# Interstate Shellfish Sanitation Conference

# *Addendum*

## to

## Proposals for Consideration

## at the

## 2013 Biennial Meeting
## January 25 – January 31, 2014

***The St. Anthony Riverwalk Hotel***
***"a national historic landmark"***

THE ST. ANTHONY
San Antonio

ISSC

**2013 Proposal Inventory**
**Substitute Proposals, Additional Information and Late Proposals**



Key:
I = Additional Information
L = Late Proposal
S = Substitute Proposal

NSSP Guide for the Control of Molluscan Shellfish

Section I. Model Ordinance

Chapter II. Risk Assessment and Risk Management

@.01 Outbreaks of Shellfish Related Illness

***Insert New Section:***

F. When the investigation outlined in Section @.01 A. indicates the illness(es) are associated with the naturally occurring pathogen *Vibrio parahaemolyticus (V.p.)* , the Authority shall determine the number of cases epidemiologically associated with implicated area and actions taken by the Authority will be based on the number of cases and the span of time as follows.

(1) When sporadic cases do not exceed a risk of one (1) illness per 100,000 servings or involves at least two (2) but not more than four (4) cases occurring within a thirty (30) day period from a hydrologically connected water body in which no two (2) cases occurred from a single harvest day, the Authority shall:

(a) Determine the extent of the hydrologically connected water body, and
(b) Issue a consumer advisory for all shellfish (or species implicated in the illness) from the implicated area; and
(c) Notify receiving States, the ISSC and the FDA Regional Shellfish Specialist that a potential health risk is associated with shellfish harvested from the implicated growing area, and

(2) When the risk exceeds one (1) illness per 100,000 servings within a thirty (30) day period or when cases exceed four (4) but not more than ten (10) over a thirty (30) day period from a hydrologically connected water body and when two (2) or more cases but less than four (4) cases occur from a single harvest day, the Authority shall:

(a) Determine the extent of the hydrologically connected water body; and

(b) Issue a consumer advisory for all shellfish (or species implicated in the illness) from the implicated growing area; and
(c) Immediately place the implicated portion(s) of the harvest area(s) in the closed status; and
(d) Notify receiving States, the ISSC, and the FDA Regional Shellfish Specialist that a potential health risk is associated with shellfish harvested from the implicated growing area; and
(e) As soon as determined by the Authority, transmit to the FDA and receiving States information identifying the dealers shipping the implicated shellfish.

(3) When the number of cases exceeds ten (10) illnesses within a thirty (30) day period from a hydrologically connected growing area or four (4) cases occurred from a single harvest date, The Authority shall:

(a) Determine the extent of the hydrologically connected water body; and
(b) Immediately place the implicated portion(s) of the harvest area(s) in the closed status; and
(c) Promptly initiate a voluntary industry recall consistent with the Recall Enforcement Policy, Title 21 CFR Part 7. The recall shall include all implicated products.

(4) When a growing area has been closed as a result of *V.p.* cases, the Authority shall keep the area closed for the following periods of time to determine if additional illnesses have occurred:

(a) The area will remain closed for a minimum of seven (7) days when sporadic cases do not exceed a risk of one (1) illness per 100,000 servings or involves four (4) or less cases occurring within a thirty (30) day period from a hydrologically connected water body in which no two (2) cases occurred from a single harvest date.

(b) The area will remain closed for a minimum of fourteen (14) days when the risk exceeds one (1) illness per 100,000 servings within a thirty (30) day period or cases exceed four (4) but not more than ten (10) cases over a thirty (30) day period from a hydrologically connected water body with two (2) or more cases but less than four (4) cases occurring from a single harvest date.
(c) The area will remain closed for a minimum of twenty-one (21) days when the number of cases exceeds ten (10) illnesses within thirty (30) days or four (4) cases occur from a single harvest date from a hydrologically connected growing area,

(5) Prior to reopening an area closed as a result of *V.p.* cases, the Authority shall:

(a) Collect and analyze samples to ensure that tdh does not exceed 10/g and trh does not exceed 10/g; or
(b) Ensure that environmental conditions have returned to levels not associated with *V.p.* cases.

(6) Shellfish harvesting may occur in an area closed as a result of *V.p.* illnesses when the Authority implements one or more of the following controls:

(a) Post harvest processing using a process that has been validated to achieve a two (2) log reduction in the levels of total *Vibrio parahaemolyticus* for

Gulf and Atlantic Coast oysters and a three (3) log reduction for Pacific Coast oysters;

(b) Restricting oyster harvest to product that is labeled for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing;

(c) Limiting the time to one (1) hour from harvest to an internal temperature of 50°.

(d) Other control measures that based on appropriate scientific studies are designed to ensure that the risk of *V.p.* illness is no longer reasonably likely to occur, as approved by the Authority.

| **Proposal for Task Force Consideration at the Interstate Shellfish Sanitation Conference 2013 Biennial Meeting** | ☒ **Growing Area** ☐ **Harvesting/Handling/Distribution** ☐ **Administrative** |
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| **Submitter:** | Food and Drug Administration |
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| **Proposal Subject:** | Dilution Guidance for Prohibited Zones Associated with Wastewater Discharges |
| **Specific NSSP Guide Reference:** | NSSP Guide Section IV. Guidance Documents<br>Chapter II. Growing Areas |
| **Text of Proposal/ Requested Action** | .16 Determining Appropriately Sized Prohibited Areas Associated with Wastewater Treatment Plants<br><br>Introduction<br><br>Molluscan shellfish are filter feeders and therefore have the ability to concentrate microorganisms from the water column, including human pathogens and toxigenic micro-algae if these organisms are present. Concentrations of microorganisms in the shellfish may be as much as 100 times greater than those found in the water, and if the microorganisms are harmful to humans, illness can result. The correlation between sewage pollution of shellfish waters and illness has been demonstrated many times. Certain shellfish-borne infectious diseases are transmitted via the fecal-oral route, with the cycle beginning with the fecal contamination of the shellfish growing waters.<br><br>In the winter of 1924-25, an oyster-borne typhoid outbreak occurred in the United States which caused a large number of illnesses and deaths (Lumsden, et al 1925). In response to this outbreak the National Shellfish Sanitation Program (NSSP) was initiated by the States, the U.S. Public Health Service, and the shellfish industry. Research at the time indicated that typhoid fever would not ordinarily be attributed to shellfish harvested from water in which not more than 50% percent of the one cc (ml) portions of water examined were positive for fecal coliform bacteria (an MPN of approximately 70 per 100 ml), provided that the areas were not subject to direct contamination with small amounts of fresh sewage which would not likely be revealed by routine bacteriological examination. As a result water quality criteria were established, namely;<br><br>(1) The area be sufficiently removed from major sources of pollution so that the shellfish are not subjected to fecal contamination in quantities which might be dangerous to public health;<br><br>(2) The area be free from pollution by even small quantities of fresh sewage; |

(3) Bacteriological examination does not ordinarily show the presence of the coli-aerogenes group of bacteria in one cc dilution of the growing area water.

Once these standards were adopted in the United States in 1925, reliance on these criteria for evaluating the safety of shellfish harvesting areas has generally proven effective in preventing major outbreaks of disease transmitted by the fecal-oral route. Today, fecal and total coliforms are used as an index of the sanitary quality of a growing area and to foretell the possible presence of fecal transmitted bacterial pathogens. The goal of the NSSP remains the same – to ensure the safety of shellfish for human consumption by preventing harvest from contaminated 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).

(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 & Burkhardt. 2010; Iwamoto et al 2010; Scallan et al. 2011; Batz et al. 2012). NoV genogroups I, II and IV and HAV are human specific 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.
The purpose of this guidance is to provide the scientific basis and recommendations for determining appropriately sized Prohibited Areas (closure zones) based on the minimum criteria established under Section II, Chapter IV. @.03 E(5) of the Model Ordinance (Section E Prohibited Classification).

Classification Requirements for Growing Areas Associated with Waste Water Treatment Plants

The NSSP Model Ordinance (MO) requires that a comprehensive sanitary survey be undertaken prior to the classification of the growing area as Approved, Conditionally Approved, Restricted, or Conditionally Restricted.

The sanitary survey must take careful recognition of any WWTPs as they represent one of the major sources of human sewage pollution. It is preferable that the shellfish growing areas be sited so far away from sewage discharges that the WWTP effluent

has no hazardous effect, because there is a direct relationship between the level of WWTP effluent dilution and the level of enteric viruses detected in the shellfish (Goblick et al. 2011).

Delineation of the Prohibited Zone around a Wastewater Treatment Plant

The NSSP MO Section II, Chapter IV. @.03 (2) (b) 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 shall 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 bacteriological or viral 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 the prohibited zone:

(1) The distance 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.

If the plant is operating outside of the normal parameters it shall be considered to be malfunctioning.

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

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

The following guidelines shall be used when assessing these factors in the dilution analysis for the closure zone:

1) Volume flow rate: For a minimally sized prohibited zone for Conditionally Approved areas managed in part based on the performance of the WWTP, the maximum monthly average flow at the WWTP recorded in the Monthly Operating Reports (MORs) maintained by the WWTP permitting authority should be used considering at a minimum the most recent two years of flow records. If the maximum monthly average flow at the WWTP from two consecutive years of flow records is within 85 – 100% of the design flow, then the design flow should

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| | be used. Thus, these flow values are appropriate when establishing a minimally sized prohibited zone when the WWTP is considered to be operating under normal operating conditions.<br><br>Additional information and historical data may be accessed on the U.S. Environmental Protection Agency (EPA) website at: http://cfpub.epa.gov/dmr/index.cfm. Consistent with the EPA regulations in 40 CFR 122.2, the maximum monthly average flow, which is typically reported in the MOR, is defined as the average ‘‘daily discharges’’ over a calendar month, calculated as the sum of all ‘‘daily discharges’’ measured during a calendar month divided by the number of ‘‘daily discharges’’ measured during that month typically expressed in units of million gallons per day (MGD). Thus, the maximum monthly average flow is defined as the highest average monthly flow (MGD) within at a minimum the most recent consecutive two years of flow records. The design flow is defined as the flow (MGD) that the WWTP is designed to discharge and can be expressed as a daily, monthly, or annual discharge. In the design of WWTPs, various flow regimes are considered such as the average flow, maximum flow and peak (instantaneous) flow. However, it is important to note that certain tolerances are allowed under EPA NPDES program and WWTPs are not necessarily expected to meet permit conditions over all flow regimes. Thus, if permit limits are expressed as a monthly average it is considered acceptable for the permitted pollutants to exceed the permit on a short term basis as long as the permit condition (monthly average) is met. It is also important to note that EPA does not have any permit limitations established for the discharge of viruses.<br><br>In the context of public health, some of these flow regimes such as when average hourly flows exceed the design flow can be associated with periods of effluent degradation leading to an increase in the viral load in the effluent. Utilizing average hourly flows and comparing against the design flow ensures that the periods when effluent degradation are most likely to occur are adequately identified and assessed. Average hourly flow rates within the most recent two years of records should be evaluated to assess the likelihood that the average hourly flows can exceed the design flow. In the absence of supporting data, the conditional area should be closed when the average hourly flow rates exceed the WWTP design flow due to the potential degradation of the virological quality of treatment. FDA studies have determined that when WWTP average hourly flow rates exceed design flow the virological quality of effluent typically degrades beyond what is considered as normal treatment. Moreover, FDA bioaccumulation studies indicate that shellfish can accumulate significant levels of viral pathogens when exposed in durations of less than one hour. However, a flow level threshold above the design flow could be determined on a case by case basis provided the virological quality of the effluent is assessed. The average hourly flow is defined as the average flow measured over an hour. More detailed flow records are typically maintained and can be accessed through the permitted WWTP. |

When conditional management based on WWTP performance is not employed the prohibited zone shall be sufficient in size to dilute the microbial loadings resulting from a WWTP malfunction (such as a sewage bypass or a loss of disinfection) to ensure the Approved area adjacent to the prohibited zone will meet the bacteriological standards for Approved area classification under all conditions including a WWTP malfunction. If the WWTP has no prior history of sewage bypasses then at a minimum a loss of disinfection malfunction shall be considered when sizing the prohibited zone. As many WWTP malfunctions occur from hydraulic overloading as a result of rainfall, snowmelt, storm events or periods of high flow, a maximum average hourly rate shall be considered when determining the size of the prohibited zone. The maximum average hourly flow is defined as the highest average hourly flow recorded within at a minimum) the most recent two consecutive years of flow records.

Location of discharge: The location of the discharge must be determined in order to define the distance from the point of effluent discharge to shellfish growing areas that could be impacted. The distance from shore and the depth of the WWTP outfall also can be used in the dilution analysis of the discharge. The location of discharge includes the location, number, size and orientation of the discharge port(s) on the outfall or its diffuser.

When determining if a WWTP within the watershed or catchment area draining to a shellfish estuary potentially impacts a shellfish growing area, in the absence of a database collected, the NSSP recommends that a worst case raw sewage discharge be assumed. In this circumstance a level of $1.4 \times 10^6$ FC/100ml assumed for a raw sewage release-requires a 100,000:1 dilution 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. A lower dilution level 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 may 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).

It should also be noted that if shellfish harvesting occurs within the zone of influence from a WWTP then these areas are subject to a WWTP Management Plan as defined in Section II Chapter IV @. 03 C.(2)(a) of the MO. Additionally, if a departure of the normal WWTP function could potentially impact a shellfish growing area then the areas affected should be managed under a conditional management plan as defined in Section II Chapter IV @. 03 C.(2)(a) of the MO.

The minimum size of a prohibited zone for a conditional area under a WWTP management plan should be determined considering both the minimum dilution

(1000:1) needed to mitigate the presence of viruses in treated effluent (or a scientifically based alternative approach) as well as the prerequisite notification time to close the conditional area during a WWTP malfunction or period of degraded effluent quality, prior to the conditional area receiving the impact from the WWTP effluent.

Performance of the WWTP: When considering the present and past performance of the WWTP, this review should include information regarding the wastewater collection system, inspection of essential plant components (including any monitoring and alarm systems), events whereby the plant exceeds its design capacity and an evaluation of the disinfection system. The plants past performance should also include a file review of the plant's Discharge Monitoring Reports, considering at a minimum, the most recent two years of permit records. When there is evidence that the WWTP exceeds design capacity, consideration should then be given to the frequency of such events and the effect this will have on the plant's ability to reduce the viral load of the effluent.

Consideration should also be given to the frequency of which the WWTP bypasses any stage of treatment or any condition that may degrade the quality of the effluent to determine the potential frequency a conditional growing area may need to close over the course of a year. This assessment will determine the feasibility of operating a conditionally managed area based on WWTP performance.

Bacteriological or viral quality of the effluent: Discharge Monitoring Reports for WWTPs should be examined and periodically monitored to assess the reliability of the disinfection systems. Any samples collected to assess the reliability of the disinfection system should be collected during the period(s) of the year that the State Shellfish Control Authority (SSCA) deems most likely to experience adverse conditions in the treatment or disinfection processes that could affect effluent quality impacting receiving waters.

Results from any bacteriological or viral sampling and analyses must be correlated with WWTP operation and evaluated in terms of the minimum treatment expected when there is a malfunction, overloading or other poor operational condition. However, 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 - the current indicator microorganisms used to assess treatment efficiency. In contrast, many human enteric viruses are not inactivated by functional WWTP systems, hence the need for an adequate dilution zone between the outfall and the shellfish resource.

Decay rate of contaminants: It should be assumed that there is no fecal coliform or viral inactivation in the effluent during possible upset conditions in the WWTP. There are a number of conditions that affect bacterial and viral inactivation, including temperature, exposure to sunlight and sedimentation levels in the water (Burkhardt et al, 2000; Lees, 2002; LaBelle, 1980; Griffen, 2003). Scientists are unsure how long viruses remain viable in the marine environment, but it is likely to be weeks or months (Younger, 2002), and enteroviruses have

been found in marine sediments suggesting that these sediments can be a source upon resuspension (Lewis, 1986). Moreover, molluscan shellfish have been found to retain viruses to a greater extent and for much longer periods than they do bacteria (Sobsey et al, 1987; Richards, 1988; Dore and Lees, 1995; Dore et al, 2000; Shieh et al, 2000).

Waste water dispersion and dilution: Dispersion of the effluent refers to the spread, location, and shape of the discharge plume with time as it leaves the WWTP outfall. Dilution of the effluent refers to the amount of receiving water that is entrained within a particular time or distance from the outfall, e.g. the dilution of the effluent within the time or distance it takes to reach the border of the prohibited zone. 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.

In poorly flushed estuaries and coastal embayments there is the potential for WWTP effluent build-up that further reduces the availability of "clean" waters to both dilute contaminant loadings and purge shellfish of contaminants (Goblick et al., 2011).

Time of waste transport to the shellfish harvest site: When there is a WWTP malfunction it is important that adequate systems are in place to officially close the harvest area before the effluent impacts the shellfish. This is a mandatory requirement for conditional management of shellfish harvest areas and all parties must agree in writing on the process steps necessary to close the harvest area after such events. Both time of travel and dilution should be considered when sizing a prohibited zone around a WWTP outfall adjacent to a conditional growing area. The overall sizing of the prohibitive zone should satisfy both a minimum dilution of 1000:1 and also factor in adequate time to respond to a malfunction event. When establishing the time of travel between the WWTP and the classified area, consideration should be given to the worst scenarios which would cause the fastest travel. For example, the peak current flows at or near the outfall during ebb tide and flood tide to determine effluent transport speeds. Current velocity information may need to be generated if such information is not available or adequate for the area of the outfall. Current velocity information can be obtained from hydrographic dye studies, drogue studies, or current meter data conducted in the vicinity of the outfall.

Location of shellfish resources: The best information that is available should be used for locating shellfish resources near the outfall. Subtidal shellfish resources may also be identified in sanitary surveys near WWTP outfalls. Therefore the SSCA must establish closure zones at WWTP outfalls in accordance with the classification requirements of the Model Ordinance..

Classification of Adjacent Waters: If the SSCA's dilution analysis determines that the shellfish water quality standards for approved waters are met at the boundary of the prohibited area during potential upset conditions, the shellfish area adjacent to the prohibited area need not be classified as Conditionally Approved and may be classified as Approved.

**Scientific Rationale for 1000:1 Dilution Guidance**

Since 1987 FDA has recommended at training courses and other venues the use of a 1000:1 dilution as the minimum level of dilution needed around a WWTP outfall to mitigate the impact of viruses for shellfish harvest areas managed conditionally based on the performance of the WWTP. It has been advised that conditional management based on WWTP performance may not be appropriate for all WWTP's that are located within proximity to shellfish harvest areas and recommended only for large, highly efficient WWTPs that are well monitored.. In 1995 this estimated level of necessary dilution was further calculated and explained by FDA using assumptions based on the most relevant scientific literature available at that time (Kohn, et al. 1995; Havelaar et al. 1993; Kapikian et al. 1990; Liu et al. 1966). Since then major advances in the detection and enumeration of NoV in wastewater and shellfish have been made, and advances in fluorometer technologies have enabled more sophisticated hydrographic dye study methods. Using these advances, FDA has conducted dye studies supplemented with the testing of shellfish sentinels for enteric viruses and their surrogates. This has afforded FDA for the first time with a means to directly determine the viral risk posed by WWTP effluent on shellfish resources. During recent years FDA has presented the findings from these studies at regional shellfish meetings, at the biennial ISSC meeting, at international scientific conferences and to international partners engaged in collaborative projects. Results from these studies are referred to herein as part of the scientific basis for the current recommended guidance.

