Proposal Subject: Guidance Document for 2 and 3 Log Reduction Method

Specific NSSP Guide Reference: Section IV. Guidance Documents Chapter IV. Naturally Occurring Pathogens .06 Guidance for 2 or 3 Log Reduction of *Vibrio parahaemolyticus* PHP Validation as an Alternative for Rapid Cooling

Text of Proposal/ Requested Action

.06 Method for Validation and Verification of a Two or Three Log Reduction of *Vibrio parahaemolyticus* (*V.p.*) in Oysters.

A. VALIDATION

1. Introduction:

Rapid refrigeration can slow the growth of *Vibrio parahaemolyticus* (*V.p.*) in recently harvested oysters. An alternative to rapid refrigeration requirements under NSSP is a post harvest process (PHP) which requires at least a two log reduction in *V.p.* levels for the Gulf and a three log reduction for the Pacific. This document provides guidance for the validation of a PHP to achieve either the two or three log reduction of *V.p.* density as appropriate.

2. Overview:

Validation of the PHP to achieve a two or three log reduction in *V.p.* levels is conducted on three harvest lots, with one initial measurement prior to PHP, or "pre-process", and ten measurements after the PHP or "post-process". This process is divided into three basic parts: 1) the pre-process *V.p* density determination of the lot, 2) determination of tube number and concentration of oyster homogenate aliquoted (inoculum) to obtain post-process *V.p.* density 3) validation and/or verification of the two or three log reduction as prescribed. Samples must be taken from three independent harvest lots to test the efficacy of the PHP process with confidence.

Although the pre-process sampling protocol requires three dilutions from one sample, post-process sampling protocol requires only a single dilution as indicated for each of the ten samples. These ten samples for each of three lots make a total of thirty samples. The number of positive tubes in each post-processed sample determines whether the sample passes or fails. The PHP is validated if no more than five of the thirty samples collected after processing fail. The PHP must be verified in each month it is performed.

The method of analysis will be the same MPN method as is utilized in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, used for the regulatory analyses for *V.p.* in shellfish as approved under the NSSP and cited in the National Shellfish Sanitation Program Guide for the Control of Molluscan Shellfish 2009 Section IV. Guidance Document Chapter II. Growing Areas.10 Approved National Shellfish Sanitation Program Laboratory Tests. *Although a* Most Probable Number (MPN) *series will be performed, an MPN/g value will not be attained or used throughout the validation process. Instead, the information used to validate and verify, and the data generated, is based on the statistical analysis of probability.*

3. Initial *V.p.* Density Determination:
For each pre-process lot, a ten-tube decimal dilution MPN is performed. The

tube code obtained establishes initial *V.p.* density on the pre-processed lot to determine how to perform the post-process lot measurements. For confidence in the initial measurement at least three dilutions are necessary. (The amount of the original sample in each dilution is one tenth as much as in the previous dilution. For example, if the lowest dilution has x grams, the next dilution has x/10, then x/100, etc.)

For a lot to be included in the validation the dilutions selected for the analysis must not result in all positive or all negative tubes. *It should be noted that in the unlikely event that the pre-processed sample tube code is not listed in the attached table, a problem in the determination of the initial V.p. level likely occurred and that the initial V.p density of the lot will have to be retested before continuing the validation study.* If unsure of the initial *V.p.* density it may be necessary to use more than three dilutions in the initial analysis. When more than three dilutions are used, the results from only three contiguous dilutions are significant in determination of the outcome. To select the three dilutions to be used, the following guidance is provided. In each example the selected dilutions are underlined in bold.

(a) When more than one of the dilutions used has all ten tubes positive, select the highest dilution (most dilute sample portion) having all ten tubes positive and the two following dilutions (i.e. 10,10,6,0).
(b) When only one of the dilutions used has all ten tubes positive, select that dilution and the two following dilutions (i.e. 10,8,4,0)
(c) When a positive tube or tubes occur in dilutions higher than the three dilutions chosen, add the number of positive tubes in the higher dilutions to the third dilution chosen (i.e. 10,9,3,1 becomes 10,9,4).
(d) When the sum of the tubes in the third dilution would exceed ten, select the three highest consecutive dilutions having at least one positive tube among them (i.e. 10,9,9,2).

4. Post PHP Process *V.p.* density determination (see attached table):

The three dilutions so determined form a tube code for the initial density of *V.p.* in the pre-processed samples. This tube code, listed in column one of the attached table in Appendix A. (see *Appendix A: Tube Code Table for Validation and Verification*), determines both the number of tubes used and the amount of inoculum in each of the post-processed samples. Once the tube code from the initial pre-process *V.p.* density measurement is obtained from the first column of the attached table, the number of tubes to be used in each of the ten post-processed samples can be obtained from the same row in the third column. Directly adjacent to column three in this same row, column four, indicates the maximum number of tubes allowed to be positive for that sample to pass.

