Proposal Subject: Post Harvest Processing

Specific NSSP Guide Reference: NSSP Guide Section III. Public Health Reasons and Explanations

Text of Proposal/ Requested Action Add a new section for Post Harvest Processing.

Chapter XVI.

Background & Performance of Post Harvest Processing (PHP) Validation/ Verification Protocols

BACKGROUND:

A post harvest process (PHP) to reduce the levels of pathogenic *vibrios* in shellfish, must be capable of reducing potentially high summer levels to a level that presents a negligible health risk. Cook et al 2002 indicated that a concentration of *Vibrio parahaemolyticus* or *Vibrio vulnificus* of 100,000 per gram was not uncommon in market oysters harvested from the Gulf Coast during summer months. A WHO/FAO (2005) risk assessment indicated that a *Vibrio vulnificus* concentration of below 30 per gram is a negligible health risk. Therefore, in an attempt to validate a post harvest process to be used throughout the year, the ISSC adopted as interim guidance, a protocol to assure that the process is capable of reducing levels of *vibrios* from an initial MPN level of 100,000/gram to <30/gram.

Obtaining an initial level of 100,000/gram was difficult to achieve consistently in some locations (even with temperature abuse) except during the hottest part of the summer. This limited the time that a validation could be conducted to 3 months of the year or less. In an attempt to allow validation during other times of the year, the ISSC proposed a validation procedure based upon a 3.52 log reduction (this is equivalent to reducing from 100,000 to 30) regardless of the initial level. A new validation protocol was developed which specified an initial level between 10,000 and 100,000 and reduction by 3.52 logs resulting in a final concentration of <30.

VALIDATION:

Validation is the initial check of a PHP to assure that the process can reduce the concentration of *V. vulnificus* and *V. parahaemolyticus* in shellfish by 3.52 logs and to levels <30 as shown in table 1. Determining the log reduction for validation uses knowledge of both the initial and final concentrations. The interval containing the initial concentration determines a test on a single sample for the final concentration. A multiple dilution test is preferred for finding a concentration and the single dilution for indicating whether the concentration is above a threshold. For the initial concentration, a serial dilution with three tubes at each of three or four dilutions was chosen. Four samples are taken to determine the initial concentration and the adjusted geometric mean is used to combine the MPN results. If four samples from a lot of shellfish with a true concentration of 100,000 per gram are examined by the MPN procedure, the probability of the geometric mean of the MPNs showing 100,000 or greater is about 50%. In an attempt to improve the probability of samples being accepted when the true concentration is 100,000 per gram, an adjustment factor of 1.3 was selected based upon examining tables of the probability of getting various results from simulated outcomes.

For a process to be validated, no more than three samples out of 30 may fail. Depending upon the initial load, failure of a single sample is determined according to the table below.

TABLE 1

AGM Interval	Grams Per Tube	Positive Tubes Allowed
59,995 or Greater	.01	2
37,174 – 59,994	.01	1
23,449 – 37,173	.1	4
12,785 – 23,448	.1	3
10,000 – 12,784	.1	2

The choice of intervals for each test in table 1 tried to keep the probabilities near the original test. The original test used .01 grams/tube and allowed 2 of the 5 tubes to have growth. It tried to test for 30 cfu/gram. At 30cfu/gram the probability of a tube with .01 grams of homogenate not having growth equals exp (-30*.01) from a Poisson. From putting this value into a binomial at a final concentration of 30 cfu/gram a single sample has a probability of passing the original test of .88656. The table gives initial concentrations which can be converted to target final concentrations by multiplying by 30/100,000 ≈ -3.52 $\log_{10}$.

A change from one test to another was done at a concentration where the probability of passing both tests was the same distance from the probability of passing the original test. For example, an initial concentration of 59,995 becomes a target final concentration of

(30/100,000) * 59,995 = 17.9985

At this target final concentration and .01 grams/tube the probability of 2 or fewer growth tubes equals .96562. The probability of 1 or fewer growth tubes equals .80751. Since .96562 - .88656 and .88656 - .80751 are equal up to rounding, the initial concentration of 59,995 was chosen as the value to change between these two tests.

Since validation tries to assure that a PHP gives the desired log reduction and gets the final concentration below 30 per gram, an operational characteristic curve is used to determine how well the process works. For an initial concentration, an operational characteristic curve indicates the probability of passing validation for various final concentrations.

The probability of passing validation for each pair depends on the initial and final concentrations. The initial concentration indicates which of the five tests in the validation procedure is used. The final concentration and the test used give the probability of the sample passing.