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 the aforementioned technical advances to prospectively assess the 1995 1000:1 dilution estimate recommendation and determine if this level of dilution is appropriate to mitigate the risk of viruses discharged in treated wastewater effluent. From 2008 through 2012 FDA conducted four additional studies (Hampton Roads, Virginia; Yarmouth, Maine; Coos Bay, Oregon; Blaine, Washington). In each of these studies, FDA evaluated male-specific coliphage (MSC) and NoV levels in shellfish together with the dilutions of WWTP effluent. The studies were designed to build a more comprehensive and in-depth understanding of viral impacts posed by WWTPs on shellfish resources.

To date, 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 the use of chlorine, and when they are operating under normal conditions. Results further indicate that in certain instances, such as when WWTPs begin to exceed their design capacity, bypass treatment, or otherwise malfunction, the 1000:1 dilution level may be inadequate and emergency closure procedures should be considered within the conditional area management plan. Under such circumstances, conditional area management plans should ensure there is sufficient time for notification to the State Shellfish Control Authority (SSCA) and for subsequent notifications closing the conditional area to harvesting.

MSC results in shellfish from the 2008-2012 studies were evaluated using 50 PFU/100 g as the threshold level of concern for MSC, since this is the level under the Model Ordinance (Section II, Chapter IV, @.03 A(5)(c)(ii)) 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. For conventional WWTPs operating under normal conditions, there were at least four occasions when dilution levels were between 700:1 and 1000:1 and MSC levels in shellfish exceeded 50 PFU/100g, but there were no occasions in which MSC levels exceeded 50 PFU/100g and dilution was greater than 1000:1. For conventional WWTPs operating under malfunction conditions, such as when flow rates exceeded the design capacity or during a treatment stage bypass, MSC levels in shellfish exceeded 50 PFU/100g in at least 13 instances in which dilution was greater than 1000:1.

When evaluating the NoV results of the 2008 – 2012 studies FDA used a value of 300 RT-PCR units of NoV/100 gram of digestive gland (digestive diverticula) as the threshold. This value was considered significant since at this level shellfish related illnesses have been reported and demonstrated by the analysis of meal remnants.

In examining the results from all the studies, there were no cases in which conventional WWTPs operating under normal conditions produced results greater than 300 NoV particles/100 g of DD in oyster sentinels when dilution levels at the associated sentinel stations were greater than 1000:1. When dilution levels were less than 1000:1, levels of NoV GII greater than 300 NoV particles/100 g of DD were detected, and on one occasion around 8000 NoV particles/100g DD were found.

On three occasions during which WWTPs were operating under malfunction conditions (as previously described), thirteen (13) oyster samples were found with NoV GII levels greater than 300 NoV particles/100 g DD when dilution was close to or greater than 1000:1. These results emphasize the critical need for sufficient notification time, meaning travel time from the WWTP discharge in 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.

In one instance, an unconventional WWTP that used membrane filtration technology rather than conventional treatment with chlorine or UV disinfection was assessed. The levels of NoV GII in shellfish sentinels near this WWTP were greater than 300 NoV particles/100 g of DD, even when dilution levels were greater than 1000:1, and on two occasions when dilution levels exceeded 10,000:1. In seven (7) instances, NoV levels at the plant were greater than 300 NoV particles/100g of DD. MSC levels were similarly high, with all six (6) samples tested having MSC levels greater than 800 PFU/100g, and in one sample greater than 10,000 PFU/100g, even though dilution levels were higher than 1000:1. This analysis demonstrates the need to assess WWTPs with unique treatment systems on a case by case basis, since some may perform better than conventional WWTPs at removing viruses and some may perform significantly worse.

The overall results of FDA's 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. 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 and hence the minimum level of dilution needed to ensure shellfish safety. However, at this time FDA does not have reliable data to justify a recommended minimum

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| | dilution less than 1000:1 or to establish any variable dilution thresholds corresponding to and dependent on such factors. It is recognized that these criteria could be determined by a State Shellfish Control Authority (SSCA) on a case by case basis, where factors of WWTP performance, disinfection method, tidal flushing, and seasonal impacts may vary. These and other factors that might influence virus levels in the shellfish can be considered by SSCAs when assessing how best to manage conditional growing areas based on WWTP performance. Using dilution levels lower than 1000:1 or other alternative approaches for managing the viral risk posed by WWTP effluents are cited in Alternate Options section (see below). 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.<br><br>Alternate Options<br><br>It is expected that the principles of this guidance shall be followed to ensure compliance with the dilution requirements of the Model Ordinance. An alternative minimum waste water dilution threshold value may be appropriate for situations in which highly effective WWTP facilities reduce the viral load of the effluent, or seasonal or geographical factors reduce the risk of viral contamination at the shellfish growing area. 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 with tertiary treatment operating under optimum design flow conditions. Regardless of the technology employed any proposed alternative minimum threshold 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<br><br>It should be noted that any alternate approach would need to consider the time of waste transport to the shellfish harvest site. As described in this guidance in geographic regions with large tidal amplitudes and/or swift tidal currents, the time of waste transport to the shellfish harvest site may be the determining factor in sizing the prohibited zone. However, there may be various strategies that could be employed to address the time of waste transport to the shellfish harvest site. For example, it may be reasonable to expect that if a facility utilized a sufficiently sized containment structure (such as the equivalent to 24-hour holding for the design capacity of the plant) in the event of a malfunction, this would allow the SSCA additional time to react to the event and take any necessary precautions. Regardless of technology or best management practices employed any proposed alternative strategy would need to be validated (ie verifying that a containment structure is properly sized and working effectively).<br><br>There are likely other alternatives in addressing the potential impact of wastewater on shellfish growing areas and approaches in validating these |

options. However, the flexibility remains with the SSCA's to determine the appropriate alternate option and validation process that can be verified.

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| **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 (if available):** | |

| **Proposal for Task Force Consideration at the Interstate Shellfish Sanitation Conference 2013 Biennial Meeting** | ☒ **Growing Area**<br>☐ **Harvesting/Handling/Distribution**<br>☐ **Administrative** |
|---|---|
| **Submitter:** | Laboratory Methods Review & Quality Assurance Committee<br>Patti Fowler, Chairperson |
| **Affiliation:** | Interstate Shellfish Sanitation Conference |
| **Address** | 209 Dawson Road<br>Suite 2<br>Columbia, SC 29223-1740 |
| **Phone:**<br>**Fax:**<br>**Email:** | 803-788-7559<br>803-788-7576<br>issc@issc.org |
| **Proposal Subject:** | Revisions to Chapter III. Requirements for the Authority |
| **Specific NSSP Guide Reference:** | 2011 NSSP Guide Section II. Model Ordinance<br>Chapter III. Laboratory |
| **Text of Proposal/ Requested Action** | **@.02 Methods.**<br><br>A. Microbiological. Methods for the analyses of shellfish and shellfish growing or harvest waters shall be:<br>(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.<br>(2) When there is an immediate or ongoing critical need for a method and no Approved NSSP Method exists, the following may be used:<br>(a) A validated AOAC, BAM, or EPA method;<br>(b) An Emergency Use Method pursuant to .02 D. (1) and (2) below.<br><br>B. Chemical and Physical. Methods for the analyses of shellfish and shellfish harvest waters shall be:<br>(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 cited in the Guidance Documents Chapter II. Growing Areas .11 Approved National Shellfish Sanitation Program Laboratory Tests. ~~Methods for the analysis of shellfish and shellfish growing or harvest waters shall:~~<br>~~(a) Be the current AOAC or APHA method for all physical and chemical measurements; and~~<br>~~(b) Express results of all chemical and physical measurements in standard units, and not instrument readings.~~<br>(2) Results shall be expressed for chemical and physical measurements in standard units and not instrument readings.<br>~~(2)~~(3) When there is an immediate or ongoing critical need for a Method and no Approved NSSP Method exists, the following may be used:<br>(a) A validated AOAC, BAM, or EPA method;<br>(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 cited in the Guidance Documents Chpater II. Growing Areas .11 Approved National Shellfish Sanitation Program Laboratory Tests. ~~The current AOAC and APHA methods used in the bioassay for paralytic shellfish poisoning toxins; and~~

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

~~(3) Approved NSSP Methods validated for use under Procedure XVI. of the Constitution, Bylaws and Procedures of the ISSC and/or cited in the Guidance Documents Chapter II. Growing Areas .11 Approved National Shellfish Sanitation Program Laboratory Tests.~~

~~(4)~~(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.

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|---|---|
| **Public Health Significance:** | This revision to Chapter III. Laboratory is necessary to clarify and guide users to the location within the Guidance Documents that lists the approved NSSP laboratory tests in .11 Approved NSSP Laboratory Tests. All approved laboratory tests are now listed in Table .11 Approved NSSP Laboratory Tests with the Guidance Document. |
| **Cost Information (if available):** | |

| **Proposal for Task Force Consideration at the Interstate Shellfish Sanitation Conference 2013 Biennial Meeting** | ☒ **Growing Area**<br>☐ **Harvesting/Handling/Distribution**<br>☐ **Administrative** |
|---|---|
| **Submitter:** | Thomas Howell |
| **Affiliation:** | Spinney Creek Shellfish, Inc. |
| **Address:** | 27 Howell Drive<br>Eliot, ME, 03903 |
| **Phone:**<br>**Fax:**<br>**Email:** | (207) 439-2719<br>(207) 439-7643<br>tlhowell@spinneycreek.com |
| **Proposal Subject:** | Male-specific Coliphage Method for Quahogs (M. mercenaria) |
| **Specific NSSP Guide Reference:** | NSSP Guide Section IV Guidance Documents Chapter II Growing Areas<br>.11 Approved Limited Use Methods for Microbiological Testing |
| **Text of Proposal/ Requested Action** | This submission presents the 'Male-specific Coliphage method for Quahogs (M. mercenaria)' for consideration as an approved limited use method for microbiological testing. At the 2009 ISSC, the 'Modified Double Agar Overlay Method for Determining Male-specific Coliphage in Soft-shelled Clams and American Oysters' was accepted as an approved limited use method for microbiological testing for re-opening growing areas after emergency closures due to sewage spills. SLV work with quahogs has demonstrated comparable performance characteristics as with soft-shelled clams and American oysters.<br><br>The requested action is to include quahogs in the footnote for MSC along with soft-shelled clams and American oysters in NSSP Guide Section IV Guidance Documents Chapter II Growing Areas .11 Approved Limited Use Methods for Microbiological Testing. |
| **Public Health Significance:** | The MSC method for quahogs was used recently by the State of New Jersey to re-open growing areas after the devastating effects of Superstorm Sandy. Increasingly, enumeration of male-specific coliphage (MSC) in soft-shelled clams, American oysters, and quahogs is needed in the NSSP to assess *viral* contamination in molluscan shellfish harvested from growing areas where fecal coliform levels in both water quality and shellfish meats may be misleading. MSC is a specialized indicator of *viral* sewage contamination, which is substantially more meaningful than fecal coliform or E. coli in evaluating the safety of shellstock harvested from growing areas potentially impacted by treated and partially treated wastewater. |
| **Cost Information (if available):** | This method for the enumeration of male-specific coliphage in soft-shelled clams, American oysters, and quahogs is inexpensive, easy to perform, and rapid, providing results within 24 hours. The cost of laboratory glassware, plastic-ware, agars, and reagents is approximately $25 per shellfish sample. In a well-equipped laboratory, the method requires 6 hours of time from initiating host to pouring plates. Hands on technician time to perform this test is significantly less on the order of 1-4 hours per test depending upon how many tests are done per day. The most expensive piece of equipment is a refrigerated centrifuge plus rotor, which costs approximately $12,000. There are no special skill sets required beyond those required to operate a state-approved shellfish laboratory under the NSSP. |

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

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

| | |
|---|---|
| Name of the New Method | Male-specific Coliphage for Quahogs (M. Mercenaria) |
| Name of the Method Developer | Thomas Howell, Spinney Creek Shellfish, Inc. |
| Developer Contact Information | Spinney Creek Shellfish, Inc.<br>27 Howel Drive<br>Eliot, ME 03903<br>(207) 439-2719<br>tlhowell@spinneycreek.com |

| Checklist | Y/N | Submitter Comments |
|---|---|---|
| **A. Need for the New Method** | | |
| Clearly define the need for which the method has been developed. | Y | |
| What is the intended purpose of the method? | Y | |
| Is there an acknowledged need for this method in the NSSP? | Y | |
| What type of method? i.e. chemical, molecular, culture, etc. | Y | Culture method for Male-specific Coliphage in Quahogs (M. Mercenaria) |

| | | |
|---|---|---|
| **B. Method Documentation** | | |
| 1. Method documentation includes the following information: | | |
| Method Title | Y | |
| Method Scope | Y | |
| References | Y | |
| Principle | Y | |
| Any proprietary aspects | N | |
| Equipment required | Y | |
| Reagents required | Y | |
| Sample collection, preservation and storage requirements | Y | |

| | | |
|---|---|---|
| Safety requirements | Y | |
| Clear and easy to follow step-by-step procedure | Y | |
| Quality control steps specific for this method | Y | |

| **C. Validation Criteria** | | |
|---|---|---|
| 1. Accuracy / Trueness | Y | |
| 2. Measurement uncertainty | Y | |
| 3. Precision characteristics (repeatability) | Y | |
| 4. Recovery | Y | |
| 5. Specificity | NA | |
| 6. Working and Linear ranges | Y | Working Range |
| 7. Limit of detection | Y | |
| 8. Limit of quantitation / Sensitivity | Y | |
| 9. Ruggedness | Y | |
| 10. Matrix effects | NA | Matrix effects were observed and modifications made to the MSC method during SLV work with soft-shelled clams and American oysters in 2008-2009. These same modifications are employed in this mehtod for quahogs. No matrix effects are anticipated |
| 11. Comparability (if intended as a substitute for an established method accepted by the NSSP) | NA | |

| **D. Other Information** | | |
|---|---|---|
| 1. Cost of the method | Y | |
| 2. Special technical skills required to perform the method | Y | |
| 3. Special equipment required and associated cost | Y | |
| 4. Abbreviations and acronyms defined | Y | |
| 5. Details of turn around times (time involved to complete the method) | Y | |
| 6. Provide brief overview of the quality systems used in the lab | Y | |

| | |
|---|---|
| Submitters Signature | Date: |
| Submission of validation data and draft method to committee | Date: |
| Reviewing members: | |
| Accepted | Date: |

| Recommendations for further work | Date: |
| --- | --- |

Comments:

**Single Laboratory Validation (SLV) Protocol**

**For Submission to the Interstate Shellfish Sanitation Conference (ISSC)**

**For Method Approval**

## Section A. Justification for New Method

| | |
|---|---|
| Name of the New Method - | Male-specific Coliphage (MSC) for Quahogs. |
| Specify the Type of Method - | Culture Method/Double Agar Overlay Method |
| Name of Method Developer - | Thomas Howell, Spinney Creek Shellfish, Inc. |
| Developer Contact Information – | Spinney Creek Shellfish, Inc.<br>27 Howell Drive<br>Eliot, Maine 03903<br>(207) 439-2719<br>(207) 439-7643 FAX<br>tlhowell@spinneycreek.com |
| Date of Submission – | November 8, 2013 |

Purpose and Intended Use of the Method.

The primary purpose and intended use of this method in the NSSP is for re-opening growing areas after emergency closures due to sewage spills. This method has been used recently to re-open growing areas after the devastating effects of Superstorm Sandy by the State of New Jersey. The method presented in this document is the same as that modified and validated for soft-shelled clams and American oyster at the 2009 ISSC in Manchester, NH. Additionally, this method can be used to verify and optimize viral depuration/relay strategies used to reduce viral contamination in quahogs harvested from growing areas impacted by wastewater treatment plant (WTP) outfall.

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

Fecal coliforms (FC), a bacterial indicator, are used for process validation for conventional depuration processes. In growing areas impacted by moderate or low-level non-point source contamination, conventional depuration methods using FC for process validation are adequate, well proven, and widely accepted by the scientific and public health community. Statistical analysis of FC samples, collected during water quality monitoring, are used to determine growing area classification. Limits on the geometric mean and 90$^{th}$ percentile are considered adequate to protect public health from the risks of viral contamination in areas not impacted by sewage and WTP pollution. However, in growing areas impacted by treated sewage, the relationship between bacterial and viral contamination can be substantially altered by the differential inactivation rates of chlorination and other disinfection methods on bacteria and

viruses. This MSC method is needed in the NSSP to evaluate viral contamination in molluscan shellfish harvested from growing areas where FC levels in both water quality and shellfish meats may be misleading. MSC is a specialized indicator of viral contamination, which is substantially more meaningful than FC in evaluating the safety of shellstock harvested from growing areas potentially impacted by treated and partially treated wastewater. Much work has been done to demonstrate that the MSC method is particularly useful and highly advantageous over FC for evaluating the efficacy of viral depuration and viral relay processes in soft-shelled clams. Continuing work is being conducted to assess the usefullness of this method for evaluating the efficacy of viral depuration and viral relay processes for American oysters and quahogs.

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

The MSC method described here has been previously validated for soft-shelled clams and American Oysters and is currently being evaluated for quahogs. Further SLV work is needed to evaluate different matrix types / other species of molluscan shellfish.

Other Comments.

**SLV work strongly suggests that this modified MSC method is appropriate (fit for purpose) for applications in Quahogs in addition to Soft-shelled clams and American oysters where a regulatory limit of 50 PFU/100gram has been established.**

**Section B. Method Documentation**

**Modified Double Agar Overlay Method**
**for Determining Male-specific Coliphage**
**In Soft-shelled Clams, American Oysters, and Quahogs (M. mercenaria)**
Nov 2013

This method for determining levels of male-specific coliphage in quahog meat is based on the method described by DeBartolomeis and Cabelli[1,2]. FDA had refined the method for oyster and hard clam meats as described in the workshop instructions, *Male-specific Bacteriophage (MSB) Workshop*, conducted in Gloucester, Massachusetts on March 9-12, 2004[3]. This original FDA (2004) method was submitted as ISSC Proposal 05-114. This method was modified again in 2008-2009 by Spinney Creek Shellfish to improve viral recovery and sensitivity for soft-shelled clams and American oysters.

Modification of the FDA (2004) Method

Spinney Creek Shellfish, Inc. (SCS) further refined these procedures for soft-shelled clam and oyster meat in 2006. In this work and in parallel work conducted by Mercuria Cumbo of the Maine Department of Marine Resources, it was observed that the extraction protocol was inadequate. The supernatant produced when soft-shelled clams and some oysters were processed was opaque and creamy while the pellet was loose and indistinct. Subsequent re-washing of the pellets in growth broth, re-processing, and re-plating showed significant levels of MSC left in the pellet, indicating poor recovery. The problem was solved by; eluting the shellfish meats with growth broth (2:1), and increasing the blending time to 180 seconds. This modification, based on EU methodology (ISO 10705-4), resulted in a clear supernatant and a distinct, firm pellet. Further experimentation and subsequent validation work confirmed that this elution approach works very well. SLV validation work conducted by (SCS) in 2009 resulted in further modification of the method to increase the limit of quantitation/sensitivity (LOQ). This increase in LOQ was achieved by plating an increased amount of supernatant (25ml) and using 10 plates. This same modified method is used for quahogs in the SLV application.