Column two of the table shows three possible dilutions of the original sample that could have been used in the initial *V.p.* density determination. If these dilutions were used to generate the tube codes in column one of the attached table, then the volume of sample to be inoculated into each of the post-process single dilution MPN tubes for the sample lot is given directly adjacent. Hence the amount to inoculate for *V.p.* density determination of post-process samples is in column five for the Gulf (2 log) and column six for the Pacific (3 log).

Since the initial density of *V.p.* may vary considerably, dilutions other than the dilutions given in column two of the table may be used. When this occurs an adjustment must be made in the volume of post-process sample inoculated into each of the single dilution MPN tubes used.

For example, the dilutions prescribed in column 2 for tube code 10, 1, 0 are 0.001, 0.0001, 0.00001. If the dilutions used were actually 0.01, 0.001, 0.0001, the amount in column five or six would be multiplied by ten. Thus, the nine tube post-process single dilution MPN would have an adjusted sample inoculum of 0.1 gram/mL (0.01 x 10) and must be used for each sample from the lot rather than the 0.01 gram/mL sample inoculum specified in column five of the table to validate the two log reduction. In the same example, to validate the three log reduction, the adjusted sample inoculum of 1.0 gram/mL (0.1 x 10) must be used for each post-process sample from the lot instead of the 0.1 gram/mL specified in column six of the table.

5. Determining validation of two or three log reduction post PHP process:
Individual post-process samples pass or fail based on the number of positive tubes which result from the single dilution MPN, as found in column four of the table. In the example above for a pre-process sample tube code of 10,1,0 using a nine tube, single dilution MPN for the analysis, column four directly across from the tube code indicates that no more than four of the nine tubes per sample may be positive for the sample to pass. For the three lots to pass and the PHP to be validated for a two or three long reduction in *V.p.* density, no more than five of the thirty individual samples from the three lots tested post-process can fail.

B. VERIFICATION

1. Initial V.p. density determination:
In each month that oysters are post harvest processed, the first lot for processing is selected for testing. The method of testing the lot is similar to the testing for validation. An initial measurement uses ten tubes at three dilution levels. This initial measurement determines the number of tubes, mass of homogenate, and number of allowed turbid growth (positive) tubes used to test the oysters after PHP processing. The table used for validation is also used for the verification process.

If the initial measurement has all negative (non turbid)tubes and the mass of inoculum in the least dilute tube contains at least 1 gram of the oyster homogenate, then the process is considered verified for that month. If the least dilute tube contains less than 1 gram of homogenate the process should be repeated with 1 gram of sample. If an all negative result is again obtained the process is considered verified for that month. If growth is observed post-process verification testing must be performed.

2. Post PHP Process *V.p.* density verification:
Post processed verification testing uses the first lot of the month. Three outcomes are possible;
(a) the process is verified for the month, or
(b) the process fails verification and the process must be revalidated, or
(c) additional testing using a subsequent lot is needed.

Four parameters determine the verification test and they are outlined in the following table. The first parameter is the number of samples taken from a lot. When the process is validated ten samples are selected from each lot; however, for verification seven samples are to be taken from the lot. The second parameter is the maximum number of growth tubes for the process to be verified with the first lot. The maximum number of samples allowed to be positive for the process to verify is 1. The third parameter is the minimum number of positive tubes that causes the process to require revalidation, which is three.

Table 1. Positive Sample Maximum and Minimum

Number of Samples	First Lot Maximum Positive for Pass	First Lot Minimum Positive for Fail	Second Lot Maximum Positive for Pass	Probability of Passing for Non-degenerate Process
7	1	3	1	96%

If the number of positive tubes in the testing of the first lot is 2, then a second lot is selected. The fourth parameter is the maximum number of positive tubes allowed for verification when the second lot is used. The following table outlines this scenario.

Table 2. Pass/Fail Schematic

Monthly Verification	
First Lot	Second Lot
7	7
6	6
5	5
Fail 4	4
3	Fail 3
Second Lot Needed 2	2
1	1
Pass 0	Pass 0

The process has a 96% probability of passing verification as long as it is working optimally; should the process degenerate in efficacy, the probability of passing significantly decreases.

Public Health Significance: In 2009, the ISSC adopted Proposal 09-208 which allows for processors to utilized shellstock that is harvested outside the *Vp* controls established as part of the States' *Vp* Plans. The proposal established a 2 log reduction requirement for the Gulf of Mexico and the Mid-Atlantic States and a 3 log reduction requirement for the Pacific Coast States. This proposal provides guidance for the validation and verification for processors choosing to use this processing option

Cost Information (if available):

Action by 2011 Task Force II: Recommended adoption of Proposal 11-211-L as submitted.

Action by 2011 General Assembly Adopted the recommendation of Task Force II on Proposal 11-211-L.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-211-L.