gram closure threshold, Alaska opened the Steamboat area to harvest on March 20, 2014. Just three days later, on March 23, 2014, sampling showed PST levels back to above the closure threshold, at 118 ug/100 grams. The Steamboat area then vacillated between open and closed status weekly until May 10, then remained open until the May 31 PST sample yielded a concentration of 528 ug/100 grams. However, the Steamboat area reopened on June 7 when the results of one sample were returned at 46 ug/100 grams.

	The high volatility of Biotoxin concentrations in shellfish sampled in the same harvest areas can be seen in the attached spreadsheet, which summarizes results of shellfish harvest area PST testing performed by the Alaskan Department of Environmental Conservation ("ADEC") in 2014. Requiring two below-regulatory level Biotoxin tests before re-opening of shellfish harvesting areas will increase confidence that Biotoxin(s) are cleared from the harvest area and that the shellfish are once again safe for human consumption. While this likely will not have a significant impact on growing areas that have fairly consistent PST levels, this will require additional testing in states that reopen areas based on a single test result in growing areas with high degrees of PST variability. Requiring two below-regulatory limit shellfish samples prior to re-opening an area closed due to Biotoxins will also increase international confidence in the safety of U.S. shellfish, avoiding future potential international bans and sanctions. For example, the proposed PSP testing standards could have avoided certain concerns raised by the Chinese government in 2013. The Middle Gravina Island growing area in Alaska was implicated in China's 2013 ban of U.S. geoduck. ADEC identifies Middle Gravina Island as an area that consistently exceeds PSP thresholds; in fact, sampling of this area in 2014 showed an average PST level of 312 ug/100 grams. However, commercial geoduck shellfish harvest for human consumption and export occurred in this harvest area in 2013 based on a sub-80 ug/100 gram sample on October 5. The previous week's sample had returned a PST level of 388 ug/100 grams, and the subsequent two samples were 385 ug/100 gram and 528 ug/100 gram, respectively. See ADEC 2013/2014 PSP Lab Results (June 10, 2014). In fact, the only PST sample below regulatory threshold for Middle Gravina Island between September 28 and December 8, 2013 was the October 5 sample. In summary, increasing the number of tests required before harvest re-opens following a Biotoxin event will reduce public health risks associated with the shellfish industry, boost international confidence in the safety of shellfish products, and minimize the potential that single anomalous readings could authorize the harvest of potentially unhealthy and dangerous shellfish product. The purpose of the proposal is to set a uniform minimum threshold for State Authority PSP testing. It appears that most State Authorities already meet or exceed the standards proposed herein. In those circumstances, the proposal would not change or alter such regulations.
Cost Information	Although costs will vary by Shellfish Authority, the costs are believed to be minimal. Most ISSC member states and provinces currently use the suggested reopening criteria or one that is already more stringent to manage Biotoxin events. Any costs associated with additional testing would be mitigated by reducing the likelihood of extensive, expensive and time-consuming recalls, international sanctions, and/or the potential repercussions in consumer confidence after illnesses occur.
Action by 2015 Task Force I	Recommends referral of Proposal 15-105 to the appropriate committee as determined by the Conference Chairman.

Proposal Subject	Using Male-Specific Coliphage as a Tool to Determine Viral Quality during Shellstock Relaying
Specific NSSP Guide Reference	Section II. Model Ordinance Chapter V. Shellstock Relaying
Text of Proposal/ Requested Action	@.01 General. The Authority shall assure that: A. The shellstock used in relaying activities is harvested from growing areas classified as conditionally approved, restricted, or conditionally restricted; B. The level of contamination in the shellstock can be reduced to levels safe for human consumption; C. The contaminated shellstock are held in growing areas classified as approved or conditionally approved for a sufficient time under adequate environmental conditions so as to allow reduction of pathogens as measured by ~~the coliform group of indicator organisms in the water~~total coliform, fecal coliform.~~;~~ ~~If~~For shellstock harvested from areas impacted by wastewater system discharges, MSC may be used as a measure for viral reduction, or poisonous or deleterious substances that may be present in shellstock to occur.~~; and~~ D. If shellstock are relayed in containers: (1) The containers are: (a) Designed and constructed so that they allow free flow of water to the shellstock; and (b) Located so as to assure the contaminant reduction required in Section C.; and (2) The shellstock are washed and culled prior to placement in the containers. @.02 Contaminant Reduction. A. The Authority shall establish species-specific critical values for water temperature, salinity, and other environmental factors which may affect the natural treatment process in the growing area to which shellstock will be relayed. The growing area to be used for the treatment process shall be monitored with sufficient frequency to identify when limiting critical values may be approached. B. The effectiveness of species-specific contaminant reduction shall be determined based on a study. The study report shall demonstrate that, after the completion of the relay activity: (1) The ~~bacteriological~~ microbiological quality of each shellfish species is the same ~~bacteriological~~ microbiological quality as that of the same species already present in the approved or conditionally approved area; or (2) Contaminant levels of poisonous or deleterious substances in shellstock do not exceed FDA tolerance levels. (3) When the source growing area is impacted by wastewater system discharge, the viral quality of each shellfish species meets the male-specific coliphage standard od 50 PFU/100gm. C. The authority may waive the requirements for a contaminant reduction study if: (1) Only microbial contaminants need to be reduced; and (2) The shellstock are relayed from a conditionally approved, restricted, or conditionally restricted area meeting the bacteriological water