**A. Apparatus and Materials.**

**Equipment and Materials for Collection and Transport of Shellfish Samples:**
4 mil plastic bags
Labels
Cooler
Gel Packs
Temperature Control Blank

**Laboratory Equipment:**
Centrifuge with rotor for 50 ml conical (or larger) tubes, 9000 x *g* performance capability, 4°C
Water bath, 50-52°C
Air Incubator, 35-37°C
Balance
Stir plate and magnetic stirring bars, sterile

Mini vortexer
Blender
Autoclave, 121°C
Refrigerator, 0–4° C
Freezer, -20°C
Thermometers, range -20–121°C
pH meter
Erlenmeyer flasks, 1 L and 2 L
Graduated cylinders, 100 ml, 500 ml and 1000 ml
600ml and 3000ml beaker
500 ml jars, autoclavable with caps
Inoculating loops (3 mm in diameter or 10 FL volume)
Bacti-cinerator
Sterile swabs
Sterile, disposable filters, 0.22 or 0.45 µm pore size
Syringes, sterile disposable; 5, 10 or 20 ml
Scrub brushes, sterile
Knives, sterile
Blender jars, sterile
Sterile plastic cups 250 ml
Pipets- 2ml, 5 ml, 10 ml
Pipet-aid
Micro-Pipettors, 100 µL, 200 µL, 1000 µL, 2500 µL
Micro-Pipet tips 200 µL, 1000 µL, 2500 µL
Pipetor Stand
Centrifuge tubes, sterile disposable 50 ml or larger
Petri dishes, sterile disposable 100 x 15 mm
Petri dish racks
Test tubes 16 x 100 mm (for soft agar)
Test tubes 16 x 150 mm, with screw caps
Test tube racks--size to accommodate tubes
Freezer vials, sterile 30 ml with screw caps
Baskets with tops to hold freezer vials
Parafilm tape
Aluminum foil

**Reagents:**
Reagent water
Glycerol- sterile
Ethanol, 70% or laboratory disinfectant
Calcium chloride, 1M
Mineral oil

**Antibiotic stocks:**
Ampicillin sodium salt (Sigma A9518)
Streptomycin sulfate (Sigma S6501)
Streptomycin and Ampicillin stock solutions (50 µg/ml each). Note: Antibiotics must always be added to liquids and media after these have been autoclaved and cooled.

**Media:**
Bottom Agar
DS Soft Agar
Growth Broth

**Bacterial Host Strain:**
*E.coli* $F_{amp}$ . *E. coli* HS(pFamp)RR (selected by Dr. Victor J. Cabelli, University of Rhode Island, Kingston, RI, USA, frozen stock ATCC # 700891).

**MSC (Coliphage) Stock:**
Type Strain - MS2, ATCC # 15597

**B. Media Composition.**

**Bottom Agar:**

| | |
|---|---|
| Tryptone | 10.0 g |
| Dextrose | 1.0 g |
| NaCl | 5.0 g |
| Agar | 15.0 g |
| DI water | 990 ml |
| Final pH | 6.7 ± 0.2 at 25°C |

1. With gentle mixing, add all the components to 990 ml of $dH_2O$ in a 2000 ml flask. Dissolve, heat until clear and boiling started.
2. Sterilize at 121°C ± 2°C for 15 minutes.
3. Temper to 50°C in the water bath.
4. Add 5 ml of Streptomycin sulfate/Ampicillin solution, aseptically to the flask (50 µg/ml each in final) and mix. Transfer to 2 – 500ml sterile jars (easier to pour plates from jars).
5. Pipet (or pour) 15 ml aliquots aseptically into sterile 100 x 15 mm Petri dishes and allow the agar to harden. Tip Petri dish lids off slightly to reduce condensation.
6. Store bottom agar plates inverted at 4°C and warm to room temperature for 1 hour before use.
7. Plates stored sealed at 4°C can be used up to 6 weeks.

**Streptomycin sulfate/Ampicillin Solution:**
1. Dissolve 0.5g of streptomycin sulfate and 0.5g of ampicillin in 50 ml of $dH_2O$ with a sterile 100 ml graduated cylinder in sterile 600 ml beaker with sterile stir bar.
2. Stir for 2 to 3 minutes, no heat.
3. Filter through sterile 0.22 µm filter.
4. Store in 5 ml aliquots in sterile 30 ml capped freezer vials at -20°C for up to one year. Label and date.
5. Allow to come to room temperature before adding and mixing in tempered bottom agar at 50°C.

**DS Soft Agar:**

| | |
|---|---|
| Tryptone | 10.0 g |
| Dextrose | 1.0 g |
| NaCl | 5.0 g |
| 1M $CaCl_2$ | 0.5 ml |
| Agar | 7.0 g |
| DI water | 500 ml |
| Final pH | 6.7 ± 0.2 |

1. With gentle mixing, add all the components to 500 ml of $dH_2O$ in a 1000 ml flask.
2. Bring flask contents to a boil.
3. Dispense in 2.5 ml aliquots into 16 x 100 ml tubes, cover and freeze (-20°C) for up to three months.
4. Sterilize prior to use at 121°C ± 2°C for 15 minutes, then temper to 50-52°C for no longer than 2 hours

**1M $CaCl_2$ Solution:**

1. Add 11.1 g of $CaCl_2$ anhydrous (FW 111.0, Dihydrate FW 147) to 100 ml
2. $dH_2O$ in a screw top bottle and dissolve or use prepared from VWR.
3. Sterilize by autoclaving at 121°C for 15 minutes.
4. Store up to three months at 4°C.
5. Use at room temperature.

**Growth Broth:**

| | |
|---|---|
| Tryptone | 10.0 g |
| Dextrose | 1.0 g |
| NaCl | 5.0 g |
| DI water | 1000 ml |

1. With gentle mixing, add all the components to 1000 ml of $dH_2O$ water in a 2000 ml flask.
2. Dissolve and dispense into sterile screw top containers.
3. Sterilize at 121°C ± 2°C for 15 minutes.
4. Store for up to three months at 4°C.

**Storage Slants:** Tryptic Soy Agar.

**C. Storage and Propagation of Host Strain, E. coli $F_{amp}$.**

**Storage:**

1. Lab stock culture – Frozen at – 80°C indefinitely (most desirable method) in broth culture containing 10% glycerol under no selective pressure. Selective pressure is reapplied when the culture is retrieved, by streaking onto Bottom Agar plates containing the two antibiotics.
2. Long-term working stock culture – Grown tryptic soy agar slant with sterile mineral oil overlay under no selective pressure and stored at room temperature in the dark for up to 2 years.
3. Long-term working stock – 6-hour grown tryptic soy agar slant and deep stab with sterile mineral oil overlay containing the two antibiotics, Ampicillin and Streptomycin (least desirable method).

4. Short-term working stock culture - Grown Bottom Agar streak plate stored at 4°C up to 3 weeks.
5. Short-term working stock culture – Grown in Growth broth and used within 6-12 hours (same day).

**Glycerol Solution, 10%:** Add 9 ml of distilled water to 1 ml of undiluted glycerol. Autoclave resulting 10% glycerol solution at 121°C for 15 minutes and use at room temperature. For storage, add 1/5th volume of 10% glycerol solution, let stand for 30 minutes, dispense 1 ml aliquots in 2 ml cryo-vials and store at -70 to –80°C (best) or at –20°C.

**Propagation:**
1. Vortex to aerate 10 ml of Growth Broth medium tempered to 35 – 37°C just prior to inoculation.
2. Transfer host strain to Growth Broth using sterile swab to collect material from several colonies off grown Bottom Agar streak plate and warmed to room temperature.
3. Gently shake to mix, then incubate at 35–37°C for 4-6 hours (turbidity=$10^7$cells/ml; O.D at 540nm=0.4).
4. Once turbidity is observed, use of the host strain broth culture (log-phased growth) may commence

**(following initial inoculation and mixing, do not shake or mix the host strain broth culture)**.

**D. Control Plates.**
1. Negative Control - Add 2.5 ml of Growth Broth and 0.2 ml host to the 2.5 ml DS Soft Agar tube.
2. Positive Control - Make serial dilutions using growth broth of the concentrated MS2 control (to grow approximately 50-100 PFU per 2.5 ml), and add 2.5 ml of appropriate MS2 dilution and 0.2 ml of host to 2.5 ml DS Soft agar.

**E. MSC Density Determinations in Soft Shelled Clam, American Oyster, and Quahog Tissues.**

**Sample Requirements.** Samples of shellstock and shucked meats are held under dry refrigerated conditions at 1–4°C. Samples must be comprised of a representative number of animals (12 to 15). Samples are analyzed within 24 hours of collection. Animals with broken shells or animals that appear dead are discarded. Sample collection bags must be properly identified with lot #, date and time of collection, collection location and collector's initials.

**Preparation of Shellfish for Analysis.** Using soap and water, analyst's hands are thoroughly scrubbed and rinsed. Using a sterile brush, shells of whole animals are scrubbed under running potable water to remove loose material from the shells. Shellfish then are placed on a clean paper towel or in an open weave basket to dry. Scrubbed, drying animals should not come in contact with each other. Once the shells of washed shellfish are dry, analysts wash their hands thoroughly with soap and water, then rinse their hands with 70% alcohol and allow to air dry. Shellfish are shucked and the meats and liquors are saved into a sterile 250 ml cups.

**Direct Analytical Technique for Soft Shelled Clams, American Oysters, and Quahogs.** For each soft shelled clam, American oyster, or quahog sample, ten (10) Bottom Agar plates and ten (10) 2.5 ml DS Soft Agar tubes are prepared. Use a 4 to 6 h culture of host strain, *E. coli* $F_{amp}$. Always begin analyses with a negative control (blank) plate and finish analyses with a positive control plate followed by a second negative control plate.

1. Shuck 12 soft shelled clams, American oysters, or quahogs into sterile 250 ml cup, tare and add to sterile blender. To make a 1:2 (wgt:vol) elution with growth broth eluent using twice the volume of the shellfish. Add to blender with sample. Homogenize by blending for 180 seconds at high speed.
2. Immediately weigh 33.0 g of homogenate from each sample into labeled sterile 50 ml centrifuge tubes after blender has stopped before foam separation can occur.
3. Centrifuge each sample for 15 min. @ 9,000-10,000 x g; 4°C.
4. Pipette off and weigh the supernatant in a new sterile 50 ml centrifuge tube.
5. Allow the supernatant to warm to RT (approximately 20-30 minutes).
6. Shake or vortex the supernatant.
7. Gently pipette 200 µL of log phase host strain, *E. coli* HS(pFamp)RR using 200 µL micro pipettor and a 200 µL pipet tip, then pipette 2500 µL aliquot of supernatant using the 2500 µL micro pipettor and a 2500 µL pipet tips, to 2.5 ml DS Soft agar tube (tempered to 52°C).
8. **Once E. coli $F_{amp}$ is added to the mixture do not shake, only gently mix contents by rolling the tube between palms.**
9. Overlay the 5.2 ml onto a Bottom Agar plate containing Streptomycin and Ampicillin (50 g/ml final concentrations). Drag the mixture into a clear area and gently swirl the plates to spread sample and agar mixture.
10. Allow plates to set then inverted and incubated for 16 - 20 hours at 35- 37°C.

**Calculations of Results**

$$\frac{\text{Total number of MSC (N)}}{\text{Total supernatant plated (25gm)}} \times \frac{\text{Weight of supernatant extracted (Ws)}}{\text{grams of sample used (11gm)}} \times 100 =$$

$$\frac{N}{25\text{ gm}} \times \frac{Ws}{11\text{ gm}} \times 100 = (0.364)(N)(Ws) = \text{PFU of MSC/100 gm}$$

*Example: Clam/Oyster plate counts - 13, 23, 12, 16, 12, 18, 17, 21, 19, 17 and 27.5 g supernatant.*

*Result = (0.364)*(168MSC)(27.5gm) = 1681 PFU of MSC/100 gm*
**0.364=100/(25 x 11)*

**F. Sample Collection and Storage.**

1. Record all pertinent information on the collection form.
2. During transportation store samples in a cooler at 0 to 10°C.
3. At laboratory, store samples in a refrigerator at 0 to 4 °C.
4. Maximum holding times for shellfish samples is up to 24 hours.

**G. Quality Assurance.**

1. Positive and negative control plates are run with MSC analyses each day.
2. Media sterility checks are made per batch and records are maintained.
3. Media log book is maintained (pH, volume, weights of each components, lot numbers, etc.).
4. An intra- and inter-laboratory performance program is developed.
5. Circular zones of clearing (typically 1 to 10 mm in diameter) in lawn of host bacteria after 16- 20 hours of incubation are counted as plaques. (Count the number of plaques on each plate.)
6. MSC determinations are reported as plaque forming unit (PFU) per 100 grams.
7. The desired range for counting is 0 to 100 PFU per plate. If the count exceeds the upper range or if the plaques are not discrete, results should be recorded as "too numerous to count" (TNTC) or >10,000 PFU of MSC/100gm.
8. Temperatures incubators are checked twice daily (at least 4 hours apart) to ensure operation within the stated limits of the method, and results are recorded in a logbook.
9. Check thermometers at least annually against a NIST-certified thermometer.
10. Calibrate the balance monthly using ASTM-certified Class 1 or 2 or NIST Class S reference weights.
11. Laboratory analysts adhere to all applicable quality control requirements set forth in the most recent version of FDA's *Shellfish Laboratory Evaluation Checklist*.
12. Calibration of micro-pipettors needs to be checked quarterly and records kept. Micro-pipettors used for handling MSC control and transferring host cells need to have a barrier tip or be dedicated to the specific use to prevent contamination

**H. Safety.**

Samples, reference materials, and equipment known or suspected to have Coliphage attached or contained must be sterilized prior to disposal.

**I. Technical Terms.**

°C - degrees Celsius
µL - microliter
g - gram
L - liter
M - molar
ml - milliliter
rpm - revolutions per minute
Ave. - average
MSC - Male-specific Coliphage, Male-specific Bacteriophage, F+ Bacteriophage
NIST - National Institute of Standards and Technology
PFU - plaque forming units
RT - room temperature
TNTC - too numerous to count
LOD - Limit of Detection
LOQ - Limit of Quantitation
Host Strain - *E.coli* $F_{amp}$ bacteria (*E.coli* HS(pFamp)RR)
Male-specific Coliphage - Viruses that infect coliform bacteria only via the F-pili.
Plaque - Clear circular zones (typically 1 to 10 mm in diameter) in lawn of host cells after incubation.

**References:**


1. Cabelli, V.J. 1988. Microbial indicator levels in shellfish, water, and sediments from the upper Narragansett Bay conditional shellfish-growing area. Report to the Narragansett Bay Project, Providence, RI.
2. DeBartolomeis, J. and V.J. Cabelli. 1991. Evaluation of an *Escherichia coli* host strain for enumeration of F male-specific Coliphages. Appl. Environ. Microbiol. 57(4):1201-1205.
3. U.S. Food and Drug Administration. 2004. Male-specific Coliphage (MSC) Workshop, conducted in Gloucester, Massachusetts on March 9-12, 2004.


**Other Information:**

This method for the enumeration of male-specific coliphage in soft-shelled clams, American oysters, and quahogs is inexpensive, easy to perform, and rapid, providing results within 24 hours. The cost of laboratory glassware, plastic-ware, agars, and reagents is approximately $25 per shellfish sample. In a well equipped laboratory, the method requires 6 hours of time from initiating host to pouring plates. Hands on technician time to perform this test is significantly less on the order of 1-4 hours per test depending upon how many tests are done per day. The most expensive piece of equipment is a refrigerated centrifuge plus rotor, which costs approximately $12,000. There are no special skill sets required beyond those required to operate a state-approved shellfish laboratory under the NSSP.

## C. Validation Criteria

**Preliminary Studies**

A master spike determination experiment was run before other SLV work was performed to evaluate the planned routine for the spike determinations. In previous SLV work with soft-shelled clams and oysters, viral clumping was identified as a problem when the master spike was evaluated using growth broth and then compared to determination of MSC levels in the soft-shelled clam and oyster matrix. The spike determination was lower than the spiked samples of clean shellfish suggesting a negative recovery (the spike determinations were underestimating the sample results). The solution was to use clean soft-shelled clam or oyster supernatant and spin down the master spike sample to break up the clumps of MSC. This was sufficient for soft-shelled clam and oyster matrix. However, with quahogs, clean quahog homogenate was superior to both quahog supernatant and soft-shelled clam supernatant in making the spike determination. Preliminary studies of viral recovery as determined by resuspending the pellet in growth broth and re-processing twice showed that the recovery was very high.

As a result of these preliminary studies, two modifications of the SLV procedures used for soft-shelled clams and oysters were needed. First, the independent spike determination was dropped and the replicate plate values were used to calculate the estimated mean spike concentration. This meant that various validiation criteria were plotted against estimated mean spike from the triplicate samples verses an independent spike concentrations. This also required that the recovery be determined by the double re-wash and replate routine to directly evaluate the viral recovery. Because we do not have an independent estimate for the spike, we calculated and used measurement uncertainty for the mean replicate plate value which will give us a range of values for LOQ and LOD rather than a single value. Consequently, the determination of linear range is not possible and working range has been substituted as a validation criteria.

**The Determination of LOD, LOQ, and Working Range** using the NSSP SOP for the Single Laboratory Validation of Marine Biotoxin and Non-MPN Based Microbiological Methods.

The SOP for the determination of LOQ, LOD, and the Working Range yields a database from which subsets of data can be use to generate other validation criteria. For this LOQ, LOD database ten trials were run for quahogs. Supplemental samples were taken at the low range with a custom low-level master spike because of problems getting determinate results at those low levels. Effort was taken to use different shellstock from a variety of growing areas over a period of time and to utitilize shellstock that had non detectable levels of MSC (no plaques in the 10 plates). Several trial batches of shellstock were held in depuration for several days to weeks prior to the validation trials to ensure no detectable levels of MSC. Table 1 below shows the trial #, growing area, harvest date, and date of analysis for shellstock used during these validation trials.

**Table 1**

| Trial # | Growing Area | Harvest Date | Date of Analysis |
|---|---|---|---|
| Quahogs | | | |
| 1 | CT268, CT | 12/17/13 | 1/8/13 |
| 2 | CT268, CT | 12/17/13 | 1/15/13 |
| 3 | Hog Island, VA | 12/24/13 | 1/21/13 |
| 4 | Hog Island, VA | 12/24/13 | 1/29/13 |
| 5 | CT431, CT | 1/28/13 | 2/4/13 |
| 5A | Barnegat Bay, NJ | 1/16/13 | 2/13/13 |
| 5B | Barnegat Bay, NJ | 2/4/13 | 2/18/13 |
| 5C | Barnegat Bay, NJ | 2/4/13 | 2/18/13 |
| 6A | Barnegat Bay, NJ | 2/4/13 | 2/18/13 |
| 6B | CT115, CT | 2/7/13 | 2/27/13 |
| 6C | Hog Island, VA | 2/21/13 | 3/4/13 |
| 6D | Hog Island, VA | 2/21/13 | 3/5/13 |
| 6E | Hog Island, VA | 2/21/13 | 3/6/13 |
| 6F | Hog Island, VA | 2/21/13 | 3/7/13 |
| 7 | New Inlet, VA | 3/7/13 | 3/12/13 |
| 8 | New Inlet, VA | 3/7/13 | 3/19/13 |
| 9 | Spinney Creek, ME | 3/21/13 | 3/27/13 |
| 10 | Spinney Creek, ME | 3/21/13 | 4/3/13 |

For each of the 10 validation trials, 12-15 shellfish were homogenized in a 2:1 eluate of growth broth to shellfish meat in accordance with the method described above. The homogenate was evenly distributed to 5 sterile beakers with Spinplus magnetic stir bars, tared and weighed. A master spike solution was prepared in growth broth and was varied in concentration during the trials. The master spike solution was on the order of $10^3$ MSC/ml. Four subsequent serial dilutions were made for each trial from the master spike at a 3:1 dilutions. This represented different spike concentrations over the working range of the method. The 5 beakers were spiked with spike concentration 1 through 5 and three aliquots of 33 grams each were taken from each

of the 5 beakers which were actively stirred to prevent separation. In this way, 3 true replicates were generated at each of the 5 spike concentrations. The 5 sets of 3 aliquots were processed and plated according to the method description above. Supplement trials 5A-5C and 6A-6F were performed using a low-level spike that was made to get some additional low-level replicates.

Table 2 below show the estimated mean spike and tabulated MSC replicate plate concentrations results in units of PFU of MSC/100gm. RSD is relative standard deviation.