The probability of passing any of the five tests in the validation procedure is calculated from the final concentrations. In addition, simulations generated outcomes from the initial concentration. The adjusted geometric means for the

MPNs of these outcomes indicate the probability of each of the five tests given the initial concentration. The product of the probability of each test times the probability of passing with the test were added over all five tests. This gives the probability a sample would pass. Calculating with a binomial gave the probability that at most 3 of the 30 samples would fail for a validation. The following table (table 2) gives the probability of passing validation with various combinations of initial and final concentrations.

TABLE 2

INITIAL CONC.	FINAL CONCENTRATIONS																
	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51
10,000	.96	.14	.00														
20,000	1	.93	.39	.04	.00												
30,000	1	.99	.89	.56	.21	.05	.01	.00									
40,000		1	.97	.85	.62	.36	.18	.07	.03	.01	.00						
50,000		1	.99	.96	.86	.69	.49	.31	.17	.09	.04	.02	.01	.00			
60,000			1	.99	.95	.87	.73	.56	.38	.24	.13	.06	.03	.01	.00		
70,000				1	.98	.93	.84	.70	.53	.36	.22	.12	.06	.03	.01	.00	
80,000				1	.99	.96	.90	.78	.63	.45	.29	.17	.09	.05	.02	.01	.00
90,000				1	.99	.97	.92	.82	.68	.51	.34	.20	.11	.06	.03	.01	.00
100,000					1	.98	.93	.84	.70	.53	.36	.23	.13	.06	.03	.01	.00

Highlighted areas represent a 3.52 log reduction between initial concentration and final concentration.

The original reason for using 30 samples for validation was to be able to select one each week for 30 weeks during the warm weather. This would have given an idea how the post harvest process performed under various conditions throughout the summer. In order for this to be more feasible for industry, this arrangement was changed to 10 measurements on a single lot on each of 3 days.

VERIFICATION:

After initial validation of a PHP, verification of the process must be done monthly. In the verification process, the output of the PHP is tested to determine if it is below 30 per gram. If a PHP fails verification, then it has to be revalidated in order to use labeling claims as approved by the ISSC. Any verification that is not excessively burdensome may miss some problems with the process. Consequently, if other evidence indicates a problem then action may be needed regardless of verification results.

Samples can be taken throughout a month on different lots of product. Although testing different lots could help find intermittent problems, a small processor during a slow month may not be able to test many different lots. Consequently, the decision of how many lots are tested for verification may be left up to the processor with the approval of the state SSCA.

In order to determine the probability of verification failures that would result in revalidation, 1000 simulations were run with each simulation mimicking nine months and counting the number of passes. Nine months represents the number of months in a year that oysters might be expected to have high vibrio counts. The count for 9 months that passed indicates how likely the post harvest process would

be of not needing revalidation.

Based upon a verification procedure that requires 30 tubes per month be tested with no more than 11 of the 30 tubes being positive for the process to be verified for that month and assuming that all months are independent and identically distributed, the table (table 3) below indicates the probability of failing verification in at least one of nine months and at least twice in nine months for various final concentrations.

TABLE 3

Final Concentration	Probability of 1 failure in 9 months	Probability of 2 failures in 9 months
20	4	0
25	17	1
30	45	11
35	76	39
40	93	73

Example: If a final concentration of 30 has been achieved by the Post Harvest Process, there is an 11% chance that revalidation will be required based upon two verification failures within a 9 month period. Likewise, at a final concentration of 30, there is a 45% chance that one failure would occur within 9 months.

Cook, D.W., P. O'Leary, J.C. Hunsucker, E.M. Sloan, J.C. Bowers, R.J. Blodgett, and A. DePaola. 2002. *Vibrio vulnificus* and *Vibrio parahaemolyticus* in U.S. retail shell oysters: A national survey June 1998 to July 1999. J. Food Prot. 65:79-87.

FAO and WHO. Risk assessment of Vibrio vulnificus in raw oysters: Interpretative summary and technical report. 2005. Rome, Italy, FAO. Microbiological Risk Assessment Series No. 8.

Public Health Significance: This information provides an explanation of the development of the validation/ verification guidance given for post harvest processing.

Cost Information (if available): No additional cost.

Action by 2009 Task Force II: Recommended adoption of Proposal 09-237 as submitted.

Action by 2009 General Assembly Adopted recommendation of 2009 Task Force II on Proposal 09-237.

Action by USFDA 02/16/2010 Concurred with Conference action on Proposal 09-237.