	quality for restricted areas used for shellstock depuration per Chapter IV. @.02 G. and Chapter IV. @.02 H.; and (3) The treatment period exceeds sixty (60) days. D. The time period shall be at least fourteen (14) consecutive days when environmental conditions are suitable for shellfish feeding and cleansing unless shorter time periods are demonstrated to be adequate. E. When container relaying is used and the Authority allows a treatment time of less than fourteen (14) days, the Authority shall require more intensive sampling including: (1) Product sampling before and after relay; and (2) Monitoring of critical environmental parameters such as temperature and salinity~~.~~; and/or (3) Male-specific coliphage monitoring before and after relay for shellstock relay from areas impacted by wastewater system discharge. F. The Authority shall establish the time period during the year when relaying may be conducted.
Public Health Significance	The ISSC held a MSC meeting in Charlotte on August 18-19, 2014, and discussed the available MSC science and knowledge. A panel of MSC experts provided MSC information and consensus regarding the use of MSC in the NSSP. (Click here to view, download, or print the MSC meeting report) Male-specific Coliphage (MSC) is a RNA virus of E. coli present in high numbers in raw sewage (on the order of 105 PFU/100gm). MSC is a good surrogate or marker for norovirus and hepatitis A viruses, which are the viral pathogens of concern in sewage. The ISSC Growing Area Classification Committee acknowledged that MSC should be considered by the ISSC as an indicator for contaminant reduction studies for relaying.
Cost Information	The use of MSC is not a requirement; rather, it is an option for States to use, so there would be no cost to States who do not choose to use it. For States that do choose to use MSC, the cost is discussed in the ISSC MSC Meeting Report, August 18-19, 2014, where it states: The MSC assay for shellfish is relatively easy to perform and the cost is roughly equivalent to that of performing fecal coliform testing. The initial cost to prepare laboratory to perform analysis, depends on the lab, and may be approximately $8000 to $10,000, if additional equipment is needed. There may also be cost associated with sample collection.
Action by 2015 Task Force I	Recommends adoption of Proposal 15-106 as amended: @.01 General. The Authority shall assure that: A. The shellstock used in relaying activities is harvested from growing areas classified as conditionally approved, restricted, or conditionally restricted; B. The level of contamination in the shellstock can be reduced to levels safe for human consumption; C. The contaminated shellstock are held in growing areas classified as approved or conditionally approved for a sufficient time under adequate environmental conditions so as to allow reduction of pathogens as measured by total coliform, fecal coliform. For shellstock harvested from areas impacted by wastewater system discharges, MSC may be used as a measure for viral reduction, or poisonous or deleterious substances that may be present in shellstock to occur. D. If shellstock are relayed in containers:

(1) The containers are:
(a) Designed and constructed so that they allow free flow of water to the shellstock; and
(b) Located so as to assure the contaminant reduction required in Section C.; and
(2) The shellstock are washed and culled prior to placement in the containers.

@.02 Contaminant Reduction.

A. The Authority shall establish species-specific critical values for water temperature, salinity, and other environmental factors which may affect the natural treatment process in the growing area to which shellstock will be relayed. The growing area to be used for the treatment process shall be monitored with sufficient frequency to identify when limiting critical values may be approached.
B. The effectiveness of species-specific contaminant reduction shall be determined based on a study. The study report shall demonstrate that, after the completion of the relay activity:
(1) The microbiological quality of each shellfish species is the same microbiological quality as that of the same species already present in the approved or conditionally approved area; or
(2) Contaminant levels of poisonous or deleterious substances in shellstock do not exceed FDA tolerance levels;or~~.~~
(3) When the source growing area is impacted by wastewater system discharge, the viral quality of each shellfish species meets the male-specific coliphage standard od 50 PFU/100gmor pre-determined levels established by the Authority based on studies conducted on regional species under regional conditions.
C. The authority may waive the requirements for a contaminant reduction study if:
(1) Only microbial contaminants need to be reduced; and
(2) The shellstock are relayed from a conditionally approved, restricted, or conditionally restricted area meeting the bacteriological water quality for restricted areas used for shellstock depuration per Chapter IV. @.02 G. and Chapter IV. @.02 H.; and
(3) The treatment period exceeds sixty (60) days.
D. The time period shall be at least fourteen (14) consecutive days when environmental conditions are suitable for shellfish feeding and cleansing unless shorter time periods are demonstrated to be adequate.
E. When container relaying is used and the Authority allows a treatment time of less than fourteen (14) days, the Authority shall require more intensive sampling including:
(1) Product sampling before and after relay; and
(2) Monitoring of critical environmental parameters such as temperature and salinity; and/~~or~~
(3) For SSCA using Male-specific coliphage monitoring before and after relay for shellstock relay from areas impacted by wastewater system discharge.

F. The Authority shall establish the time period during the year when relaying may be conducted.

Proposal Subject	Ineffective Model Ordinance Requirement
Specific NSSP Guide Reference	Section II. Model Ordinance Chapter VI. Shellfish Aquaculture
Text of Proposal/ Requested Action	@ .02 Seed Shellstock. ~~A. The Authority shall establish the submarket size for each species of shellfish in accordance with Section .01 B. and Section .01 C.~~ ~~B.~~ All sources of seed shall be sanctioned by the Authority. .01 Exceptions. ~~The following~~ Hatchery activities are exempt~~ed~~ from these requirements.~~:~~ ~~A. Hatcheries;~~ ~~B. Nursery products which do not exceed ten (10) percent of the market weight; and~~ ~~C. Nursery products which are six (6) months or more growing time from market size.~~ .03 Seed Shellstock. Seed may come from any growing area, or from any growing area in any classification, provided that: A. The source of the seed is sanctioned by the Authority~~.~~; and ~~B. Seed from growing areas or growing areas in the restricted or prohibited classification have acceptable levels of poisonous or deleterious substances; and~~ ~~C~~B. Seed from growing areas or growing areas in the prohibited classification are cultured for a minimum of six (6) months. .05 Land Based Aquaculture. A. Operational Plan. Each land based aquaculture facility shall have a written operational plan. The plan shall be approved by the Authority prior to its implementation and shall include: (1) A description of the design and activities of the culture facility; (2) The specific site and boundaries in which shellfish culture activities will be conducted; (3) The types and locations of any structures, including rafts, pens, cages, nets, tanks, ponds, or floats which will be placed in the waters; (4) The species of shellfish to be cultured and harvested; (5) If appropriate, the source and species of other organisms to be cultured in any polyculture systems; (6) Procedures to assure that no poisonous or deleterious substances are introduced into the activities; (7) A program of sanitation, maintenance, and supervision to prevent contamination of the final shellfish products; (8) A description of the water source, including the details of any water treatment process or method, if necessary; (9) A program to maintain water quality, which includes collection of microbial water samples and their method of analysis and routine temperature and salinity monitoring. The bacterial indicator monitored shall be the same as used for monitoring growing areas; ~~(10) Collection of information on the microbial and chemical quality of shellfish harvested from the aquaculture site;~~ (1~~1~~0) Collection of data concerning the quality of food production (algae or other) used in the artificial harvest system; (1~~2~~1) Maintenance of the required records; and (1~~3~~2) How shellstock will be harvested, processed if applicable, and sold.