**Table 2 – Tabulated Results of the Quahog Validation Trials**

| Trial # | Estimated Mean Spike value (PFU/100gm) | MSC Repicate Plate Concentrations (PFU/100gm) | Log of Replicate MSC Plates | RSD |
|---|---|---|---|---|
| **1** | 17788 | 17729 | 4.249 | 0.0092 |
| | | 16213 | 4.210 | |
| | | 19421 | 4.288 | |
| | | | | |
| | 5105 | 4501 | 3.653 | 0.0233 |
| | | 4479 | 3.651 | |
| | | 6335 | 3.802 | |
| | | | | |
| | 1976 | 2373 | 3.375 | 0.0220 |
| | | 1795 | 3.254 | |
| | | 1761 | 3.246 | |
| | | | | |
| | 452 | 389 | 2.590 | 0.0229 |
| | | 454 | 2.657 | |
| | | 514 | 2.711 | |
| | | | | |
| | 68 | 97 | 1.987 | 0.0975 |
| | | 43 | 1.633 | |
| | | 65 | 1.813 | |
| **2** | 21724 | 21470 | 4.332 | 0.0042 |
| | | 20971 | 4.322 | |
| | | 22731 | 4.357 | |
| | | | | |
| | 4277 | 4650 | 3.667 | 0.0099 |
| | | 4234 | 3.627 | |
| | | 3946 | 3.596 | |
| | | | | |
| | 1298 | 1188 | 3.075 | 0.0109 |
| | | 1321 | 3.121 | |
| | | 1384 | 3.141 | |
| | | | | |
| | 414 | 399 | 2.601 | 0.0180 |
| | | 377 | 2.576 | |

| | | | | |
|---|---|---|---|---|
| | | 465 | 2.667 | |
| | | | | |
| | 97 | 54 | 1.732 | 0.1010 |
| | | 119 | 2.076 | |
| | | 119 | 2.076 | |

| | | | | |
|---|---|---|---|---|
| **3** | 10470 | 9360 | 3.971 | 0.0103 |
| | | 11149 | 4.047 | |
| | | 10900 | 4.037 | |
| | | | | |
| | 2890 | 2671 | 3.427 | 0.0088 |
| | | 3060 | 3.486 | |
| | | 2939 | 3.468 | |
| | | | | |
| | 871 | 743 | 2.871 | 0.0285 |
| | | 800 | 2.903 | |
| | | 1069 | 3.029 | |
| | | | | |
| | 225 | 230 | 2.362 | 0.0178 |
| | | 244 | 2.387 | |
| | | 202 | 2.305 | |
| | | | | |
| | 51 | 77 | 1.886 | 0.1700 |
| | | 55 | 1.740 | |
| | | 22 | 1.342 | |

| | | | | |
|---|---|---|---|---|
| **4** | 10255 | 10203 | 4.009 | 0.0065 |
| | | 10899 | 4.037 | |
| | | 9664 | 3.985 | |
| | | | | |
| | 2397 | 2500 | 3.398 | 0.0073 |
| | | 2446 | 3.388 | |
| | | 2245 | 3.351 | |
| | | | | |
| | 1000 | 879 | 2.944 | 0.0160 |
| | | 1035 | 3.015 | |
| | | 1085 | 3.035 | |
| | | | | |
| | 301 | 279 | 2.446 | 0.0126 |
| | | 322 | 2.508 | |
| | | 302 | 2.480 | |
| | | | | |
| | 50 | 54 | 1.732 | 0.0336 |
| | | 54 | 1.732 | |
| | | 43 | 1.633 | |

| | | | | |
|---|---|---|---|---|
| **5** | 6056 | 6257 | 3.796 | 0.0034 |
| | | 5997 | 3.778 | |
| | | 5914 | 3.772 | |
| | | | | |
| | 1539 | 1534 | 3.186 | 0.0168 |
| | | 1352 | 3.131 | |
| | | 1731 | 3.238 | |
| | | | | |
| | 476 | 515 | 2.712 | 0.0321 |
| | | 539 | 2.732 | |
| | | 375 | 2.574 | |
| | | | | |
| | 103 | 121 | 2.083 | 0.0348 |
| | | 88 | 1.944 | |
| | | 99 | 1.996 | |
| | | | | |
| **5A** | 61 | 43 | 1.633 | 0.0875 |
| | | 53 | 1.724 | |
| | | 86 | 1.934 | |
| | | | | |
| | 60 | 94 | 1.973 | 0.3136 |
| | | 74 | 1.869 | |
| | | 11 | 1.041 | |
| **5B** | 52 | 21 | 1.322 | 0.1836 |
| | | 83 | 1.919 | |
| | | 52 | 1.716 | |
| **5C** | 59 | 42 | 1.623 | 0.1147 |
| | | 93 | 1.968 | |
| | | 42 | 1.623 | |
| | | | | |
| | 62 | 72 | 1.857 | 0.1317 |
| | | 31 | 1.491 | |
| | | 83 | 1.919 | |

| | | | | |
|---|---|---|---|---|
| **6A** | 57 | 64 | 1.806 | 0.0581 |
| | | 43 | 1.633 | |
| | | 65 | 1.813 | |
| **6B** | 79 | 75 | 1.875 | 0.1180 |
| | | 118 | 2.072 | |
| | | 43 | 1.633 | |
| **6C** | 36 | 53 | 1.724 | 0.1257 |
| | | 22 | 1.342 | |
| | | 32 | 1.505 | |
| **6D** | 17 | 22 | 1.342 | 0.1786 |
| | | 11 | 1.041 | |
| | | | | |

| | | | | |
|---|---|---|---|---|
| | 15 | 22 | 1.342 | 0.1522 |
| | | 11 | 1.041 | |
| | | 11 | 1.041 | |
| **6E** | 22 | 32 | 1.505 | 0.1815 |
| | | 11 | 1.041 | |
| | | 22 | 1.342 | |
| | 18 | 22 | 1.342 | 0.1399 |
| | | 22 | 1.342 | |
| | | 11 | 1.041 | |
| **6F** | 32 | 43 | 1.633 | 0.1260 |
| | | 21 | 1.322 | |
| | | 21 | 1.322 | |
| | 21 | 21 | 1.322 | 0.1811 |
| | | 11 | 1.041 | |
| | | 32 | 1.505 | |

| | | | | |
|---|---|---|---|---|
| **7** | 8295 | 9036 | 3.956 | 0.0088 |
| | | 8103 | 3.909 | |
| | | 7745 | 3.889 | |
| | | | | |
| | 1914 | 2141 | 3.331 | 0.0187 |
| | | 1627 | 3.211 | |
| | | 1974 | 3.295 | |
| | | | | |
| | 528 | 549 | 2.740 | 0.0147 |
| | | 474 | 2.676 | |
| | | 562 | 2.750 | |
| | | | | |
| | 108 | 151 | 2.179 | 0.0750 |
| | | 97 | 1.987 | |
| | | 76 | 1.881 | |
| | | | | |
| | 18 | 22 | 1.342 | 0.1399 |
| | | 22 | 1.342 | |
| | | 11 | 1.041 | |

| | | | | |
|---|---|---|---|---|
| **8** | 6885 | 7515 | 3.876 | 0.0091 |
| | | 6430 | 3.808 | |
| | | 6710 | 3.827 | |
| | | | | |
| | 1700 | 1883 | 3.275 | 0.0132 |
| | | 1552 | 3.191 | |
| | | 1664 | 3.221 | |
| | | | | |
| | 464 | 491 | 2.691 | 0.0091 |
| | | 439 | 2.642 | |

| | | | | |
|---|---|---|---|---|
| | | 462 | 2.665 | |
| | | | | |
| | 86 | 75 | 1.875 | 0.0278 |
| | | 96 | 1.982 | |
| | | 86 | 1.934 | |
| | | | | |
| | 21 | 11 | 1.041 | 0.1811 |
| | | 21 | 1.322 | |
| | | 32 | 1.505 | |

| | | | | |
|---|---|---|---|---|
| **9** | 6341 | 6672 | 3.824 | 0.0051 |
| | | 6149 | 3.789 | |
| | | 6203 | 3.793 | |
| | | | | |
| | 1633 | 1594 | 3.202 | 0.0126 |
| | | 1802 | 3.256 | |
| | | 1502 | 3.177 | |
| | | | | |
| | 437 | 392 | 2.593 | 0.0167 |
| | | 480 | 2.681 | |
| | | 438 | 2.641 | |
| | | | | |
| | 87 | 141 | 2.149 | 0.1165 |
| | | 54 | 1.732 | |
| | | 65 | 1.813 | |
| | | | | |
| | 18 | 11 | 1.041 | 0.1399 |
| | | 22 | 1.342 | |
| | | 22 | 1.342 | |

| | | | | |
|---|---|---|---|---|
| **10** | 6468 | 6969 | 3.843 | 0.0076 |
| | | 6174 | 3.791 | |
| | | 6260 | 3.797 | |
| | | | | |
| | 1356 | 1766 | 3.247 | 0.0349 |
| | | 1106 | 3.044 | |
| | | 1196 | 3.078 | |
| | | | | |
| | 517 | 474 | 2.676 | 0.0223 |
| | | 603 | 2.780 | |
| | | 474 | 2.676 | |
| | | | | |
| | 82 | 75 | 1.875 | 0.0337 |
| | | 75 | 1.875 | |
| | | 97 | 1.987 | |
| | | | | |

| | | | | |
|---|---|---|---|---|
| | 36 | 43 | 1.633 | 0.2544 |
| | | 11 | 1.041 | |
| | | 54 | 1.732 | |

To precisely determine the LOD and LOQ, it is necessary to convert the data to log coefficient of variation and log estimated mean spike and to run the log linear regression. Graphs 1 show this log linear regression from the quahog data. The LOQ of the method may be found at the point of intersection of the log estimated mean spike and the log coefficient of variation of –1.0 (or its antilog, 10%). The LOD may be found at the point of intersection of the log estimated mean spike and the log coefficient of variation of –0.477 (or its antilog of, 33%). Taking the antilog of the spike concentrations at these points of intersection gives the LOQ and LOD, respectively. Graph 1 indicates the LOQ and LOD for the quahogs to be 43 PFU/100gm and 4 PFU/100gm, respectively. Table 3 shows the results of the log linear regression.

Graph 1 - The LOQ and LOD of Quahogs.

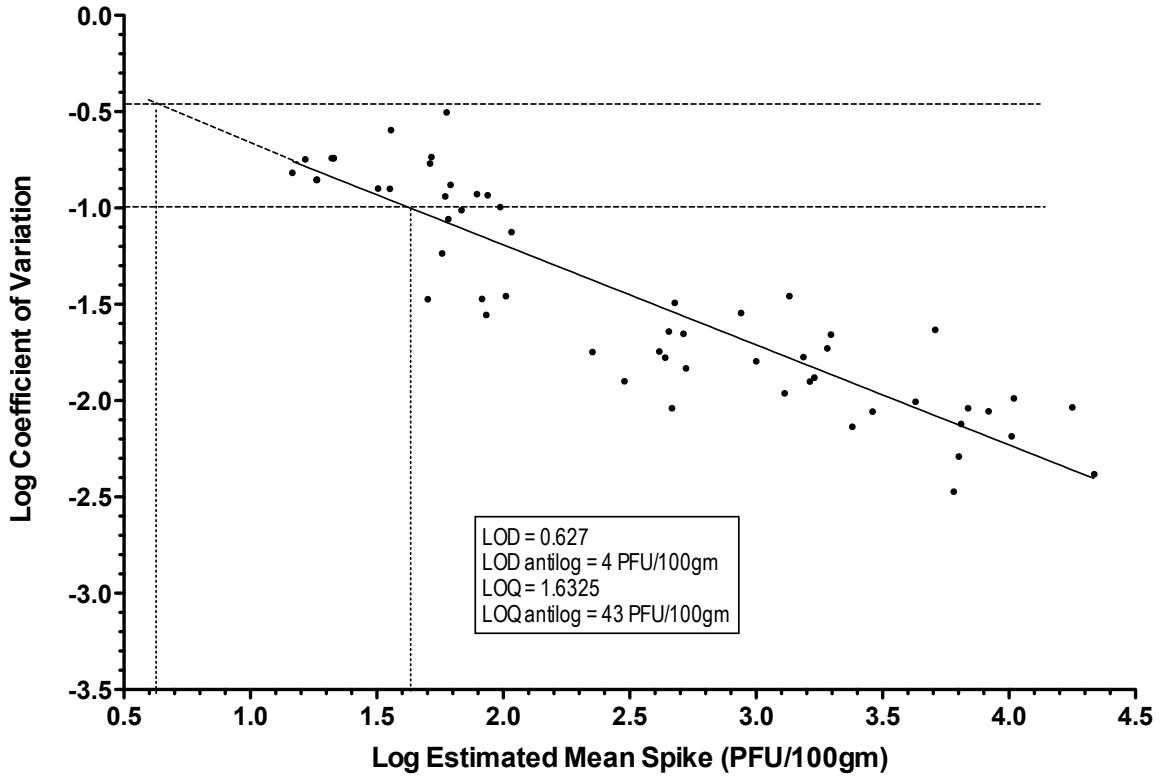


Table 3 – Results of the Log Linear Regression and Calculation of LOQ and LOD

| | |
|---|---|
| Best-fit values | |
| Slope | -0.5193 ± 0.03312 |
| Y-intercept when X=0.0 | -0.1524 ± 0.08902 |
| X-intercept when Y=0.0 | -0.2934 |
| 1/slope | -1.926 |
| 95% Confidence Intervals | |
| Slope | -0.5857 to -0.4529 |
| Y-intercept when X=0.0 | -0.3308 to 0.02605 |
| X-intercept when Y=0.0 | -0.7250 to 0.04479 |
| Goodness of Fit | |
| R square | 0.8145 |
| Sy.x | 0.2352 |
| Is slope significantly non-zero? | |
| F | 245.8 |
| DFn, DFd | 1.000, 56.00 |
| P value | <0.0001 |
| Deviation from zero? | Significant |

**LOQ = Antilog [-1.926 (-1.0 + 0.1524)] = 42.90**
**LOD = Antilog [-1.926 (-0.478 + 0.1524)] = 4.25**

## Measurement Uncertainty

In this SLV, an independent estimate of spike concentration was not used. Therefore, the LOQ and LOD had to be determined as a range of values determined as the measurement uncertainty. Measurement Uncertainty was determined by subtracting the log replicate plate values from the log estimated mean spike, then calculating the 95% confidence limits of the mean difference. Table 4 shows these statistics from the quahogs.

Table 4 – Measurement Uncertainty for Quahogs.

| | | antilog |
|---|---|---|
| Number of values | 172 | |
| Mean | 0.0178 | **1.042** |
| Std. Deviation | 0.288 | |
| Std. Error | 0.009816 | |
| Lower 95% CI of mean | -0.00158 | **0.996** |
| Upper 95% CI of mean | 0.03718 | **1.089** |

From the regression, the LOQ intercept of -1.0 on the y-axix (log coefficient of variation) of Graph 1 and Table 3 equals 1.63248 on the x-axis (log estimated mean spike). The LOD intercept at -.0478 on the y-axix of Graph 1 and Table 3 equals 0.62711 on the x-axis. Substracting the lower limit of the measurement uncertainty log value -0.00158 from the LOD log value of 0.62711 equals 0.6287. The antilog of which is the lower limit of 4.25 for LOD.

Adding the upper limit of the measurement uncertainty log value of 0.03718 to the LOD log value of 0.62711 equals 0.66429. The antilog of which is the upper limit of 4.62 for LOD. Substracting the lower limit of the measurement uncertainty log value -0.00158 from the LOQ log value of 1.63248 equals 1.6341. The antilog of which is the lower limit of 43.06 for LOQ. Adding the upper limit of the measurement uncertainty log value of 0.03718 to the LOD log value of 1.63248 equal 1.6697. The antilog of which is the upper limit of 46.74 for LOQ.

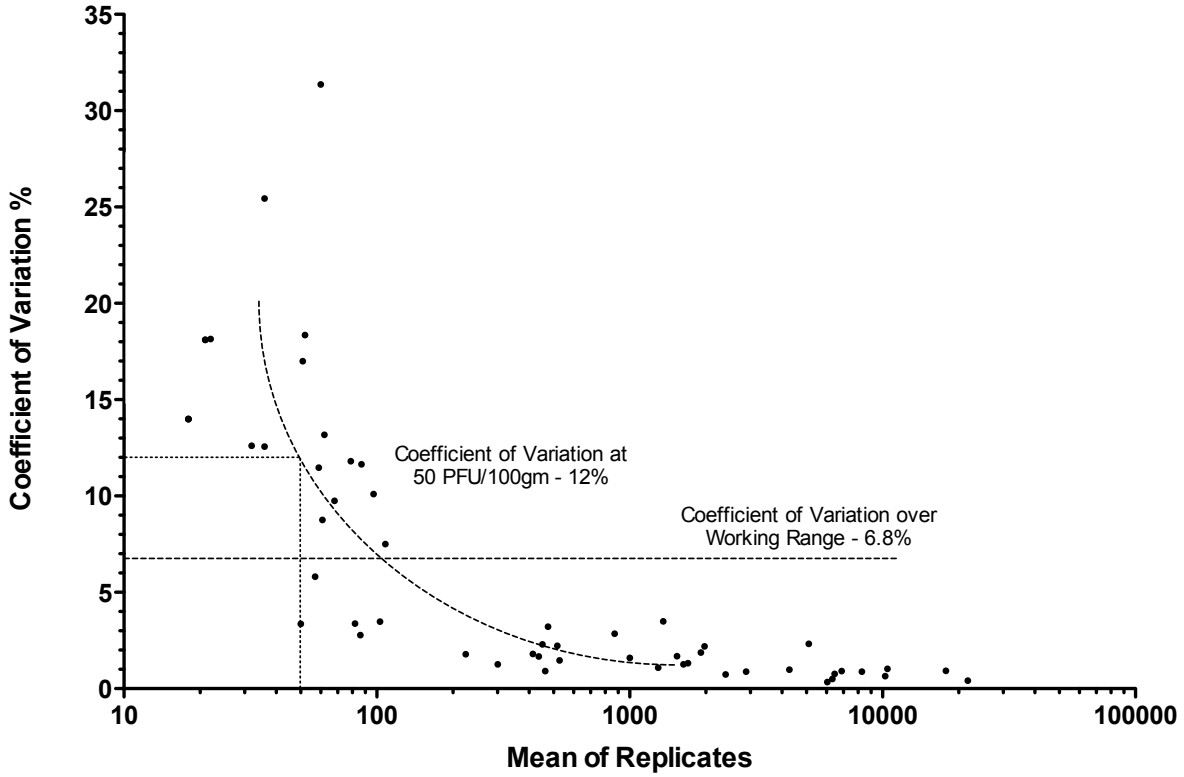
In summary, the LOD for quahogs ranges from 4.25 to 4.65 PFU/100gram. The LOQ for quahogs ranges from 43.06 to 46.74. As a result, a conservative estimate for the LOD and LOQ for quahogs was chosen to be 5 and 47 PFU/100gm, respectively. The upper working range is estimated to be approximately 200 PFU per plate or 20,000 PFU/100gm. In summary, the method has a working range of 5 PFU/100gm to 20,000PFU/100gm for quahogs. This method is fit for purpose with respect to a regulatory level of 50 PFU/100gm as the LOQ is less than the regulatory level.

**Data Summary: Quahogs**
Working range of the method as implemented 5 to 20,000 PFU/100gm
The limit of detection of the method as implemented 5 PFU/100gm
The limit of quantitation/sensitivity of the method as implemented 47 PFU/100gm

**The Determination of Accuracy/Trueness** is based upon the NSSP SOP for the Single Laboratory Validation of Marine Biotoxin and Non-MPN Based Microbiological Methods using the more robust databases acquired from the determination of the LOQ/LOD/Linear Range. Because we do not have an independant estimate of spike concerntration in this SLV, The Accuracy/Trueness can not be calculated.

**The Determination of the Precision and Recovery** is based upon the NSSP SOP for the Single Laboratory Validation of Marine Biotoxin and Non-MPN Based Microbiological Methods using the more robust data set acquired from the determination of the LOQ/LOD/Linear Range. To examine the precision over the working range of the method, a simple graphical approach was followed. The coefficients of variation were determined from the log transformed replicate data (50 sets of three true replicates) and were plotted verses the mean of the triplicate results (non log transformed data). The results are shown in Graph 2 for quahogs.

Graph 2 - Coefficient of Variability (%) of Replicates verses Mean of Replicate for Quahogs.




In Graph 2 above, the coefficient of variation at 50PFU/100gm level was determined graphically (approximately 12% for Quahogs) and shows the precision at this regulatory point. As expected, the precision decreases as the LOQ and LOD are approached. The mean, minimum, and maximum coefficient of variations as determined over the working range for quahogs appear in Table 5 below.

Table 5 – Mean, Minimum, and Maximum Coefficient of Variation over the Working Ranges.

Average Coefficient of Variation = 6.81%
Minimum Coefficient of Variation = 0.34%
Maximum Coefficient of Variation = 31%

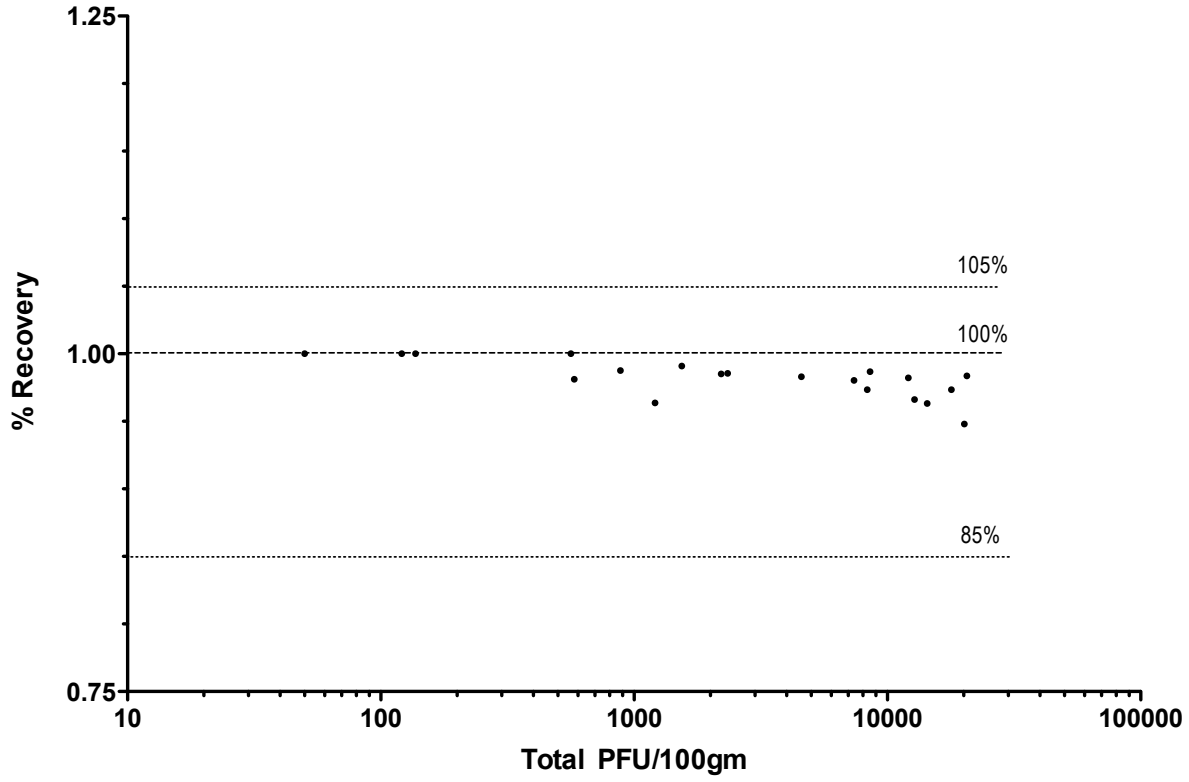
To determine the recovery of the method, a routine of re-washing the pellet into growth broth, then re-processing and re-plating twice (until depletion) was employed to directly determine the recovery. Supplemental samples 11 through 21 were spiked at lower levels to assure that recovery was consistent at low to high range concentrations along the working range. Table 6 show this recovery data for quahogs. The viral extraction demonstrated by this routine for this method varies from 94.8% to 100%.

Table 6 - Direct Recovery to Depletion for Quahogs.

| | MSC Recovered | | | |
|---|---|---|---|---|
| **Trial #** | 1 ml of Master Spike 33 gm homogenate (PFU/100gm) | Rewash Pellet and process (PFU/100gm) | Rewash 2nd Pellet and process (PFU/100gm) | Total PFU's |
| **1** | 13834 **96.32%** | 495 **3.45%** | 33 **0.23%** | 14362 |
| **2** | 19093 **94.80%** | 1026 **5.09%** | 22 **0.11%** | 20141 |
| **3** | 20289 **98.37%** | 336 **1.63%** | 0 **0.00%** | 20625 |
| **4** | 17433 **97.35%** | 463 **2.59%** | 11 **0.06%** | 17907 |
| **5** | 8424 **98.68%** | 113 **1.32%** | 0 **0.00%** | 8537 |
| **7** | 8117 **97.35%** | 221 **2.65%** | 0 **0.00%** | 8338 |
| **8** | 12357 **96.61%** | 434 **3.39%** | 0 **0.00%** | 12791 |
| **9** | 7232 **98.03%** | 145 **1.97%** | 0 **0.00%** | 7377 |
| **10** | 11889 **98.22%** | 216 **1.78%** | 0 **0.00%** | 12105 |
| **11** supplemental | 4497 **98.30%** | 78 **1.70%** | 0 **0.00%** | 4575 |
| **12** supplemental | 2176 **98.51%** | 22 **1.00%** | 11 **0.50%** | 2209 |
| **13** supplemental | 2306 **98.55%** | 34 **1.45%** | 0 **0.00%** | 2340 |
| **14** supplemental | 1528 **99.09%** | 0 **0.00%** | 14 **0.91%** | 1542 |
| **15** supplemental | 1167 **96.37%** | 33 **2.73%** | 11 **0.91%** | 1211 |
| **16** supplemental | 570 **98.11%** | 11 **1.89%** | 0 **0.00%** | 581 |
| **17** supplemental | 563 **100.00%** | 0 **0.00%** | 0 **0.00%** | 563 |
| **18** supplemental | 872 **98.75%** | 11 **1.25%** | 0 **0.00%** | 883 |
| **19** supplemental | 50 **100.00%** | 0 **0.00%** | 0 **0.00%** | 50 |
| **20** supplemental | 121 **100.00%** | 0 **0.00%** | 0 **0.00%** | 121 |
| **21** supplemental | 137 **100.00%** | 0 **0.00%** | 0 **0.00%** | 137 |

The average percent recovery of the method as implemented by this laboratory is calculated by averaging the above results and is reported at 98.2% with the sequential rewashing routine. Graph 3 shows the % Recovery verses Total PFU's and shows consistently high recovery over the working range.

Graph 3 - % Recovery verses Total PFU's over the Working Range




**Data Summary: Quahogs**

- Is the precision of the method under study consistent through the working range? **No, it varies as expected as the method approaches the LOD**
- The coefficient of variation of the test method as implemented is **6.8%** .
- Is the recovery of the method under study consistent through the working range? **Yes, it is consistently high over the working range**
- What is the overall percent recovery of the method under study? **98.2%**

**Ruggedness** was determined using the NSSP SOP for the Single Laboratory Validation of Marine Biotoxin and Non-MPN Based Microbiological Methods.

Different lots of agar, tryptone, and host E-coli culture and were prepared well in advance of the trials. Ten different harvest lots of quahogs were used for these analyses. Table 7 shows the data, data analysis, and the results of the paired t-test for quahogs.

Table 7 - Determination of the Method Ruggedness for Quahogs.

| Media A PFU/100gm | Media B PFU/100gm | Log Media A | Log Media B |
|---|---|---|---|
| 3309 | 3451 | 3.5197 | 3.5379 |
| 5224 | 5660 | 3.7180 | 3.7528 |
| 664 | 617 | 2.8222 | 2.7903 |
| 123 | 157 | 2.0899 | 2.1959 |
| 1985 | 2600 | 3.2978 | 3.4150 |
| 346 | 592 | 2.5391 | 2.7723 |
| 110 | 143 | 2.0414 | 2.1553 |
| 3485 | 3056 | 3.5422 | 3.4852 |
| 4316 | 3959 | 3.6351 | 3.5976 |
| 1902 | 1792 | 3.2792 | 3.2533 |
| | Skew | -0.7036 | -0.7246 |
| | Variance | 0.4019 | 0.3388 |
| | Ratio of Larger Var to Lower Var | 1.1862 | |

skew between -2 and 2 indicates symmetry
Ratio of Varieances < 2 indicates homogeneity of variance

| Paired t-test (Media A verses Media B) | |
|---|---|
| P value | 0.1442 |
| P value summary | ns |
| Are means signif. different? (P < 0.05) | No |
| One- or two-tailed P value? | Two-tailed |
| t, df | t=1.600 df=9 |
| Number of pairs | 10 |

**Data Summary: Quahogs**
Value for the test of symmetry of the distribution of Media A data -.7036
Value for the test of symmetry of the distribution of Media B data -.7246
Variance of Media A data .4019
Variance of Media B data .3388
Ratio of the larger to the smaller of the variances of Media A and Media B 1.1862
Is there a significant difference between Media A and Media No

**Acknowledgement**

This Spinney Creek Shellfish, Inc. Single Laboratory Validation study was the culmination of previous work conducted by the FDA, Captain William Burkhardt, Dauphin Island Laboratory, AL and presented at the 2005 Conference in proposals 05-105, 05-114, and 05-113. Many thanks go to Ms. Mercuria Cumbo, Microbiologist, Maine Department of Marine Resources for her initial and patient instruction of the FDA method to Spinney Creek Shellfish, Inc. personnel. Ms. Cumbo was the first to observe viral extraction problems with soft-shelled clams and was instrumental in modifying this method to improve extraction efficiencies. Her constant technical assistance and direction throughout the SLV study was instrumental in the success of this project. Many thanks as well to Ms. Linda Chandler, FDA, College Park, MD who advised us in the modification of the method and well as constant oversight with the SLV study. Ms. Chandler's helpful insight into the SOP's, technical expertise, and review of the SLV results and document was pivotal in the completion to the project. Partial support was received from the New Hampshire Sea Grant College Program under Grant No. NA10OAR4170082 (CFDA No. 11.417) from the National Oceanic and Atmospheric Administration. Many thanks to Dr. Stephen H. Jones of the University of New Hampshire and the University staff for providing guidance and assisting with this opportunity. The findings, opinions and recommendations expressed in this report are those of the author and not necessarily those of University or of the Federal Awarding Agency. Finally, special thanks are due to Laura Stadig, Spinney Creek Shellfish, Inc. She worked tirelessly and precisely over many months, to execute the tedious task of performing the SLV laboratory work. Thanks to all, this was at all levels a group effort.

NSSP Guide for the Control of Molluscan Shellfish

Section I. Model Ordinance

Chapter II. Risk Assessment and Risk Management

@.01 Outbreaks of Shellfish Related Illness

***Insert New Section:***

F. When the investigation outlined in Section @.01 A. indicates the illness(es) are associated with the naturally occurring pathogen *Vibrio parahaemolyticus (V.p.)* , the Authority shall determine the number of cases epidemiologically associated with implicated area and actions taken by the Authority will be based on the number of cases and the span of time as follows.

(1) When sporadic cases do not exceed a risk of one (1) illness per 100,000 servings or involves at least two (2) but not more than four (4) cases occurring within a thirty (30) day period from a hydrologically connected water body in which no two (2) cases occurred from a single harvest day, the Authority shall:

(a) Determine the extent of the hydrologically connected water body, and
(b) Issue a consumer advisory for all shellfish (or species implicated in the illness) from the implicated area; and
(c) Notify receiving States, the ISSC and the FDA Regional Shellfish Specialist that a potential health risk is associated with shellfish harvested from the implicated growing area, and

(2) When the risk exceeds one (1) illness per 100,000 servings within a thirty (30) day period or when cases exceed four (4) but not more than ten (10) over a thirty (30) day period from a hydrologically connected water body and when two (2) or more cases but less than four (4) cases occur from a single harvest day, the Authority shall:

(a) Determine the extent of the hydrologically connected water body; and

(b) Issue a consumer advisory for all shellfish (or species implicated in the illness) from the implicated growing area; and
(c) Immediately place the implicated portion(s) of the harvest area(s) in the closed status; and
(d) Notify receiving States, the ISSC, and the FDA Regional Shellfish Specialist that a potential health risk is associated with shellfish harvested from the implicated growing area; and
(e) As soon as determined by the Authority, transmit to the FDA and receiving States information identifying the dealers shipping the implicated shellfish.

(3) When the number of cases exceeds ten (10) illnesses within a thirty (30) day period from a hydrologically connected growing area or four (4) cases occurred from a single harvest date, The Authority shall:

(a) Determine the extent of the hydrologically connected water body; and
(b) Immediately place the implicated portion(s) of the harvest area(s) in the closed status; and
(c) Promptly initiate a voluntary industry recall consistent with the Recall Enforcement Policy, Title 21 CFR Part 7. The recall shall include all implicated products.

(4) When a growing area has been closed as a result of *V.p.* cases, the Authority shall keep the area closed for the following periods of time to determine if additional illnesses have occurred:

(a) The area will remain closed for a minimum of seven (7) days when sporadic cases do not exceed a risk of one (1) illness per 100,000 servings or involves four (4) or less cases occurring within a thirty (30) day period from a hydrologically connected water body in which no two (2) cases occurred from a single harvest date.

(b) The area will remain closed for a minimum of fourteen (14) days when the risk exceeds one (1) illness per 100,000 servings within a thirty (30) day period or cases exceed four (4) but not more than ten (10) cases over a thirty (30) day period from a hydrologically connected water body with two (2) or more cases but less than four (4) cases occurring from a single harvest date.
(c) The area will remain closed for a minimum of twenty-one (21) days when the number of cases exceeds ten (10) illnesses within thirty (30) days or four (4) cases occur from a single harvest date from a hydrologically connected growing area,

(5) Prior to reopening an area closed as a result of *V.p.* cases, the Authority shall:

(a) Collect and analyze samples to ensure that tdh does not exceed 10/g and trh does not exceed 10/g; or
(b) Ensure that environmental conditions have returned to levels not associated with *V.p.* cases.

(6) Shellfish harvesting may occur in an area closed as a result of *V.p.* illnesses when the Authority implements one or more of the following controls:

(a) Post harvest processing using a process that has been validated to achieve a two (2) log reduction in the levels of total *Vibrio parahaemolyticus* for

Gulf and Atlantic Coast oysters and a three (3) log reduction for Pacific Coast oysters;

(b) Restricting oyster harvest to product that is labeled for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing;

(c) Limiting the time to one (1) hour from harvest to an internal temperature of 50°.

(d) Other control measures that based on appropriate scientific studies are designed to ensure that the risk of *V.p.* illness is no longer reasonably likely to occur, as approved by the Authority.

PUBLIC HEALTH RATIONALE

The ISSC stakeholders have worked hard since the 1990s, using a number of science and policy tools to mitigate the public health effects associated with *Vibrio* species, most notably *Vibrio vulnificus* (Vv) and *Vibrio parahaemolyticus* (Vp). As a result, the Model Ordinance has slowly evolved with different requirements for Vv and Vp. These controls include the use of Vv Control Plans (VVCP) and Vp Control Plans (VPCP) which vary from state to state. States requiring Vv controls generally must implement more restrictive harvest controls than states which only require Vp control plans. Additionally, risk per serving standards associated with VVCP require corrective actions that are absent in VPCP. This disparity creates an economic advantage for industry in states with less stringent requirements and potentially favors higher exposure to more risky product. This proposal will provide a level playing field for the shellfish industry by unifying the controls for Vp and Vv.

To-date, the Model Ordinance requirements have not been effective in reducing the number of cases of Vv and Vp. FoodNet data (Figure 1 below) indicates that vibriosis has more than doubled since the baseline years of 1996-98 while illnesses from all other major foodborne pathogens have either been stable or in most cases decreased during this same period [3]. COVIS data provided to ISSC supports similar increases in vibriosis in the US as observed with FoodNet. Vv and Vp Control Plans are not achieving expected illness reductions. In fact, Vv illnesses have exceeded the ISSC baseline each of the three years since the VVCP was implemented in 2010 and reported Vp illnesses have increased four of the five years since implementation of the VPCP in 2008. There have also been 49 deaths due to Vv since 2010 and 21 due to Vp since 2008 [8, 11].

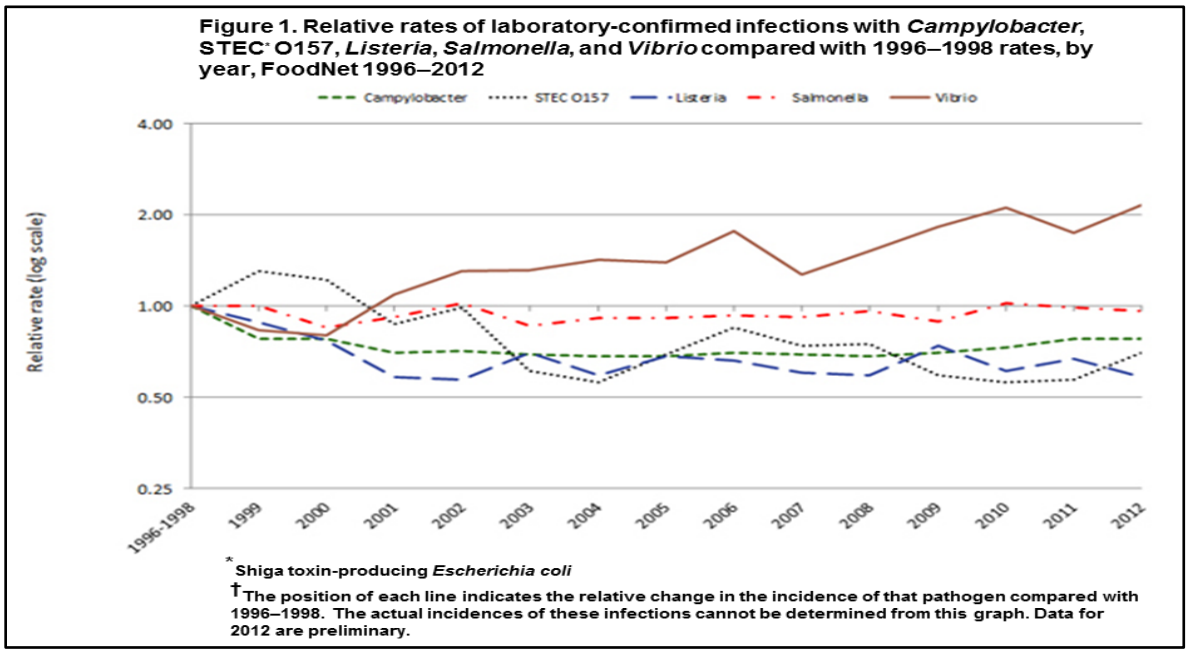



**Figure 1. Relative rates of laboratory-confirmed infections with *Campylobacter*, STEC* O157, *Listeria*, *Salmonella*, and *Vibrio* compared with 1996–1998 rates, by year, FoodNet 1996–2012**

*Shiga toxin-producing *Escherichia coli*

†The position of each line indicates the relative change in the incidence of that pathogen compared with 1996–1998. The actual incidences of these infections cannot be determined from this graph. Data for 2012 are preliminary.