Public Health Significance	Chapter VI. @.02 A.: This requirement to establish the submarket size of shellfish does not make sense with regard to its linked requirement to establish submarket size in accordance with 01.B and 01.C which provide exemptions for nursery products. As written, this is an unclear requirement and has no purpose in this Chapter. Chapter VI. .01 B. and C.: It is impossible to get this information and to verify for each facility this is very ineffective. Chapter VI. .03 B.: No acceptable level of poison. Chapter VI. .05 A. (10): Requirement already addressed by other requirements. The contaminant level of the shellfish has already been controlled in accordance with requirements that seed shellfish not be contaminated with poisonous and deleterious substances and the that requirement for aquaculture sites to be controlled for poisonous and deleterious substances and the requirement that the aquaculture site water quality be maintained.
Cost Information	
Action by 2015 Task Force I	Recommends adoption of Proposal 15-107 as amended: @ .02 Seed Shellstock. A. The Authority shall establish the submarket size for each species of shellfish. ~~A.~~B. All sources of seed shall be sanctioned by the Authority. .01 Exceptions. Hatchery activities are exempt from these requirements. .03 Seed Shellstock. Seed may come from any growing area, or from any growing area in any classification, provided that: A. The source of the seed is sanctioned by the Authority; and B. Seed from growing areas or growing areas in the prohibited classification are cultured for a minimum of six (6) months. .05 Land Based Aquaculture. A. Operational Plan. Each land based aquaculture facility shall have a written operational plan. The plan shall be approved by the Authority prior to its implementation and shall include: (1) A description of the design and activities of the culture facility; (2) The specific site and boundaries in which shellfish culture activities will be conducted; (3) The types and locations of any structures, including rafts, pens, cages, nets, tanks, ponds, or floats which will be placed in the waters; (4) The species of shellfish to be cultured and harvested; (5) If appropriate, the source and species of other organisms to be cultured in any polyculture systems; (6) Procedures to assure that no poisonous or deleterious substances are introduced into the activities; (7) A program of sanitation, maintenance, and supervision to prevent contamination of the final shellfish products; (8) A description of the water source, including the details of any water treatment process or method, if necessary;

	(9) A program to maintain water quality, which includes collection of microbial water samples and their method of analysis and routine temperature and salinity monitoring. The bacterial indicator monitored shall be the same as used for monitoring growing areas; (10) Collection of data concerning the quality of food production (algae or other) used in the artificial harvest system; (11) Maintenance of the required records; and (12) How shellstock will be harvested, processed if applicable, and sold.

Proposal Subject	PCOX Method Status
Specific NSSP Guide Reference	Section IV. Guidance Documents Chapter II. Growing Areas .11 Approved Laboratory Tests
Text of Proposal/ Requested Action	This request is for a change in the status of the PCOX method for determining paralytic shellfish poisoning (PSP) toxins from "Approved Limited Use" to "Approved". This change would be reflected by: 1. Adding the PCOX method to NSSP Section IV Guidance Documents Chapter II. Growing Areas .11 Approved NSSP Laboratory Tests, Table 2. Approved Methods for Marine Biotoxin Testing with Biotoxin Type: Paralytic Shellfish Poisoning (PSP), Application: Growing Area Survey & Classification, Sample Type: Shellfish, and Application: Controlled Relaying Sample Type: Shellfish; and 2. Deleting the PCOX method from NSSP Section IV Guidance Documents Chapter II. Growing Areas .11 Approved NSSP Laboratory Tests, Table 4. Approved Limited Use Methods for Marine Biotoxin Testing. The PCOX method for paralytic shellfish poisoning toxins (PSTs) was developed by the Canadian Food Inspection Agency (CFIA) and National Research Council Canada (NRCC) using post-column oxidation and fluorescence detection (PCOX). This method was optimized, tested, and used extensively in the authors' laboratory before the formal validation process was initiated to ensure that it could perform in the "real-life" setting of a regulatory monitoring laboratory. The method performed well, and was subjected to a single-laboratory validation (SLV) study [1]. The data generated in the SLV study was used to support proposal 09-104 to the Interstate Shellfish Sanitation Conference (ISSC) to approve the PCOX method for official use; the result of this proposal was that the method was approved as a Type IV method. The PCOX method was implemented for screening PST levels in shellfish at the Canadian Food Inspection Agency Dartmouth Laboratory in November, 2009, following ISSC approval; all samples were analysed using the PCOX method, and results leading to regulatory action were confirmed by mouse bioassay (MBA), AOAC OMA 959.08[2]. The method was next subjected to an international collaborative inter-laboratory study [3]. This collaborative study was successful, and the results were used to support the approval of the PCOX method as an AOAC official method of analysis (OMA), First Action status – OMA 2011.02 [4]. All MBA analyses for PSTs were eliminated in CFIA laboratories when the PCOX method was granted OMA, First Action status in April, 2011, and the PCOX method was considered a quantitative, regulatory method, without the need for MBA confirmation of results. The PCOX method was promoted to AOAC OMA, Final Action status in 2013 in response to positive method performance feedback from users. The PCOX method has been used to analyse almost 50,000 shellfish samples since it was implemented in Canada in November, 2009, with the Canadian Food Inspection Agency (CFIA) Dartmouth Laboratory completing almost 19,000 of those tests. This large dataset from CFIA laboratories provides an opportunity to verify performance characteristics with routine use over an extended period of time. A summary of QC performance at the CFIA Dartmouth Laboratory is shown in Table 1 below. These data demonstrate excellent precision (CV of <10% for average total PSTs) and accuracy (102 ± 17% for total PSTs) in method performance examined over a span of five and a half years, including multiple instruments, multiple analysts, and numerous batches of reagents. Additional data from other CFIA laboratories reveal similar results for >1500 additional QC points. The performance characteristics of the method were also evaluated and confirmed as part of a ring