The cost of vibriosis to society is significant. Economists and epidemiologists can provide formulas for estimating the acute health costs of morbidity and mortality factors (human illness and deaths). There are also significant costs associated with the public health responses required; case investigations, trace back to harvest areas, closure and opening protocols and product recalls. However, the costs to the oyster and clam industries also include the loss of customer and consumer confidence, both in the US and export markets such as the European Union. The efforts by the ISSC to date to control vibriosis have been unsuccessful. This evidenced by petitions from consumer advocates, audits by GAO and refusal of product by international trading partners [2, 4, 9].

There are likely several reasons for the increasing incidence of vibriosis, including improved clinical diagnosis and illness surveillance systems, increased raw shellfish consumption patterns, expanded seasonal and geographical range of illness and the emergence of highly virulent strains. For example, the introduction of the US West Coast outbreak strain of Vp into the Long Island Sound in 2012 caused the largest oyster-associated outbreak ever reported along the Atlantic Coast, tripling 2012 Atlantic Vp cases relative to the previous 5-year mean [10, 12] . This outbreak strain re-emerged in the same area in 2013 and illnesses expanded geographically from MA to VA by July [12]. The 2013 Vp case count to-date far exceeds 2012 figures for the entire season and is likely to increase considering the long lag between harvest and illness reporting and because the 2013 season continues. Numerous outbreaks, area closures and recalls have disrupted the industry and brought negative publicity about deteriorating shellfish safety.

**Figure 2. Estimated Atlantic (North and Mid-Atlantic States) servings for summer (July-Sept) season assuming 50% raw consumption and 200g per serving.** Imputed serving estimates for 2012 and 2013 are estimates based on recent (5 years) of data and considering early season closures due to Vp outbreaks.

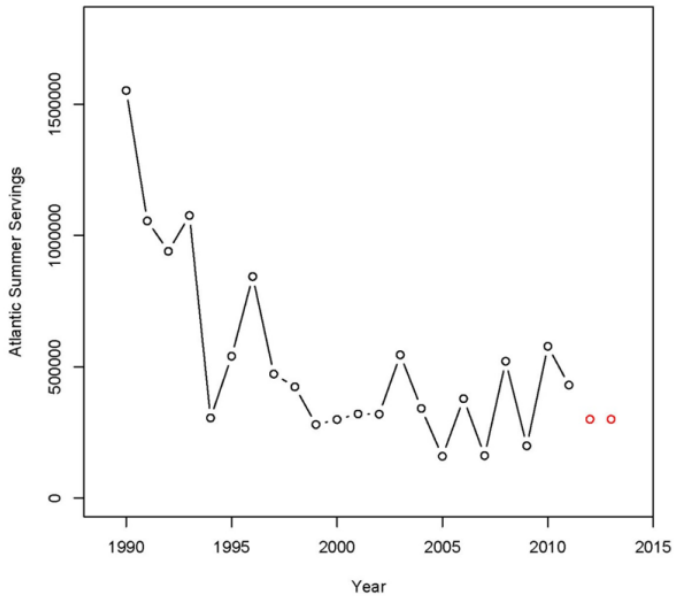



Figure 2 indicates relatively stable shellfish production in the Atlantic region since 2000 and projects 2012 and 2013 servings based on average harvest from 2007-2011. Figures 3 and 4 highlight the increase in illnesses and risk since 2012 after the introduction of the Pacific NW outbreak strain.

**Figure 3: Oyster Associated Vp illnesses associated with summer (July – Sept) harvest from Atlantic (North and Mid-Atlantic) States from 1997 to the present.** Vp Illnesses for 2012 and 2013 estimated to be 28 and 54, respectively. Estimates for 2013 are based on incomplete data.

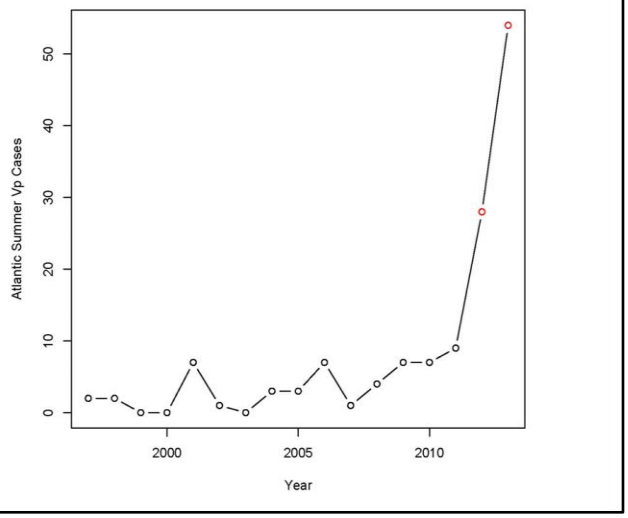
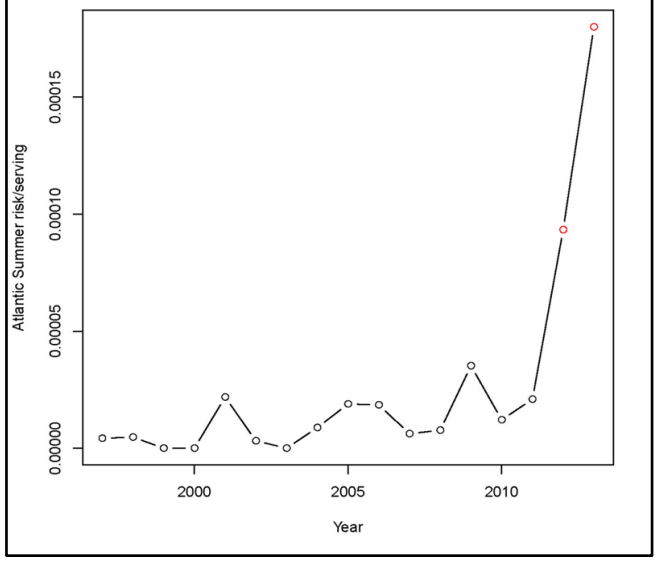



**Figure 4. Estimated Risk per serving for Atlantic summer oysters assuming no under-reporting or under-diagnosis.**




Sound scientific information is available on the conditions required to prevent growth of the vibrio pathogens: Vv ≤55°F and Vp ≤50°F [5]. As with other foodborne pathogens, the risk of vibrio illness increases relative to the exposure to the organism. In other words, the more vibrio bacteria consumed the higher the chance the shellfish consumer will become ill. For example, FDA and FAO/WHO risk assessments for Vp assume a doubling of risk each time the bacteria doubles [1, 7]. The FAO/WHO Vv risk assessment assumes that the risk increases about 1.5-fold for each doubling [6]. Generation times for Vp can be as fast as one hour when ambient temperatures are around 90°F and almost as fast for Vv.

Immediate cooling upon harvest would prevent post-harvest vibrio growth, maintain levels present at the time of harvest, and provide enhanced public health protection relative to the current VVCPs and VPCPs. This approach is consistent with the international guidance put forward in the Codex Alimentarius guidance for bivalve mollusks[5] and industry cooling practices with other seafood products that are inherently less risky. Immediate cooling at the time of harvest is considered to be the best management practice, offering significant risk reduction, which can be used in the process of harvesting shellfish that are to be consumed raw.

While exploring the practicality of immediate cooling, FDA has undertaken field studies on board small harvesting vessels. These studies demonstrated that oysters coming from warm harvest waters (80-90°F) can be cooled to less than 50°F within 30 minutes using an ice slurry system without significant hindrance of crew harvesting activity. Frequently asked questions regarding the cost, risks and benefits of using ice are listed in Appendix A.

The public health benefit from immediate cooling at harvest time would be significant. Tables 1 and 2 depict the estimated benefits of cooling for Vp and Vv, respectively.

**Table 1. Estimated Benefits of Rapid Cooling *Vibrio Parahaemolyticus* based on reported and laboratory confirmed illnesses without the adjustments for under-reporting or under-diagnosis.**

| Region | Reported Illnesses/year (Baseline 2008-2011) | Predicted # of Reported Illnesses/year(Rapid Cooling) | Predicted %-Reduction in Reported Illness | Predicted Cost* of Reported Illness (Baseline) (Millions) | Predicted Cost* of Reported Illness (Rapid Cooling) (Millions) |
|---|---|---|---|---|---|
| Atlantic | 20.1 | 1.0 | 95% | 0.95 | 0.047 |
| Gulf | 16.4 | 1.6 | 90% | 0.78 | 0.076 |
| PNW | 131 | 7.9 | 94% | 6.22 | 0.38 |
| **TOTAL** | **167.5** | **10.5** | **94%** | **7.95** | **0.50** |

*Cost per reported illness determined as $47,500 by combining Ralston's cost estimates for each of 3 illness severity classes (2=seek physician ($500), 3=hospitalization ($10,000), 4=death ($5,000,000)) with probabilities of each severity class among reported illnesses (2=seek physician (77.8%), 3=hospitalization (21.3%), 4=death (0.9%) as determined by Scallan et al.

**Table 2. Estimated Benefits of Rapid Cooling *Vibrio vulnificus* based on reported and laboratory confirmed illnesses without the adjustments for under-reporting or under-diagnosis.**

| Gulf State | Predicted # of reported Illness (Baseline) | Predicted # of reported Illness (Rapid Cooling) | Predicted % Reduction in Reported Illness | Predicted Cost* of Reported Illness (Baseline) (Millions) | Predicted Cost* of Reported Illness (Rapid Cooling) (Millions) |
|---|---|---|---|---|---|
| Texas | 4.1 | 3.0 | 27% | 7.2 | 5.3 |
| Louisiana | 11.7 | 9.3 | 20% | 20.6 | 16.4 |
| Florida | 2.3 | 1.3 | 41% | 4.0 | 2.4 |
| **TOTAL** | **18.1** | **13.6** | **25%** | **31.8** | **24.1** |

*Cost per reported illness determined as 1.76 million by combining Ralston's cost estimates for each of 3 illness severity classes (2=seek physician ($500), 3=hospitalization ($10,000), 4=death ($5,000,000) with probabilities of each severity class among reported illnesses (2=seek physician (8.7%), 3=hospitalization (56.3%), 4=death (35%) as determined by Scallan et al.; predicted number of reported cases for baseline and immediate cooling scenarios in selected states (TX, LA, FL) were determined using the Vv calculator assuming: (a) baseline time-to-refrigeration, cooldown time and oyster temperatures at harvest equal to that specified in Vv management plans in effect in each state (TX, LA, FL); (b) 1.46 million Gulf oyster servings per year consumed by at risk individuals distributed by month as specified in Vv management plans; (c) 21% of Gulf servings attributed to TX, 57% attributed to LA, and 10% attributed to FL (based on NMFS landings data).

The FDA Dauphin Island scientific team is currently working on a number of projects associated with oyster cooling practices. At the time of writing this rationale the results and conclusions from these projects are not available. This information will be available at the 2013 ISSC meeting.

Aside from the projected reduction in morbidity and mortality numbers, there will be further positive effects associated with acceptance of this proposal. This proposal would unify and simplify the controls for Vp and Vv and provide a level playing field for all of industry. There likely also would be a gain in trust by national and international customers and consumer advocacy groups. While immediate cooling is not as effective as Post Harvest Processing (PHP) or closures, it is far less disruptive to the nation's commercial shellfish industry than those approaches and offers a control strategy generally available to all the shellfish industry.

As with any regulatory policy, implementation will be critical for success. There will need to be ownership by the industry and verification by State regulators that the policy is being actively implemented. To implement this proposal, if adopted, industry will be required to make some changes to their harvesting vessels and ensure that they have access to the resources that enable immediate cooling such as containers to maintain shellfish at cooled temperatures. Additional obstacles, such as the availability of "approved" ice supplies may need to be overcome. Therefore, it may be appropriate for the ISSC to consider a stepped process to allow industry to achieve full compliance over 2 years.

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12. Newton A. COVIS Summary Data. 2013.

## Appendix A
## On Board Oyster Icing: Frequently Asked Questions

What are vibrios and why are they a problem?

- Vibrios are naturally occurring bacteria commonly found in oysters during warm months
- Vibrios can cause diseases ranging from diarrhea to death

Why is rapid cooling of oysters needed?

- Vibrios present at harvest can grow until oysters are cooled to 50F
- As the vibrios double so does the risk of illness

Is it feasible to cool oysters rapidly on small harvest boats?

- Ice is the most effective means for rapid chilling of oysters on-board small boats
- Either layering ice with oysters or dipping in ice slurries are effective cooling methods

How much ice is needed and what is the cost?

- One bushel of ice in a slurry produced with 90°F seawater can cool 2 bushels of oysters
- Reuse of the ice slurry can reduce ice usage to 1 bushel of ice for 4 bushels of oysters
- The additional cost for purchase of ice is approximately $1/bushel or 80# sack

Is it safe to reuse ice slurries for repeated dipping of oysters?

- FDA research indicates that dipping oysters for 10-20 minutes does not allow any bacteria from the ice slurries to enter the shell and contaminate the meats

Will ice slurries kill oysters?

- Oyster dipped in ice slurries survive over a 2-week period as well as with conventional refrigeration

What new equipment and boat modifications are needed?

- Dipping container (5-gallon bucket, ice chest, plastic drum)
- Cold storage container (external ice chest, insulated hull with lid)

What are the benefits from rapid cooling?

- Reduced risk of illness
- Fewer closures from outbreaks
- Potentially higher prices for oysters produced under best management practices
- Longer harvest periods

- Prevents delays for out of state shipments

| **Proposal for Task Force Consideration at the Interstate Shellfish Sanitation Conference 2013 Biennial Meeting** | ☐ **Growing Area** ☒ **Harvesting/Handling/Distribution** ☐ **Administrative** |
|---|---|
| **Submitter:** | Kenichi Wiegardt/Pacific Rim Shellfish Sanitation Conference |
| **Affiliation:** | Pacific Rim Shellfish Sanitation Conference |
| **Address** | Shelton, Washington |
| **Phone:**<br>**Fax:**<br>**Email:** | (360) 244-3099<br><br>Oysterman73@hotmail.com |
| **Proposal Subject:** | *Panopea generosa* as Species Exempted from Shellstock Storage Critical Control Point |
| **Specific NSSP Guide Reference:** | NSSP Guide Section II. Model Ordinance<br>Chapter XIII. Shellstock Shipping .01 Critical Control Points<br>B. Shellstock Storage Critical Control Point - Critical Limits. |
| **Text of Proposal/ Requested Action** | (5) Product intended for relay, wet storage, or depuration, or either geoduck clams (*Panopea generosa*), or *Mercenaria sp* which are~~is~~ being cooled utilizing an Authority approved tempering plan are exempt from the requirement listed above in .01 B. (4) above.**[C]**<br><br>Implementation is to begin three (3) months after concurrence by FDA. This achieves the goal of not waiting until publication of the new NSSP Guide and takes into account the requirement that FDA approve all changes adopted at the ISSC Biennial Meeting, while minimizing unnecessary loss of geoduck product. |
| **Public Health Significance:** | The geoduck clam (*Panopea generosa*) was until 2010, referred to by the extinct clam name of *Panopea abrupta*. The optimum handling, keeping and shipping temperature is 47° to 52° Fahrenheit (8.3°-11.1° Celsius). The lower temperatures contained in the shellstock critical control point at Chapter XIII. .01. B. (4) would cause significant mortality in this product. |
| **Cost Information (if available):** | There is no projected cost for this proposal. There is expected cost savings associated with this proposal due to the high loss of product expected with compliance. |

| **Proposal for Consideration at the Interstate Shellfish Sanitation Conference 2013 Biennial Meeting** | ☐ **Growing Area** ☒ **Harvesting/Handling/Distribution** ☐ **Administrative** |
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| **Submitter:** | Lawrence Colby |
| **Affiliation:** | Borough of Highlands Depuration Committee |
| **Address** | 171 Bay Avenue<br>Highlands, NJ 07732 |
| **Phone:**<br>**Fax:**<br>**Email:** | 732-872-1224<br>unavailable<br>larrycolby@comcast.net |
| **Proposal Subject:** | Accounting of Shellfish Quantities in Depuration Facilities |
| **Specific NSSP Guide Reference:** | NSSP Section II Model Ordinance Chapter XV. Depuration |
| **Text of Proposal/ Requested Action** | Chapter XV. Depuration<br><br>Requirements for the Authority<br><br>[Note: The Authority must meet the requirements of this section even if the Authority does not formally adopt this Chapter in regulation.]<br><br>A. Prior to authorizing depuration, the Authority shall develop and maintain an effective program to:<br>(1) Control shellstock harvesting by special license in accordance with Chapter VIII. @.01 C.;<br>(2) Control shellstock transportation between the harvest area and the depuration facility to prevent shellstock from being illegally diverted to direct marketing;<br>(3) Approve the design and construction of the depuration facility or activity including subsequent changes;<br>B. If shellstock is transported interstate to be depurated, the Authorities in both States shall execute a memorandum of agreement to provide adequate control measures to prevent diversion prior to depuration.<br>C. The Authority shall review and approve the Depuration Plant Operating Manual prior to granting depuration certification.<br>D. The Authority shall review the depuration plant performance index and other records as part of the monthly inspections to verify that the process and CCP are effective and the process verification analysis is being performed properly.<br>E. The Authority shall maintain adequate records for each depuration facility. The following records for each facility shall be kept for the period of five years:<br>(1) Inspection reports and reviews of the plant performance in accordance to Section D. (above);<br>(2) Current Depuration Plant Operations Manual for each dealer (Section .03)~~.~~<u>; and</u><br>(3) <u>Precise inventory control and bio-security, before and after the depuration process.</u><br>F. The Authority shall assure that each dealer has procedures to assure that no shellstock which has not been depurated is removed from the depuration facility without the direct supervision of the Authority. |

Chapter XV. Depuration

Requirements for the Dealer

.03 Other Model Ordinance Requirements

I. Plant Operations Manual. The dealer shall prepare a written Depuration Plant Operations Manual (DPOM) according to Minimum Requirements of a Depuration Plant Operations Manual (below); and update the DPOM as necessary. A copy of the DPOM shall be kept in a location readily accessible to the trained personnel responsible for the depuration activity. The minimum requirements for a Depuration Plant Operations Manual shall address:
   (1) Introduction including:
      (a) Status of document (to create, revise, or update DPOM);
      (b) Ownership and principal(s) involved with operation of facility;
      (c) Address and phone number of owners and principles; and
      (d) Summary of proposed use of the depuration facility including statement of objectives of the operation of the plant, species to be processed, proposed periods of facility operation, proposed sources of shellfish, including potential harvest areas, and maximum capacity of plant.
   (2) Description of the facility including:
      (a) Site plan drawings;
      (b) Facility layout including detailed schematic of the entire depuration system;
      (c) Schematic drawing of process;
      (d) Product flow diagram showing product movement through facility (may be combined with Section 01 B. (3);
      (e) Statement that construction materials and fabrication will meet the requirements of Section 03 E. (1) and (2); and
      (f) Schematic of seawater delivery and distribution system.
   (3) Design specifications of depuration unit including:
      (a) Depuration tank diagram including tank dimensions and construction details, influent and effluent locations, operating water level, and typical container configuration;
      (b) Process water system describing type of system (flow-through or recirculating), pretreatment and filtration systems, disinfection system, and hydraulic schematic;
      (c) Shellfish containers construction and material meets Section .04 and Section .08 of this Chapter; and
      (d) List of equipment including washing, culling, and packing equipment, material handling equipment, and cleaning and sanitation equipment.
   (4) Laboratory to be utilized for microbial analyses (in house, government agency, private commercial);
   (5) Depuration process monitoring including:
      (a) Sampling protocols including frequency of sampling, number of samples, sampling locations, and methodology for process water analyzing, incoming shellstock, depurated shellstock, and growing waters;

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| | (b) Monitoring equipment maintenance and calibration procedures and copy of activity log forms that will be used for data entry;<br>(c) Process water monitoring protocol for physical and chemical parameters; and<br>(d) Data analysis and evaluation.<br>(6) Standard Operating Procedure for:<br>(a) Receiving and holding;<br>(b) Washing, culling, and placement of undepurated product in process tanks;<br>(c) Depuration unit operation;<br>(d) Monitoring of depuration unit operation;<br>(e) Removal of depurated product from process tanks;<br>(f) Storage parameters and procedures;<br>(g) Labeling/tagging procedures;<br>(h) Plant cleaning and sanitation; and<br>(i) Data analysis.<br>(j) Recall procedures.<br>(7) Record Keeping. List categories of information that will be recorded. Include copies of proposed forms to be used in each category. A single form may be used for several categories if properly designed.<br>(a) Shipping and receiving records;<br>(b) Plant Operation Log, including provisions for recording the values for chemical and physical parameters;<br>(c) Maintenance and Sanitation Log(s);<br>(d) Laboratory records; and<br>(e) Counts of shellfish before and after the depuration process, specifically including the total number, or volume of shellfish. Shellfish sold by the piece after depuration shall be counted by the piece upon landing. If sold by volume, then volume would be recorded at landing. |
| **Public Health Significance:** | To ensure that all product delivered to the depuration plant is properly placed into the depuration process it is critical that counts and amounts of shellfish are properly counted and volumes properly assessed upon receipt. Harvester allegations of missing or diverted shellfish imply that some product may be diverted from the process. |
| **Cost Information (if available):** | Since plant operators typically count product after the process, counting at the beginning instead should not impact the cost of the operation. |

| **Proposal for Task Force Consideration at the Interstate Shellfish Sanitation Conference 2013 Biennial Meeting** | ☐ **Growing Area** ☒ **Harvesting/Handling/Distribution** ☐ **Administrative** |
|---|---|
| **Submitter:** | US Food and Drug Administration |
| **Affiliation:** | US Food and Drug Administration |
| **Address** | Center for Food Safety and Applied Nutrition<br>5100 Paint Branch Parkway<br>College Park, Maryland 20740 |
| **Phone:**<br>**Fax:**<br>**Email:** | 240-402-2300<br>301-436-2601<br>paul.distefano@fda.hhs.gov |
| **Proposal Subject:** | *Vibrio parahaemolyticus* Control Plan for Hard Clams (*Merceneria merceneria)* |
| **Specific NSSP Guide Reference:** | NSSP Guide Section II Chapter II Risk Assessment and Risk Management Section @.06 *Vibrio parahaemolyticus* Control Plan |
| **Text of Proposal/ Requested Action** | @.06 *Vibrio parahaemolyticus* Control Plan<br><br>A. Risk Evaluation.<br>Every State from which oysters or hard clams (*Merceneria merceneria*) are harvested shall conduct a *Vibrio parahaemolyticus* risk evaluation annually. The evaluation shall consider each of the following factors, including seasonal variations in the factors, in determining whether the risk of *Vibrio parahaemolyticus* infection from the consumption of oysters or hard clams harvested from an area (hydrological, geographical, or growing) is reasonably likely to occur: (For the purposes of this section, "reasonably likely to occur" shall mean that the risk constitutes an annual occurrence)<br>(1) The number of *Vibrio parahaemolyticus* cases epidemiologically linked to the consumption of oysters or hard clams commercially harvested from the State; and<br>(2) Levels of total and tdh+ *Vibrio parahaemolyticus* in the area, to the extent that such data exists; and<br>(3) The water temperatures in the area; and<br>(4) The air temperatures in the area; and<br>(5) Salinity in the area; and<br>(6) Harvesting techniques in the area; and<br>(7) The quantity of harvest from the area and its uses i.e. shucking, half-shell, PHP.<br>B. Control Plan<br>(1) If a State's *Vibrio parahaemolyticus* risk evaluation determines that the risk of *Vibrio parahaemolyticus* illness from the consumption of oysters or hard clams harvested from a growing area is reasonably likely to occur, the State shall develop and implement a *Vibrio parahaemolyticus* Control Plan; or<br>(2) If a State has a shellfish growing area in which harvesting occurs at a time when average monthly daytime water temperatures exceed those listed below, the State shall develop and implement a *Vibrio parahaemolyticus* Control Plan. The average water temperatures representative of harvesting conditions (for a period not to exceed |

thirty (30) days) that prompt the need for a Control Plan are:

(a) Waters bordering the Pacific Ocean: 60°F.

(b) Waters bordering the Gulf of Mexico and Atlantic Ocean (NJ and south): 81°F.

(c) However, development of a Plan is not necessary if the State conducts a risk evaluation, as described in Section A. that determines that it is not reasonably likely that *Vibrio parahaemolyticus* illness will occur from the consumption of oysters or hard clams harvested from those areas.

(i) In conducting the evaluation, the State shall evaluate the factors listed in Section A. for the area during periods when the temperatures exceed those listed in this section;

(ii) In concluding that the risk is not reasonably likely to occur, the State shall consider how the factors listed in Section A. differ in the area being assessed from other areas in the state and adjoining states that have been the source of shellfish that have been epidemiologically linked to cases of *Vibrio parahaemolyticus* illness; or

(3) If a State has a shellfish growing area that was the source of oysters or hard clams that were epidemiologically linked to an outbreak of *Vibrio parahaemolyticus* within the prior five (5) years, the State shall develop and implement a *Vibrio parahaemolyticus* Control Plan for the area.

(4) For States required to implement *Vibrio parahaemolyticus* Control Plans, the Plan shall include the administrative procedures and resources necessary to accomplish the following:

(a) Establish one or more triggers for when control measures are needed. These triggers shall be the temperatures in Section B. (2) where they apply, or other triggers as determined by the risk evaluation.

(b) Implement one or more control measures to reduce the risk of *Vibrio parahaemolyticus* illness at times when it is reasonably likely to occur. The control measures may include: (i) Post harvest processing using a process that has been validated to achieve a two (2) log reduction in the levels of total *Vibrio parahaemolyticus* for Gulf and Atlantic Coast oysters and hard clams and a three (3) log reduction for the Pacific Coast oysters;

(i)

(ii) Closing the area to oyster and/or hard clam harvest;

(iii) Restricting oyster and/or hard clams harvest to product that is labeled for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing;

(iv) Limiting time from harvest to refrigeration to no more than five (5) hours, or other times based on modeling or sampling, as determined by the Authority in consultation with FDA;

(v) Limiting time from harvest to refrigeration such that the levels of total *Vibrio parahaemolyticus* after the completion of initial cooling to 60°F (internal temperature of the oysters or hard clams) do not exceed the average

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| | levels from the harvest water at time of harvest by more than 0.75 logarithms, based on sampling or modeling, as approved by the Authority;<br>(vi) Other control measures that based on appropriate scientific studies are designed to ensure that the risk of *V.p.* illness is no longer reasonably likely to occur, as approved by the Authority.<br>(c) Require the original dealer to cool oysters and/or hard clams to an internal temperature of 50°F (10°C) or below within ten (10) hours or less as determined by the Authority after placement into refrigeration during periods when the risk of *Vibrio parahaemolyticus* illness is reasonably likely to occur. The dealer's HACCP Plan shall include controls necessary to ensure, document and verify that the internal temperature of oysters and/or hard clams has reached 50°F (10°C) or below within ten (10) hours or less as determined by the Authority of being placed into refrigeration. Oysters or hard clams and/or hard clams without proper HACCP records demonstrating compliance with this cooling requirement shall be diverted to PHP or labeled *"for shucking only"*, or other means to allow the hazard to be addressed by further processing.<br>(d) Evaluate the effectiveness of the Plan.<br>(e) Modify the Control Plan when the evaluation shows the Plan is ineffective, or when new information is available or new technology makes this prudent as determined by the Authority.<br>(f) Optional cost benefit analysis of the *Vibrio parahaemolyticus* Control Plan.<br>C. The Time When Harvest Begins For the purpose of time to temperature control, time begins once the first shellstock harvested is no longer submerged. |
| **Public Health Significance:** | Hard clams, of the species *Mercenaria mercenaria,* from the Atlantic coast have been increasingly implicated in *Vibrio parahaemolyticus* illnesses in recent years and now constitute a significant risk second to oysters with regard to reported illnesses in the US. In order to reduce the incidence of *Vibrio parahaemolyticus* illnesses, States with a history of illnesses associated with hard clams harvested from their growing areas, and states where a risk evaluation has determined that the risk of *Vibrio parahaemolyticus* is reasonably likely, need to develop and implement a *Vibrio parahaemolyticus* control plan aimed at reducing the incidence of illness to no more than 1 illness in 100,000 servings. |
| **Cost Information (if available):** | |

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| **Submitter:** | US Food and Drug Administration |
| **Affiliation:** | US Food and Drug Administration |
| **Address** | Center for Food Safety and Applied Nutrition<br>5100 Paint Branch Parkway<br>College Park, Maryland 20740 |
| **Phone:**<br>**Fax:**<br>**Email:** | 240 402-2300<br>301 436-2601<br>paul.distefano@fda.hhs.gov |
| **Proposal Subject:** | *Vibrio parahaemolyticus* Control Plan Water Temperatures |
| **Specific NSSP Guide Reference:** | NSSP Guide Section II Chapter II Risk Assessment and Risk Management Section<br>@.06 *Vibrio parahaemolyticus* Control Plan<br>B. Control Plan (2) |
| **Text of Proposal/ Requested Action** | @.06 *Vibrio parahaemolyticus* Control Plan<br><br>A. Risk Evaluation.<br>Every State from which oysters are harvested shall conduct a *Vibrio parahaemolyticus* risk evaluation annually. The evaluation shall consider each of the following factors, including seasonal variations in the factors, in determining whether the risk of *Vibrio parahaemolyticus* infection from the consumption of oysters harvested from an area (hydrological, geographical, or growing) is reasonably likely to occur: (For the purposes of this section, "reasonably likely to occur" shall mean that the risk constitutes an annual occurrence)<br>(1) The number of *Vibrio parahaemolyticus* cases epidemiologically linked to the consumption of oysters commercially harvested from the State; and<br>(2) Levels of total and tdh+ *Vibrio parahaemolyticus* in the area, to the extent that such data exists; and<br>(3) The water temperatures in the area; and<br>(4) The air temperatures in the area; and<br>(5) Salinity in the area; and<br>(6) Harvesting techniques in the area; and<br>(7) The quantity of harvest from the area and its uses i.e. shucking, half-shell, PHP.<br>B. Control Plan<br>(1) If a State's *Vibrio parahaemolyticus* risk evaluation determines that the risk of *Vibrio parahaemolyticus* illness from the consumption of oysters harvested from a growing area is reasonably likely to occur, the State shall develop and implement a *Vibrio parahaemolyticus* Control Plan; or<br>(2) If a State has a shellfish growing area in which harvesting occurs at a time when average monthly daytime water temperatures exceed those listed below, the State shall develop and implement a *Vibrio parahaemolyticus* Control Plan. The average water temperatures representative of harvesting conditions (for a period not to exceed thirty (30) days) that prompt the need for a Control Plan are:<br>(a) Waters bordering the Pacific Ocean: 60°F.<br>(b) Waters bordering the Gulf of Mexico and Atlantic Ocean (NJ and |

south): 81°F.

(c) Waters bordering the Atlantic Ocean (NY and north): 60°F.

(~~c~~d) However, development of a Plan is not necessary if the State conducts a risk evaluation, as described in Section A. that determines that it is not reasonably likely that *Vibrio parahaemolyticus* illness will occur from the consumption of oysters harvested from those areas.

(i) In conducting the evaluation, the State shall evaluate the factors listed in Section A. for the area during periods when the temperatures exceed those listed in this section;

(ii) In concluding that the risk is not reasonably likely to occur, the State shall consider how the factors listed in Section A. differ in the area being assessed from other areas in the state and adjoining states that have been the source of shellfish that have been epidemiologically linked to cases of *Vibrio parahaemolyticus* illness; or

(3) If a State has a shellfish growing area that was the source of oysters that were epidemiologically linked to an outbreak of *Vibrio parahaemolyticus* within the prior five (5) years, the State shall develop and implement a *Vibrio parahaemolyticus* Control Plan for the area.

(4) For States required to implement *Vibrio parahaemolyticus* Control Plans, the Plan shall include the administrative procedures and resources necessary to accomplish the following:

(a) Establish one or more triggers for when control measures are needed. These triggers shall be the temperatures in Section B. (2) where they apply, or other triggers as determined by the risk evaluation.

(b) Implement one or more control measures to reduce the risk of *Vibrio parahaemolyticus* illness at times when it is reasonably likely to occur. The control measures may include: (i) Post harvest processing using a process that has been validated to achieve a two (2) log reduction in the levels of total *Vibrio parahaemolyticus* for Gulf and Atlantic Coast oysters and a three (3) log reduction for the Pacific Coast oysters;

(i)

(ii) Closing the area to oyster harvest;

(iii) Restricting oyster harvest to product that is labeled for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing;

(iv) Limiting time from harvest to refrigeration to no more than five (5) hours, or other times based on modeling or sampling, as determined by the Authority in consultation with FDA;

(v) Limiting time from harvest to refrigeration such that the levels of total *Vibrio parahaemolyticus* after the completion of initial cooling to 60°F (internal temperature of the oysters) do not exceed the average levels from the harvest water at time of harvest by more than 0.75 logarithms, based on sampling or modeling, as approved by the Authority;

(vi) Other control measures that based on appropriate scientific studies are designed to ensure that the risk of *V.p.* illness is no longer reasonably likely to occur, as approved by the Authority.

(c) Require the original dealer to cool oysters to an internal temperature of 50°F (10°C) or below within ten (10) hours or less as determined by the Authority after placement into refrigeration during periods

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| | when the risk of *Vibrio parahaemolyticus* illness is reasonably likely to occur. The dealer's HACCP Plan shall include controls necessary to ensure, document and verify that the internal temperature of oysters has reached 50°F (10°C) or below within ten (10) hours or less as determined by the Authority of being placed into refrigeration. Oysters without proper HACCP records demonstrating compliance with this cooling requirement shall be diverted to PHP or labeled *"for shucking only"*, or other means to allow the hazard to be addressed by further processing.<br>(d) Evaluate the effectiveness of the Plan.<br>(e) Modify the Control Plan when the evaluation shows the Plan is ineffective, or when new information is available or new technology makes this prudent as determined by the Authority.<br>(f) Optional cost benefit analysis of the *Vibrio parahaemolyticus* Control Plan.<br>C. The Time When Harvest Begins For the purpose of time to temperature control, time begins once the first shellstock harvested is no longer submerged. |
| **Public Health Significance:** | Presently Chapter II. Section @.06 B. (2) does not include a water temperature for New York and north. |
| **Cost Information (if available):** | |

| **Proposal for Task Force Consideration at the Interstate Shellfish Sanitation Conference 2013 Biennial Meeting** | ☐ **Growing Area** ☒ **Harvesting/Handling/Distribution** ☐ **Administrative** |
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| **Submitter:** | US Food and Drug Administration |
| **Affiliation:** | US Food and Drug Administration |
| **Address** | Center for Food Safety and Applied Nutrition<br>5100 Paint Branch Parkway<br>College Park, Maryland 20740 |
| **Phone:**<br>**Fax:**<br>**Email:** | 240 402-2300<br>301 436-2601<br>paul.distefano@fda.hhs.gov |
| **Proposal Subject:** | *Vibrio parahaemolyticus* Control Plan Risk Per Serving |
| **Specific NSSP Guide Reference:** | NSSP Guide Section II Chapter II Risk Assessment and Risk Management Section<br>@.06 *Vibrio parahaemolyticus* Control Plan<br>New D. |
| **Text of Proposal/ Requested Action** | @.06 *Vibrio parahaemolyticus* Control Plan<br><br>A. Risk Evaluation.<br>Every State from which oysters are harvested shall conduct a *Vibrio parahaemolyticus* risk evaluation annually. The evaluation shall consider each of the following factors, including seasonal variations in the factors, in determining whether the risk of *Vibrio parahaemolyticus* infection from the consumption of oysters harvested from an area (hydrological, geographical, or growing) is reasonably likely to occur: (For the purposes of this section, "reasonably likely to occur" shall mean that the risk constitutes an annual occurrence)<br>(1) The number of *Vibrio parahaemolyticus* cases epidemiologically linked to the consumption of oysters commercially harvested from the State; and<br>(2) Levels of total and tdh+ *Vibrio parahaemolyticus* in the area, to the extent that such data exists; and<br>(3) The water temperatures in the area; and<br>(4) The air temperatures in the area; and<br>(5) Salinity in the area; and<br>(6) Harvesting techniques in the area; and<br>(7) The quantity of harvest from the area and its uses i.e. shucking, half-shell, PHP.<br>B. Control Plan<br>(1) If a State's *Vibrio parahaemolyticus* risk evaluation determines that the risk of *Vibrio parahaemolyticus* illness from the consumption of oysters harvested from a growing area is reasonably likely to occur, the State shall develop and implement a *Vibrio parahaemolyticus* Control Plan; or<br>(2) If a State has a shellfish growing area in which harvesting occurs at a time when average monthly daytime water temperatures exceed those listed below, the State shall develop and implement a *Vibrio parahaemolyticus* Control Plan. The average water temperatures representative of harvesting conditions (for a period not to exceed |

thirty (30) days) that prompt the need for a Control Plan are:

(a) Waters bordering the Pacific Ocean: 60°F.

(b) Waters bordering the Gulf of Mexico and Atlantic Ocean (NJ and south): 81°F.

(c) However, development of a Plan is not necessary if the State conducts a risk evaluation, as described in Section A. that determines that it is not reasonably likely that *Vibrio parahaemolyticus* illness will occur from the consumption of oysters harvested from those areas.

(i) In conducting the evaluation, the State shall evaluate the factors listed in Section A. for the area during periods when the temperatures exceed those listed in this section;

(ii) In concluding that the risk is not reasonably likely to occur, the State shall consider how the factors listed in Section A. differ in the area being assessed from other areas in the state and adjoining states that have been the source of shellfish that have been epidemiologically linked to cases of *Vibrio parahaemolyticus* illness; or

(3) If a State has a shellfish growing area that was the source of oysters that were epidemiologically linked to an outbreak of *Vibrio parahaemolyticus* within the prior five (5) years, the State shall develop and implement a *Vibrio parahaemolyticus* Control Plan for the area.

(4) For States required to implement *Vibrio parahaemolyticus* Control Plans, the Plan shall include the administrative procedures and resources necessary to accomplish the following:

(a) Establish one or more triggers for when control measures are needed. These triggers shall be the temperatures in Section B. (2) where they apply, or other triggers as determined by the risk evaluation.