study on PSTs in oyster tissue organized by a laboratory in the United Kingdom [5]. Accuracy has also been evaluated through successful participation in CFIA and international proficiency testing programs by all three CFIA laboratories using the PCOX method. These performance characteristics exceed those specified by Codex [6] for quantitative chemical methods; recovery guidelines at these concentration are 80-110% with ≤44% RSD and repeatability guidelines for these concentration are <15% RSD.

Table 1: CFIA Dartmouth Laboratory summary of QC performance from November, 2009 – June, 2015

		GTX1	GTX3	STX	TOTAL PST
In-house reference material 1	n	520			
	Average	24[b]	29[b]	139[b]	264[b]
	Standard Deviation	3.3	2.3	11.9	17.0
	% RSD	13%	8%	9%	6%
In-house reference material 2	n	504			
	Average	45[b]	50[b]	62[b]	244[b]
	Standard Deviation	3.4	2.3	6.2	12.8
	% RSD	8%	5%	10%	5%
SPIKE RECOVERY (%)	n	1024			
	Average	100%[a]	100%	98%	102%
	Standard Deviation	38%[a]	10%	15%	17%
	Concentration Range [b,c]	3-11[a]	7-10	28-61	57-92[d]

[a] higher variability is observed because spiking levels are below the method LOD
[b] µg STXdiHCl eq/100g
[c] multiple spiking solutions were used over time; range reflects minimum and maximum spiking levels
[d] including only individual toxins that were above the method LOD

The method is also being used outside of Canada. The Norwegian School of Veterinary Science (NSVS) completed a validation study before implementing the PCOX method for all samples in January, 2013. Again, the performance of the method in the Norwegian laboratory was consistent with results from the collaborative study. It is also worth noting that all CFIA laboratories and the NSVS are accredited to ISO 17025 and maintain the PCOX method on their scope of accreditation. Within the United States, Maine has completed validation studies and been approved to use the PCOX method for regulatory samples since April, 2014, and Alaska has completed validation studies [7] and is currently awaiting final FDA approval to implement the method for regulatory testing (but currently uses the method for non-regulatory samples). Oregon has recently expressed interest in the method as well. Chilean laboratories at the University of Chile plan to validate the PCOX method and transition from MBA to the PCOX method in the near future. The method is also being used for non-routine or research purposes in New Zealand (Cawthron Laboratory), the United Kingdom (CEFAS laboratory), Ireland (Marine Institute), Chile (University of Chile), the United States (e.g., Alaska Environmental Health Laboratory, US FDA), and Canada (NRCC).

Training has been requested and delivered to groups in the United States (2010) and Europe (2012), and scientists from the Maine Department of Marine Resources and

Bigelow Laboratory for Ocean Sciences were hosted for training at the CFIA Dartmouth Laboratory (2012). There was also interest in a training course organized by the China Section of AOAC International, but logistical difficulties have prevented the course from taking place thus far.

Feedback from participants in the collaborative study was very positive, and most laboratories experienced no problems with the method; however, like all methods, there are limitations and weaknesses. One weakness of the method is that it cannot resolve neosaxitoxin (NEO) from decarbamoylneosaxitoxin (dcNEO), or gonyautoxin-6 (GTX6) from gonyautoxin-4 (GTX4). The inability to resolve these toxins is an issue for samples contaminated by *Gymnodinium catenatum*, in which dcNEO and GTX6 are often present. This challenge is being examined, and the European Union Reference Laboratory for Marine Biotoxins has expressed interest in collaborating to overcome it. Another weakness of the method is the LC column, which suffers from a short lifespan. An alternative column has been proposed, but research continues to find a more suitable replacement. A weakness of all chemical PST methods is the unavailability of analytical standards for some toxins (such as GTX6, and C3/C4). The unavailable toxins are uncommon in North American toxin profiles (these toxins are common in samples contaminated by *Gymnodinium catenatum*) and have very low toxicity. These challenges are included here to provide a complete description of the method, and also to highlight that these issues are not serious enough to prevent implementation of the method. Research will continue to improve the robustness and flexibility of the method to make it easier to implement in different laboratories.

The PCOX method is more sensitive than the MBA, and can be used to provide earlier warning of rising PST levels in shellfish. This earlier warning capacity can be used to focus additional sampling and increase the probability of detecting toxin levels before they exceed the regulatory limit [8], resulting in increased food safety, and fewer product recalls for industry.

The ISSC terminology describing method status has been updated since the PCOX method was approved in 2009, and the PCOX method status is currently "Approved Limited Use"; however, there are currently no clear statements of what "limited use" means for this method. The method has been successfully implemented for regulatory samples in multiple accredited laboratories for several years, and performance data from these laboratories agree with those generated during the original inter-laboratory study. The status of this method should be changed to "Approved" to reflect the fact that this method is no longer in limited use, and no critical limitations to the method have been identified. This change would also be consistent with the changes resulting from adoption of Proposal 13-309, which recognizes AOAC OMA status when considering proposed methods that are demonstrated as fit-for-purpose.