(b) Implement one or more control measures to reduce the risk of *Vibrio parahaemolyticus* illness at times when it is reasonably likely to occur. The control measures may include: (i) Post harvest processing using a process that has been validated to achieve a two (2) log reduction in the levels of total *Vibrio parahaemolyticus* for Gulf and Atlantic Coast oysters and a three (3) log reduction for the Pacific Coast oysters;

(i)

(ii) Closing the area to oyster harvest;

(iii) Restricting oyster harvest to product that is labeled for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing;

(iv) Limiting time from harvest to refrigeration to no more than five (5) hours, or other times based on modeling or sampling, as determined by the Authority in consultation with FDA;

(v) Limiting time from harvest to refrigeration such that the levels of total *Vibrio parahaemolyticus* after the completion of initial cooling to 60°F (internal temperature of the oysters) do not exceed the average levels from the harvest water at time of harvest by more than 0.75 logarithms, based on sampling or modeling, as

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| | approved by the Authority;<br>(vi) Other control measures that based on appropriate scientific studies are designed to ensure that the risk of *V.p.* illness is no longer reasonably likely to occur, as approved by the Authority.<br>(c) Require the original dealer to cool oysters to an internal temperature of 50°F (10°C) or below within ten (10) hours or less as determined by the Authority after placement into refrigeration during periods when the risk of *Vibrio parahaemolyticus* illness is reasonably likely to occur. The dealer's HACCP Plan shall include controls necessary to ensure, document and verify that the internal temperature of oysters has reached 50°F (10°C) or below within ten (10) hours or less as determined by the Authority of being placed into refrigeration. Oysters without proper HACCP records demonstrating compliance with this cooling requirement shall be diverted to PHP or labeled *"for shucking only"*, or other means to allow the hazard to be addressed by further processing.<br>(d) Evaluate the effectiveness of the Plan.<br>(e) Modify the Control Plan when the evaluation shows the Plan is ineffective, or when new information is available or new technology makes this prudent as determined by the Authority.<br>(f) Optional cost benefit analysis of the *Vibrio parahaemolyticus* Control Plan.<br>C. The Time When Harvest Begins For the purpose of time to temperature control, time begins once the first shellstock harvested is no longer submerged.<br><u>D. States implementing a Vibrio parahaemolyticus Control Plan shall determine the level of protection afforded by calculating the observed risk per serving based on the number of annual illnesses attributed to shellfish harvested from the state and the state's annual oyster and/or hard clam production. Modify the Control Plan when the observed risk per serving is greater than 1 illness per 100,000 servings.</u> |
| **Public Health Significance:** | In the absence of a requirement for states to determine the observed risk per serving, it is not possible to verify that the level of protection offered by state Control Plans is consistent with the level of protection (≤1 illness per 100,000 servings) intended by time and temperature controls as defined by the *Vibrio parahaemolyticus* risk calculator. Requiring states to determine the observed risk per serving using annual illness data and annual production data will allow the ISSC to gauge the success of state control plans and engage states in developing additional controls where necessary. During periods of unacceptable risk, further restrictions on time and temperature controls, or other equivalent measures, should be considered to reduce risk to an acceptable level. |
| **Cost Information (if available):** | |

| **Proposal for Task Force Consideration at the Interstate Shellfish Sanitation Conference 2013 Biennial Meeting** | ☐ **Growing Area**<br>☒ **Harvesting/Handling/Distribution**<br>☐ **Administrative** |
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| **Name of Submitter:** | ISSC Executive Board |
| **Affiliation:** | Interstate Shellfish Sanitation Conference |
| **Address Line 1:**<br>**Address Line 2:**<br>**City, State, Zip** | 209 Dawson Road<br>Suite 2<br>Columbia, SC 29223 |
| **Phone:**<br>**Fax:**<br>**Email:** | 803-788-7559<br>803-788-7576<br>issc@issc.org |
| **Proposal Subject:** | Implementation Date for Harvester and Dealer Training Requirements |
| **Specific NSSP Guide Reference:** | NSSP Guide Section II<br>Chapter VIII Control of Shellfish Harvesting .01 General A. and<br>Chapter X General Requirements for Dealers .04 Certification Requirements |
| **Text of Proposal/ Requested Action** | Change the implementation date for the harvester and dealer training requirements adopted in Proposal 09-212 from January 1, 2014 to January 1, 2015. |
| **Public Health Significance:** | In 2013 the ISSC Voting Delegates adopted Proposal 09-212 which requires training for harvesters and dealers. The Voting Delegates established an implementation date of January 1, 2014, for these training requirements. States are not prepared at this time to implement these requirements and a later implementation date is being suggested. |
| **Cost Information (if available):** | |

| **Proposal for Consideration at the Interstate Shellfish Sanitation Conference 2013 Biennial Meeting** | ☐ **Growing Area** ☒ **Harvesting/Handling/Distribution** ☐ **Administrative** |
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| **Submitter:** | US Food and Drug Administration |
| **Affiliation:** | US Food and Drug Administration |
| **Address** | Center for Food Safety and Applied Nutrition<br>5100 Paint Branch Parkway<br>College Park, MD 20710 |
| **Phone:**<br>**Fax:**<br>**Email:** | 240-402-2300<br>301-436-2601<br>Melissa.Evans@fda.hhs.gov |
| **Proposal Subject:** | Guidance for Submission of Post-Harvest Process Validation Studies |
| **Specific NSSP Guide Reference:** | NSSP Guide Section II Model Ordinance Chapter XVI. and Section IV Guidance Documents Chapter IV. |
| **Text of Proposal/ Requested Action** | Add a new Section .05 Template for Submission of Post-Harvest Process Validation Studies as follows:<br><br>In the *National Shellfish Sanitation Program* (NSSP) *Model Ordinance Chapter XVI: Post Harvest Processing* (PHP) it states that if a dealer elects to utilize a PHP for the purpose of making safety added labeling claims they must conduct a validation study to demonstrate the ability of the PHP to reduce the target pathogen(s) to acceptable levels. Specifics on target levels and approved methods of detection for pathogens are found in the Model Ordinance. All laboratory analysis must be performed by a laboratory that has been evaluated by FDA or an FDA certified LEO and found to "conform" or "provisionally conform" with the requirements of the National Shellfish Sanitation Program (NSSP) Model Ordinance Chapter III and supporting Guidance Documents. Results of the validation study should be submitted in the following format for review and consideration by state and federal shellfish control authorities. For validation of *Vibrio vulnificus* or *Vibrio parahaemolyticus* methods, checklist may be used as a guide.<br><br>1) TITLE OF PHP METHOD VALIDATED<br>2) SUMMARY<br>3) OBJECTIVES (Study Purpose)<br>a) Detailed description of the PHP method validated.<br>b) Target pathogen(s) and prescribed reduction.<br>4) METHOD OF ANALYSIS<br>a) Post-Harvest Process description.<br>i) Identify temperatures, weights or other pertinent information for the PHP method. Methods of mollusk preparation, for example acclimation to temperature or salinity, include all details. All variables that could affect the outcome of the PHP must be detailed.<br>ii) Identify number of animals used in study and number of trials performed.<br>b) Laboratory: (Pre and post processing pathogen measurement and description of analytical procedure)<br>i) Initial pathogen levels and pathogen detection model: microbiological |

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| | or chemical analysis.<br>(1) How was initial pathogen load achieved, i.e. naturally occurring population, inoculation or thermal abuse.<br>(2) Provide adjusted Geometric Mean (AGM) calculations and unit of measure appropriate for target (i.e.: MPN/g for Vibrio or coliforms, CFU/100g for Elevated Temperature Coliform Plates (ETCP fecals).<br>(3) Analytical methodology used for pathogen quantification and confirmation. This method must be recognized in the NSSP Guide for the Control of Molluscan Shellfish (Accepted methods listed in Section IV. Guidance Documents Chapter II.10 Approved National Shellfish Sanitation Program Laboratory Tests: Microbiological and Biotoxin Analytical Methods.)<br>ii) Post Process Product Analysis: microbiological or chemical analysis<br>(1) Quantify pathogen level(s) in processed product utilizing the same analytical method used to attain initial load.<br>c) Validation Outcome:<br>i) Provide specific information regarding outcome measurements. Metric used to validate method (these will vary depending on targeted pathogen and are located in the Model Ordinance). Documentation that process achieved target reduction.<br>5) RESULTS<br>a) Graphs, tables and charts outlining the validation study results.<br>i) Data from validation demonstration; levels achieved in post process.<br>ii) Pathogen measurements (for example: AGM interval, grams per tube and the number of positive tubes as per the guidance document for verification/validation).<br>6) CONCLUSIONS:<br>a) Demonstrate reduction of the target pathogen to NSSP established standards.<br>7) APPENDIX<br>a) Tables or graphical interpretations of data.<br>8) OPTIONAL INFORMATION<br>a) If appropriate, include optional items such as interpretation of confounding factors or applicable industry limitations.<br>b) Acknowledgements, for example funding sources, technical help or bibliography. |
| **Public Health Significance:** | The purpose of this proposal is to provide guidance for dealers conducting post-harvest processing validation studies for the purposes of labeling shellfish as outlined in Model Ordinance Chapter XVI. |
| **Cost Information (if available):** | |

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| | **Draft- Checklist for Submission of Post-Harvest Process Validation Studies for *Vibrio vulnificus* and *Vibrio parahaemolyticus*** |
| | **Explanation of PHP Method Validated** |
| | 1. Method name |
| | 2. Specific information about machinery, equipment, or supplies necessary to perform the method of PHP is provided |
| | 3. Standard operating procedures: Detailed description of the PHP method validated is |
| | 4. What are the specific issues that must be accounted for during processing? For example, is there a limit to number of shellfish, spacing, hold times that are considered part of the |
| | 5. Internal quality control measures for equipment calibration, maintenance, repair and for performance checks are explained. |
| | **Objectives to be Accomplished** |
| | 1. Does the process reduce the level of *Vibrio vulnificus* and/or *Vibrio parahaemolyticus* in the process to non-detectable (<30MPN/gram) and achieve a minimum 3.52 log reduction? |
| | 2. Was the process validated by demonstrating that the process will reliably achieve the appropriate reduction in the target pathogen(s) in a study as outlined in Guidance Documents Chapter IV, Naturally Occurring Pathogens. |
| | **Method of Analysis** |
| | 1. Was laboratory analysis performed by a laboratory that has been evaluated by FDA or an FDA certified LEO and found to "conform" or "provisionally conform" with the requirements of the National Shellfish Sanitation Program (NSSP) Model Ordinance Chapter III and supporting Guidance Documents? |
| | 2. Are all variables that could affect the outcome of the PHP identified: temperatures, weights or other pertinent information? |
| | **Pre Processed Samples to attain initial levels** |
| | 1. Microbiological testing for initial levels was done by a 3-tube MPN using appropriate dilutions (10-1 to 10-6). |
| | 2. Was the initial level of Vibrios for each lot of shellfish used in the validation 10,000 MPN per gram or greater based on the adjusted geometric mean (AGM) of the MPNs/g of four |
| | 3. How were the zero hour levels achieved: through naturally occurring Vibrio levels in shellfish, time/temperature abuse, inoculation? (Inoculation is not preferred) |
| | **Enumeration of or Processed Samples** |
| | 1. Does a sample consist of a composite of 10 to 12 oysters processed at one time from one |
| | 2. Is there data on ten processed samples obtained on each of three processing days (total of 30 samples)? |
| | 3. Microbiological testing for processed samples was done with a single dilution five-tube MPN, inoculating with either 0.01 g or 0.1 g of shellfish. |
| | 4. Are only analytical methods to determine Vibrio levels previously endorsed by the ISSC as indicated in Model Ordinance Chapter XVI. Post-Harvest Processing? |
| | 5. Was microbiological testing for processed samples done with a single dilution five-tube MPN, inoculating with either 0.01 g or 0.1 g of shellfish per tube? |
| | 6. **For the process to be validated, no more than three samples out of 30 may fail**. Failure is based on the Guide for the Control of Molluscan Shellfish 2009 Section IV. Guidance Documents Chapter IV. Naturally Occurring Pathogens .04 Post Harvest Processing (PHP) Validation/Verification Guidance for *Vibrio vulnificus* and *Vibrio parahaemolyticus.* |

| **Proposal for Consideration at the Interstate Shellfish Sanitation Conference 2013 Biennial Meeting** | ☐ **Growing Area** ☒ **Harvesting/Handling/Distribution** ☐ **Administrative** |
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| **Submitter:** | US Food and Drug Administration |
| **Affiliation:** | US Food and Drug Administration |
| **Address** | Center for Food Safety and Applied Nutrition<br>5100 Paint Branch Parkway<br>College Park, MD 20710 |
| **Phone:**<br>**Fax:**<br>**Email:** | 240-402-2300<br>301-436-2601<br>Melissa.Evans@fda.hhs.gov |
| **Proposal Subject:** | Guidelines for Primary Certified Shellfish Processors on Using Controls for Irradiation of Containers of Molluscan Shellfish Pre-labeled with Vibrio Reduction Language |
| **Specific NSSP Guide Reference:** | Section IV. Guidance Documents<br>Chapter III. Harvesting, Handling, Processing, and Distribution |
| **Text of Proposal/ Requested Action** | Add New Section .09<br><br>.09 Irradiation Pre-labeling Guidance<br><br>This document provides guidance to primary certified shellfish processors involved in transferring pre-labeled shellfish to be processed at irradiation post-harvest process (PHP) facilities.<br><br>Vibrios are highly sensitive to ionizing radiation. The National Shellfish Sanitation Program (NSSP) recognizes Vibrio reduction processes such as irradiation and provides general requirements for dealers using them. For irradiation the following guidelines provide additional detail:<br><br>• All shellfish irradiation facilities and shellfish processors using an irradiation facility to PHP shellfish must be recognized by their State Shellfish Control Authority (SSCA) as a certified PHP facility and comply with NSSP Model Ordinance Chapter XVI.<br><br>• Irradiation facilities must utilize a process that has been validated in accordance with the NSSP to achieve a reduction of *V.v.* and/or *V.p.* to less than 30 MPN/g. The process shall not irradiate shellfish to an absorbed dose of greater than 5.5 kGy, as provided by 21 CFR § 179.26. While the size of the container of shellfish does not affect the ability of the process to provide the proper dose of irradiation to all shellfish in a process batch, once a process has been validated it is essential that all containers be of uniform size with the same number of containers on each pallet. This is also important for purposes of product tracking and control. Each processor wishing to use an irradiation facility that has already been recognized and validated in accordance with the NSSP does not have to revalidate the irradiation process being used. Further, if a NSSP recognized irradiation facility conducts verification sample testing, processors using that facility to PHP shellfish may use those verification |

sample results to fulfill their NSSP verification requirements.

- The shellfish processor and the irradiation facility must have implemented a Hazard Analysis Critical Control Point (HACCP) plan approved by the respective SSCAs for the PHP process that ensures the target pathogen(s) in shellfish are consistently reduced to levels recognized as safe in the NSSP Model Ordinance.

- Once the irradiation process is completed containers of irradiated shellfish should be segregated from other shellfish or seafood products.

Under 21 CFR § 179.26(c), molluscan shellfish that are irradiated must bear a specific logo and a statement specifying that the shellfish have been treated by irradiation or treated with radiation. However, PHP irradiation facilities that irradiate shellfish may not have the capability to also label the shellfish as irradiated; such facilities can only irradiate the shellfish, not label them. As such, the primary processor may pre-label the pallets of shellfish as irradiated and may also provide a statement detailing Vibrio reduction.

For dealers who ship shellfish to an irradiation facility in containers that have been pre-labeled as irradiated with vibrio reduction information the following guidelines provide additional detail:

- A signed agreement should be in place between the irradiation facility and the primary certified shellfish dealer specifying the post office addresses of each party and outlining the specifications needed to ensure that the pre-labeled containers of shellfish do, in fact, undergo the validated irradiation process set forth within the agreement.

- Both the primary shellfish dealer and the irradiation facility must each have an implemented HACCP plan to ensure that shellfish pre-labeled as irradiated undergo the validated irradiation process set forth in the agreement.

- The agreement should provide for transport of the shellfish in sealed trucks and the transport should be secured with a tamperproof seal at the primary certified dealer and a record should be made of the seal number.

- The agreement should also establish that the oyster shellstock is washed, sorted, and placed into pre-labeled containers by the primary certified shellfish dealer.

- The agreement should specify how to palletize pre-packaged and pre-labeled oyster containers.

- Pallets of oyster containers shall be clearly labeled with the words "TO BE IRRADIATED."

- The number of pre-labeled containers should be documented in a HACCP record and in an additional record to be provided to the operator at the irradiation facility. This transport should be limited to pallets of shellfish to be irradiated and no other seafood or shellfish products.

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| | • When the transport arrives, the irradiation facility operator may remove the seal, record the number of containers, verify the number of containers in the transport matches the record provided by the primary certified dealer and then record the number of containers in the irradiation facility's HACCP record.<br><br>• The irradiation facility operator shall record all other required HACCP receiving critical limit information in HACCP records.<br><br>• Irradiated shellfish shall be placed in cooler storage or on transports maintained at the appropriate temperature (cooler maintained at 45 degrees and transport pre-chilled to 45 degrees).<br><br>• Irradiated shellfish shall be segregated from other seafood or shellfish products.<br><br>• The irradiation facility shall also have implemented a HACCP plan that includes the critical control points for receiving, the irradiation process, and refrigerated storage. |
| **Public Health Significance:** | Vibrio bacteria are predominately found in estuarine environments and naturally present in most shellfish. Most cases of disease attributed to Vibrio species are associated with the consumption of raw molluscan shellfish, particularly raw oysters and hard clams. Vibrio-related sicknesses can cause severe illness, including mortality. The most common Vibrio species found in shellfish are *Vibrio vulnificus* (*V.v.*) and *Vibrio parahaemolyticus (V.p.*). *V.v.* is associated with 95 percent of all seafood-related deaths in the United States. Thus, Vibrio species in uncooked molluscan shellfish provide a significant public health risk which may be minimized by enabling industry to streamline this process for irradiation PHP. |
| **Cost Information (if available):** | |

| **Proposal for Task Force Consideration at the Interstate Shellfish Sanitation Conference 2013 Biennial Meeting** | ☐ **Growing Area**<br>☒ **Harvesting/Handling/Distribution**<br>☐ **Administrative** |
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| **Submitter:** | Lori A. Howell |
| **Affiliation:** | Spinney Creek Shellfish, Inc. |
| **Address** | 27 Howell Drive<br>Eliot, Maine 03903 |
| **Phone:**<br>**Fax:**<br>**Email:** | 207-439-2719, Ext. 2<br>207-439-7643<br>lahowell@spinneycreek.com |
| **Proposal Subject:** | Eliminate requirements for the Authority to retain records of a trade secret or proprietary nature. Such records to be available at the dealer's place of business during normal business hours. |
| **Specific NSSP Guide Reference:** | NSSP Guide Section II. Model Ordinance<br>Chapter V. Shellstock Relaying @.01 General D.;<br>Chapter V. Shellstock Relaying @.02 Contaminant Reduction B.; and<br>Chapter XV. Depuration Requirements for the Authority E. (1) and (2) |
| **Text of Proposal/ Requested Action** | Chapter V. @.01<br>D. The ~~Authority~~ dealer shall retain records covering all aspects of the establishment of the heat shock process.<br><br>Chapter V. @.02<br>B. The person responsible for conducting the study ~~Authority~~ shall retain the written study report indefinitely.<br><br>Chapter XV. Requirements for the Authority<br><br>~~E. The Authority shall maintain adequate records for each depuration facility. The following records for each facility shall be kept for the period of five years: (1) Inspection reports and reviews of the plant performance in accordance to Section D. (above); (2) Current Depuration Plant Operations Manual for each dealer (Section .03).~~<br><br>Delete all other elements that require the Authority to keep on file or retain records of a trade secret or proprietary nature. Such records will be required to be maintained at the dealer facility and available to the authority for review during normal business hours. |
| **Public Health Significance:** | There is no cost to the Authority to eliminate these requirements. |
| **Cost Information (if available):** | Freedom of Information Act (and similar state act) requests can be time consuming, costly, and detract from public health activities of the Authority. Industry should be required to make records available to the Authority at the dealer's facility during normal business hours. Requiring the Authority to collect and maintain such records that may be subject to Freedom of Information Act release undermines the relationship of industry and regulators and further serves as a disincentive for businesses to conduct research, innovate and develop new products, processes and procedures |