1. Van de Riet, J.M., et al., *Liquid Chromatographic Post-Column Oxidation Method for Analysis of Paralytic Shellfish Toxins in Mussels, Clams, Scallops,and Oysters: Single-Laboratory Validation.* Journal of AOAC International, 2009. **92**(6): p. 1690-1704.
2. INTERNATIONAL, A., *Method **959.08***, in *Official Methods of Analysis, 19th Ed.* 2012, AOAC INTERNATIONAL: Gaithersburg, MD.
3. Van de Riet, J., et al., *Liquid Chromatography Post-Column Oxidation (PCOX) Method for the Determination of Paralytic Shellfish Toxins in Mussels, Clams, Oysters, and Scallops Collaborative Study.* Journal of AOAC International, 2011. **94**(4): p. 1154-1176.

	4. INTERNATIONAL, A., *Method **2011.02***, in *Official Methods of Analysis, 19th Ed.* 2012, AOAC INTERNATIONAL: Gaithersburg, MD. 5. Turner, A.D., et al., *Interlaboratory Comparison of Two AOAC Liquid Chromatographic Fluorescence Detection Methods for Paralytic Shellfish Toxin Analysis through Characterization of an Oyster Reference Material.* Journal of AOAC International, 2014. **97**(2): p. 380-390. 6. Commission, C.A., *Procedural Manual, 23rd edition.* 2015. 7. Hignutt, J.E., *Suitability of Postcolumn Oxidation Liquid Chromatography Method AOAC 2011.02 for Monitoring Paralytic Shellfish Toxins in Alaskan Shellfish—Initial Pilot Study versus Mouse Bioassay and In-House Validation.* Journal of AOAC International, 2014. **97**(2): p. 293-298. 8. Rourke, W.A. and C.J. Murphy, *Animal-Free Paralytic Shellfish Toxin Testing—The Canadian Perspective to Improved Health Protection.* Journal of AOAC International, 2014. **97**(2): p. 334-338.
Public Health Significance	The detection limit for PSTs by the MBA method is 40 µg STX diHCl eq/100g, while that of the sum of individual PSTs are significantly lower using the PCOX method - <10 µg STX diHCl eq/100g. This lower detection limit improves food safety and minimizes closures in southwestern New Brunswick, Canada, where PST levels in the Bay of Fundy are chronically high and can change very rapidly. Since the PCOX method has been implemented, the local CFIA office has determined that harvest sites with PST levels >35 µg STX diHCl eq/100g should be sampled a second time in the same week instead of waiting to sample the site the following week; by contrast, those same samples would show no toxin by the MBA method and sampling would be delayed until the regularly scheduled sample the following week. This delay potentially leaves harvest areas with increasing PST levels open over the weekend and beginning of the following week; this could lead to illnesses, food safety investigations, and product recalls that are now prevented because of the lower detection limits of the PCOX method. This information has been used to maintain harvest areas in an open status longer – an advantage for the shellfish harvesting industry - and simultaneously close the harvest areas before toxin levels exceed the regulatory limits. This change in sampling frequency has resulted in fewer food safety investigations and product recalls and was not possible before the PCOX method was implemented because the MBA method does not have enough sensitivity to detect low levels of PSTs.
Cost Information	There should be no direct cost implications to this change. It may make the transition from the MBA to the PCOX method slightly easier for laboratories not currently using the latter, or for those gearing up for PST testing for the first time. The PCOX method is less expensive than the MBA if capital purchases (LC systems) are averaged over the life of the equipment.
Action by 2015 Laboratory Method Review Committee	Recommended adoption of Proposal 15-108 as submitted.
Action by 2015 Task Force I	Recommends adoption of 2015 Laboratory Method Review Committee recommendation on Proposal 15-108.

Proposal Subject	PSP HPLC-PCOX Species Expansion
Specific NSSP Guide Reference	Section IV. Guidance Documents Chapter II Growing Areas .11 Approved NSSP Laboratory Tests
Text of Proposal/ Requested Action	4. Approved Limited Use Methods for Marine Biotoxin Testing PCOX This submission presents data to support the use of PCOX method for Quahogs (M. mercenaria and A. icelandica), Surf Clams (S. solidissima), Geoducks (P. generosa), Butter Clams (S. giganteus), Little Neck Clams (P. stamineais), and Razor Clams (S. patula) for regulatory paralytic shellfish toxin (PST) testing. Results of the 2009 Interstate Shellfish Sanitation Conference (ISSC) proposal 09-104 concluded the PCOX method approved for official use as a Type IV method; subsequently after single laboratory validation (SLV) and collaborative studies, ISSC proposal 13-309 accepted PCOX method as an AOAC official method of analysis (OMA) in 2013. Currently PCOX is an "Approved for Limited Use" method for mussel, clam, oyster and scallop. SLV work will be presented for quahogs, surf clams, geoducks, butter clams, little neck clams, and razor clams that demonstrates comparable performance characteristics for these species as with mussels, clams, oysters, and scallops using the PCOX method. The cost and challenges associated with maintaining both the MBA and PCOX methods for these species are high; differing laboratory skill sets are required and state laboratories have limited budgets and staff resources. Additionally, the recent shortage of the NIST saxitoxin standard used for MBA proficiencies is of concern if laboratories are expected to maintain MBA for verification purposes for these species. The requested action is being made and data presented for the purpose of inclusion of quahogs, surf clams, geoducks, butter clams, little neck clams, and razor clams as approved species (by addition to the footnote that includes mussels, clams, oysters, and scallops or as the ISSC deems appropriate) within the NSSP Guide Section IV Guidance Documents Chapter II. Growing Areas .11 Laboratory Tests Methods Table, Methods for Marine Biotoxin Testing with Biotoxin Type: Paralytic Shellfish Poisoning (PSP), Application: Growing Area Survey & Classification Sample Type: Shellfish, And Application: Controlled Relaying Sample Type: Shellfish.
Public Health Significance	The PCOX method was developed to provide a rapid, high throughput chemical assay that would eliminate the need to sacrifice animals, AOAC mouse bioassay (MBA), for toxin detection. There is a worldwide move to replace assays that use live animals as test subjects. Laboratories currently using PCOX for regulatory PST testing have found that the lower detection limits of the PCOX method allow for better early warning therefore better management of PST closures and significantly improved public health decision-making. The addition of the proposed species will allow regulatory laboratories to move away from the costliness of maintaining MBA and eliminate the need to sacrifice animals as well as improve management of species specific closure decision–making.
Cost Information	Total consumable costs for the analysis is estimated at $10/sample. A chemistry laboratory will usually be equipped with an LC system and a post column reactor to carry out the analysis. Total capital costs for the instrumentation required for the analysis is approximately $120,000. Although the upfront investment for instrumentation is high, the removal of care, maintenance, and cost of mice quickly offsets this expenditure.
Action by 2015 Laboratory Method Review Committee	Recommended that Proposal 15-109 be referred to an appropriate committee as determined by the Conference Chair for evaluation of data and until additional data are received.
Action by 2015 Task Force I	Recommends adoption of 2015 Laboratory Method Review Committee recommendation on Proposal 15-109.

<table>
<tr><td>Proposal Subject</td><td>Laboratory Method for Vibrio parahaemolyticus (V.p.)
Enumeration and Detection through MPN and Real-Time PCR</td></tr>
<tr><td>Specific NSSP Guide Reference</td><td>Section IV. Guidance Documents
Chapter II. Growing Areas
.11 Approved NSSP Laboratory Tests</td></tr>
<tr><td>Requested Action</td><td>This method was developed by William A. Glover (Washington State Public Health Laboratories) and is being submitted by the ISSC Executive Board. The Executive Board granted interim approval to this method on March 13, 2015. The Executive Board is submitting this proposal to comply with Article V. Section 1. of the ISSC Constitution, Bylaws, and Procedures.</td></tr>
<tr><td>Text of Proposal</td><td>Submitted by method developer William A. Glover (Washington State Public Health Laboratories)

5. Approved Methods for Vibrio Enumeration
<table>
<tr><th></th><th>Vibrio Indicator Type:</th><th>Application:
PHP
Sample Type:
Shucked</th></tr>
<tr><td>EIA[1]</td><td>Vibrio vulnificus (V.v.)</td><td>X</td></tr>
<tr><td>MPN[2]</td><td>Vibrio vulnificus (V.v.)</td><td>X</td></tr>
<tr><td>SYBR Green 1 QPCR-MPN[5]</td><td>Vibrio vulnificus (V.v.)</td><td>X</td></tr>
<tr><td>MPN[3]</td><td>Vibrio parahaemolyticus (V.p.)</td><td>X</td></tr>
<tr><td>PCR[4]</td><td>Vibrio parahaemolyticus (V.p.)</td><td>X</td></tr>
<tr><td>MPN and PCR[6]</td><td>Vibrio parahaemolyticus (V.p.)</td><td>X</td></tr>
</table>
Footnotes:
[1] EIA procedure of Tamplin, et al, as described in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, 1992.
[2] MPN method in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, followed by confirmation using biochemical analyses or by the DNA -alkaline phosphatase labeled gene probe (vvhA).
[3] MPN format with confirmation by biochemical analysis, gene probe methodology as listed in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, or a method that a State can demonstrate is equivalent.
[4] PCR methods as they are listed in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, or a method that a State can demonstrate is equivalent.
[5]Vibrio vulnificus, ISSC Summary of Actions 2009. Proposal 09-113, Page 123.
[6]William A. Glover, II, Ph.D. D9ABMM), MT(ASCP) Food and Shellfish Bacteriology Laboratory (FSBL) at the Washington State Public Health Laboratories (WAPHL)</td></tr>
<tr><td>Public Health Significance</td><td>The purpose of this method is to provide laboratories supporting the NSSP the ability to rapidly quantify Vibrio parahaemolyticus (V.p.) from oysters using a high throughput real-time PCR protocol.

The Food and Shellfish Bacteriology Laboratory (FSBL) at the Washington State Public Health Laboratories (WAPHL) tests on average over 200 oyster samples per year for Vibrio parahaemolyticus (V.p.)Culture based assays for the enumeration of V.p. take four days or longer and require the Kanagawa test (media based) to detect pathogenicity. Due to the large number of samples and need for accurate and timely results, the FSBL at the WAPHL has tested Pacific oysters (Crassostrea gigas) for (V.p.) using a MPN based real-time PCR assay for over 10 years. The real-time PCR assay utilized by the FSBL at the WAPHL has gone through redesigns and improvements by various scientists at the WAPHL based on new published literature, clinical V.p. case data, experiences in WA State over the course of</td></tr>
</table>

	a season or seasons, and requests from the Office of Shellfish & Water Protection for enhanced detection of pathogenic *V.p.* strains and additional surveillance capabilities. The real-time PCR assay redesigned and implemented in 2009 and utilized through the 2013 Vp monitoring season (June – September) was designed to detect *V.p.* using the species-specific thermolabile hemolysin gene (tlh) and virulent *V.p.* using the thermostable direct hemolysin gene (tdh). This assay was designed for high throughput in a 384-well based format. Additionally, the tlh and tdh targets were redesigned yielding amplicons between 50-150 base pairs. This is optimal for real-time PCR and is known to produce consistent results1. Validation of the assay and concept of a "molecular MPN" was conducted using FERN guidelines and was compared to the FDA BAM method. This assay served as the backbone for which further improvements and redesigns were made in 2013.
Cost Information	
Action by 2015 Laboratory Methods Review Committee	Recommended that Proposal 15-110 be referred to an appropriate committee as determined by the Conference Chair to await completed SLV data.
Action by 2015 Task Force I	Recommends adoption of 2015 Laboratory Methods Review Committee recommendation on Proposal 15-110.

<table>
<tr><td>Proposal Subject</td><td>MPN-Real-Time PCR for Pathogenic V.p.</td></tr>
<tr><td>Specific NSSP Guide Reference</td><td>Section IV. Guidance ,
Chapter II. Growing Areas
.11 Approved NSSP Laboratory Tests</td></tr>
<tr><td>Requested Action</td><td>This method was developed by Jessica Jones (FDA Gulf Coast Seafood Laboratory) and is being submitted by the ISSC Executive Board. The Executive Board granted interim approval to this method on March 13, 2015. The Executive Board is submitting this proposal to comply with Article V. Section 1. of the ISSC Constitution, Bylaws, and Procedures.</td></tr>
<tr><td>Text of Proposal</td><td>Submitted by method developer Jessica Jones (FDA Gulf Coast Seafood Laboratory)

5. Approved Methods for Vibrio Enumeration

<table>
<tr><th></th><th>Vibrio Indicator Type:</th><th>Application:
PHP Sample Type:
Shucked</th><th>Application:
Reopening</th></tr>
<tr><td>EIA[1]</td><td>Vibrio vulnificus (V.v.)</td><td>X</td><td></td></tr>
<tr><td>MPN[2]</td><td>Vibrio vulnificus (V.v.)</td><td>X</td><td></td></tr>
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<tr><td>MPN[3]</td><td>Vibrio parahaemolyticus (V.p.)</td><td>X</td><td></td></tr>
<tr><td>PCR[4]</td><td>Vibrio parahaemolyticus (V.p.)</td><td>X</td><td></td></tr>
<tr><td>MPN-Real Time PCR[6]</td><td>tdh+ and trh+ Vibrio parahaemolyticus (V.p.)</td><td>X</td><td>X</td></tr>
</table>
Footnotes:

[1] EIA procedure of Tamplin, et al, as described in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, 1992.
[2] MPN method in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, followed by confirmation using biochemical analyses or by the DNA -alkaline phosphatase labeled gene probe (vvhA).
[3] MPN format with confirmation by biochemical analysis, gene probe methodology as listed in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, or a method that a State can demonstrate is equivalent.
[4] PCR methods as they are listed in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, or a method that a State can demonstrate is equivalent.
[5]Vibrio vulnificus, ISSC Summary of Actions 2009. Proposal 09-113, Page 123.
[6]MPN-real time PCR method for the tdh and trh genes for total V. parahaemolyticus as described in Kinsey et al., 2015.</td></tr>
<tr><td>Public Health Significance</td><td>The current NSSP method for enumeration of tdh+ Vp requires a minimum of four days from receipt of sample to results reporting. Currently, there is no NSSP-approved method for enumeration of trh+ Vp. At the 2013 conference, proposal 13-202 was adopted which requires testing for the presence of tdh and trh prior to reopening of growing areas closed as a result of Vp illnesses [Chapter II @.01.F(5)]. This proposed MPN-real-time PCR method provides results in as little as 24h from receipt of sample. Availability of this more rapid method will facilitate reopening decision making.</td></tr>
<tr><td>Cost Information</td><td>This method costs ~$120 per sample for laboratory consumables, supplies, and reagents. Most equipment needed for testing is standard microbiology equipment, but purchase of a heat block (~$400) and/or centrifuge (~$2,500) may be necessary. Purchase of a real-time PCR instrument will be required ($30,000-$45,000). Additional costs for a laboratory would vary based on their operational overhead and labor.</td></tr>
<tr><td>Action by 2015 Laboratory Method</td><td>Recommended that Proposal 15-111 be adopted and direct the Executive Office to request the submitter revise the SOP so that the BAM MPN calculator be used for determination</td></tr>
</table>

Review Committee	of MPN values.
Action by 2015 Task Force I	Recommends adoption of 2015 Laboratory Methods Review Committee recommendation on Proposal 15-111.

<table>
<tr><td>Proposal Subject</td><td>Direct Plating Method for trh</td></tr>
<tr><td>Specific NSSP Guide Reference</td><td>Section IV. Guidance Documents
Chapter II. Growing Areas
.11 Approved NSSP Laboratory Tests</td></tr>
<tr><td>Requested Action</td><td>This method was developed by Jessica Jones (FDA Gulf Coast Seafood Laboratory) and is being submitted by the ISSC Executive Board. The Executive Board granted interim approval to this method on March 13, 2015. The Executive Board is submitting this proposal to comply with Article V. Section 1. of the ISSC Constitution, Bylaws, and Procedures.</td></tr>
<tr><td>Text of Proposal</td><td>Submitted by method developer Jessica Jones (FDA Gulf Coast Seafood Laboratory)

5. Approved Methods for Vibrio Enumeration

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<tr><td>PCR[4]</td><td>Vibrio parahaemolyticus (V.p.)</td><td>X</td><td></td></tr>
<tr><td>Direct Plating[6]</td><td>trh+ Vibrio parahaemolyticus (V.p.)</td><td>X</td><td>X</td></tr>
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Footnotes:

[1] EIA procedure of Tamplin, et al, as described in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, 1992.
[2] MPN method in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, followed by confirmation using biochemical analyses or by the DNA -alkaline phosphatase labeled gene probe (vvhA).
[3] MPN format with confirmation by biochemical analysis, gene probe methodology as listed in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, or a method that a State can demonstrate is equivalent.
[4] PCR methods as they are listed in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, or a method that a State can demonstrate is equivalent.
[5]Vibrio vulnificus, ISSC Summary of Actions 2009. Proposal 09-113, Page 123.
[6]Direct plating method for trh as described in Nordstrom et al., 2006.</td></tr>
<tr><td>Public Health Significance</td><td>Scientific evidence suggests that the presence of the trh gene in V. parahaemolyticus (Vp) is correlated with higher virulence. Additionally, at the 2013 conference, proposal 13-202 was adopted which requires testing for the presence of trh prior to reopening of growing areas closed as a result of Vp illnesses [Chapter II @.01.F(5)]. Currently, there are no NSSP approved methods for enumeration of trh. This method is a needed option for testing following Vp illness closures.</td></tr>
<tr><td>Cost Information</td><td>This method costs ~$5 per test for laboratory consumables, supplies, and reagents. Most equipment needed for testing is standard microbiology equipment, but purchase of a specialized water bath or environmental chamber may be necessary at a cost of ~$3,000-$5,000. Additional costs for a laboratory would vary based on their operational overhead and labor.</td></tr>
<tr><td>Action by 2015 Laboratory Methods Review</td><td>Recommended that Proposal 15-112 be referred to an appropriate committee as determined by the Conference Chair to further review the data submitted.</td></tr>
</table>

Committee	
Action by 2015 Task Force I	Recommends adoption of 2015 Laboratory Methods Review Committee recommendation on Proposal 15-112.

<table>
<tr><td>Proposal Subject</td><td>MPN-Real-Time PCR for Total Vp</td></tr>
<tr><td>Specific NSSP Guide Reference</td><td>Section IV. Guidance Documents
Chapter II. Growing Areas
.11 Approved NSSP Laboratory Tests</td></tr>
<tr><td>Requested Action</td><td>This method was developed by Jessica Jones (FDA Gulf Coast Seafood Laboratory) and is being submitted by the ISSC Executive Board. The Executive Board granted interim approval to this method on March 13, 2015. The Executive Board is submitting this proposal to comply with Article V. Section 1. of the ISSC Constitution, Bylaws, and Procedures.</td></tr>
<tr><td>Text of Proposal</td><td>Submitted by method developer Jessica Jones (FDA Gulf Coast Seafood Laboratory)

5. Approved Methods for Vibrio Enumeration

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<tr><td>MPN-Real Time PCR[6]</td><td>Vibrio parahaemolyticus (V.p.)</td><td>X</td><td>X</td></tr>
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Footnotes:

[1] EIA procedure of Tamplin, et al, as described in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, 1992.
[2] MPN method in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, followed by confirmation using biochemical analyses or by the DNA -alkaline phosphatase labeled gene probe (vvhA).
[3] MPN format with confirmation by biochemical analysis, gene probe methodology as listed in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, or a method that a State can demonstrate is equivalent.
[4] PCR methods as they are listed in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, or a method that a State can demonstrate is equivalent.
[5]Vibrio vulnificus, ISSC Summary of Actions 2009. Proposal 09-113, Page 123.

[6]MPN-real time PCR method for the tlh gene for total V. parahaemolyticus as described in Kinsey et al., 2015.</td></tr>
<tr><td>Public Health Significance</td><td>The current NSSP method for enumeration of Vp requires a minimum of four days from receipt of sample to results reporting. The MPN-real-time PCR method provides results in as little as 24h from receipt of sample. At the 2013 conference, proposal 13-202 was adopted which requires testing prior to reopening of growing areas closed as a result of Vp illnesses [Chapter II @.01.F(5)]. Availability of this more rapid method will facilitate reopening decision making.</td></tr>
<tr><td>Cost Information</td><td>This method costs ~$100 per sample for laboratory consumables, supplies, and reagents. Most equipment needed for testing is standard microbiology equipment, but purchase of a heat block (~$400) and/or centrifuge (~$2,500) may be necessary. Purchase of a real-time PCR instrument will be required ($30,000-$45,000). Additional costs for a laboratory would vary based on their operational overhead and labor.</td></tr>
</table>

Action by 2015 Laboratory Methods Review Committee	Recommended adoption of Proposal 15-113 as submitted and direct the Executive Office to request the submitter revise the SOP so that the BAM MPN calculator be used for determination of MPN values.
Action by 2015 Task Force I	Recommends adoption of 2015 Laboratory Methods Review Committee recommendation on Proposal 15-113.

Proposal Subject	Pre-Proposal for Male-Specific Coliphage Enumeration in Wastewater by Direct Double-Agar Overlay Method
Specific NSSP Guide Reference	Section IV. Guidance Documents Chapter II. Growing Areas .11 Approved NSSP Laboratory Tests
Requested Action	The submitter of the pre-proposal requests approval to submit a full proposal to the ISSC for approval of the analytical method for use in the NSSP.
Text of Proposal	Submitted by the developer Kevin Calci (FDA Gulf Coast Seafood Laboratory) Proposed Use of the Method: This method is applicable for the enumeration of MSC wastewater influent, effluent and sewage contaminated surface waters. The method will directly determine the quantity of MSC in wastewater to provide information of the viral reduction efficiencies of wastewater treatment plants. Method is also applicable for the analysis of surface source waters as part of a shoreline survey. Description of Method: This method employs E. coli HS (pFamp) RR as a male-specific coliphage host in a direct double agar overlay for the quantification of plaque forming units. All sample volumes are plated in triplicate. Briefly, 2.5ml of sample is mixed with 2.5ml of soft agar and 0.2ml of Famp host and then poured onto bottom agar petri plate. One ml of the sample is serially diluted down to 1:10 and 1:100. Those two dilutions are then plated by placing 2.5ml of sample is mixed with 2.5ml of soft agar and 0.2ml of Famp host and then poured onto bottom agar petri plate. The plates are incubated at 35-37°C for 16-20 h. Under indirect light the plaque forming units are counted. The working range of the 9 plate method would be 14pfu/1OOml to 1.0 x 106 pfu/1 OOml.
Public Health Significance	Scientific consensus at the MSC informational meeting supported the use of MSC to evaluated wastewater treatment plant viral reduction efficiency to better inform the SSCA's conditional management plans impacted by wastewater treatment plant operations. This method would identify a consistent and accurate measure of MSC load in wastewater influent, effluent and surface waters.
Cost Information	
Action by 2015 Laboratory Methods Review Committee	Recommended that Proposal 15-114 be referred to an appropriate committee as determined by the Conference Chair to await SLV data.
Action by 2015 Task Force I	Recommends adoption of 2015 Laboratory Methods Review Committee recommendation on Proposal 15-114.