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.01 *Vibrio* Risk Management for Oysters

Background

Current information concerning *Vibrio vulnificus*, which is responsible for several shellfish associated illnesses and deaths each year can be found in Watkins and McCarthy (1994).

A small number of shellfish-borne illnesses have also been associated with bacteria of the genus *Vibrio* (Bonner, 1983; Blake *et al.*, 1979; Morris, 1985; Joseph *et al.*, 1982; Roderick, 1982). The *Vibrios* are free-living aquatic microorganisms, generally inhabiting marine and estuarine waters (Joseph *et al.*, 1982; Spira, 1984; Colwell 1984; Bachman, 1983). Among the marine *Vibrios* classified as pathogenic are strains of non-01 *Vibrio cholerae*, *V. parahaemolyticus*, and *V. vulnificus* (Bachman, 1983; Desmarchelier, 1984; Blake, 1980). All three species have been recovered from coastal waters in the United States and other parts of the world (Joseph, 1982; Colwell, 1984; Blake, 1980; DePoala, 1981; Madden, 1982; Davey, 1982; Oliver, 1983; Tamplin, 1982; NIH, 1984). These and other *Vibrios* have been detected in some environmental samples recovered from areas free of overt sewage contamination and coliform (Bonner, 1983; Joseph, 1982; Spira, 1984).

In general, shellfish-borne vibrio infections have tended to occur in coastal areas in the summer and fall when the water was warmer and vibrio counts were higher (Bonner, 1983; Morris, 1985; Joseph, 1982). *V. parahaemolyticus* and non-01 *V. cholerae* are commonly reported as causing diarrhea illness associated with the consumption of seafood including shellfish (Bonner, 1983; Blake, 1979; Morris, 1985; Joseph, 1982; Baross and Liston, 1970; Morris, 1981). In contrast, *V. vulnificus* has been related to two distinct syndromes: wound infections, often with tissue necrosis and bacteria, and primary septicemia characterized by fulminant illness in individuals with severe chronic illnesses such as liver disease, hemochromatosis, thalassemia major, alcoholism or malignancy (Bonner *et al.*, 1983; Tacket, 1984). Increasing evidence shows that individuals with such chronic diseases are susceptible to septicemia and death from raw seafood, especially raw oysters (Bonner *et al.*, 1983; Blake, 1979; Morris, 1985; Rodrick, 1982; Bachman, 1983; Blake, 1980; Oliver, 1983; NIH, 1984; Tacket, 1984; Oliver 1982; FDA, 1985). Shellfish-borne vibrio infections can be prevented by cooking seafood thoroughly, keeping them from cross contamination after cooking, and eating them promptly or storing them at hot (60°C or higher) or cold (4°C or lower) temperatures. If oysters and other seafood are to be eaten raw, consumers are probably at lower risk to vibrio infection during months when seawater is cold than when it is warm (Blake, 1983 and 1984).

.02 *Vibrio vulnificus* Management Plan

The voting delegates at the 1999 Annual Meeting in New Orleans created the *Vibrio* Management Committee (VMC). Subsequently, *Vibrio vulnificus* and *Vibrio parahaemolyticus* subcommittees have been charged to develop appropriate illness control measures for these two pathogens. The VMC provides guidance and oversight to the subcommittees. Subcommittee recommendations are reviewed by the VMC before submittal to Task Forces. At the 2001 annual meeting, Task Forces reviewed the VMC's recommendation of reducing the rate of etiologically confirmed shellfish-borne *Vibrio vulnificus* septicemia with the intention to submit the recommendation to the voting delegates. The goal is to reduce the rate of illness reported in California, Florida, Louisiana and Texas due to the consumption of commercially harvested raw or undercooked oysters by 40 percent, for years 2005 and 2006 (average) and by 60 percent for years 2007 and 2008 (average) from the average illness rate for the years 1995 - 1999 of 0.306/million. The list of states may be adjusted if after a thorough review, epidemiological and statistical data demonstrates that it would be appropriate. The rate of illness shall be calculated as the number of illnesses adjusted for population. This adjustment will be performed in consultation with statisticians and epidemiologists from California, Florida, Louisiana and Texas and Federal agencies. The baseline data and all future data for measuring illness reduction shall be the reported illnesses in the California, Florida, Louisiana and Texas for the period 1995 to 1999, inclusive, as compiled by the Southeast Regional Office of the U.S. Food and Drug Administration. The data used for measuring goal attainment shall begin with 2002 data. For the purpose of maintaining an accurate count of the number of illnesses report by each state (California, Florida, Louisiana and Texas), the following will apply:

- (a) Illness cases counted are those reported by California, Florida, Louisiana and Texas;
- (b) Each illness case is recorded under the state that reports it;
- (c) Each case is not counted more than once; and
- (d) In the event more than one report per case is filed, the case is recorded under the state of diagnosis.

The formula for calculating the rate of illness is as follows:

$$\frac{\text{number of cases}}{\text{population}}$$

The V.v. subcommittee members will include, at a minimum, balanced representation from industry and state shellfish control authorities from *Vibrio vulnificus* Illness Source States California, Florida, Louisiana and Texas, FDA, NOAA, EPA, CDC, state epidemiologists; as well as industry and shellfish control representatives from other regions. *Vibrio vulnificus* Illness Source States are those states reporting two (2) or more etiologically confirmed shellfish-borne *Vibrio vulnificus* illnesses since 1995 traced to the consumption of commercially harvested raw or undercooked oysters that originated from the waters of that state. Etiologically confirmed means those cases in which laboratory evidence of a specific agent is obtained and specified criteria are met.

Recognizing the increasing importance and roles for the Committee, leadership will be expanded and structured in a similar manner as stated in the ISSC By-Laws for Task Forces (reference: ISSC By-Law, Article I Task Forces). The VMC Chair shall alternately be selected from a state shellfish control authority and from industry. The Board Chairman, with approval of the Board, shall appoint a VMC

Chair and Vice-Chair. If the VMC Chair represents a state shellfish control authority, the Vice-Chair shall be an industry representative. At the end of the VMC Chair's term of office, the Vice Chair will become Chairman and a new Vice Chair will be appointed who represents the same segment of the Conference as the outgoing VMC Chair. A VMC Chair and Vice Chair should be appointed before October 1, 2001 in order to be consistent with plans for annual VMC meetings and with the effective date of *Vibrio vulnificus* Risk Management Plans. Likewise, the term of office shall be for (2) years.

The VMC will meet at least annually to develop and approve annual VMC work plans for *Vibrio vulnificus* illness reduction and review progress. A series of work plans, each covering a one-year period shall be adopted. The first work plan and progress review period will cover a seventeen-month period from August 1, 2001 to December 31, 2003 followed subsequently by annual work plans. Work plans will include goals, tasks, performance measures and assessment methods to track and achieve progress towards the illness reduction goals. The work plans will be developed by the VMC and approved by the VMC membership. The chair of the VMC will deliver a written annual progress report, including a summary of the previous year's progress made in the education program, to the ISSC March executive board meeting. The report shall be made available to the general membership. The annual work plan structure, outlined below, provides adaptive management and assures consistent progress towards the illness reduction goals. If annual assessment of progress towards achieving the illness rate reduction goals show inadequate progress the VMC shall incorporate actions into current and subsequent work plans to assure success in achieving those goals. In addition, if annual review shows inadequate progress the VMC will develop issues for deliberation at the 2005 biennial meeting to consider actions such as:

- increased educational efforts,
- limited harvest restriction,
- reduction in time from harvest to refrigeration,
- phased-in post-harvest treatment requirements, or
- other equivalent controls.

Work plans developed by the VMC shall include the following elements and shall define the administrative procedures and resources necessary for accomplishment (i.e. establishment and maintenance):

(a) An ISSC Consumer Education Program targeted toward individuals who consume raw oysters and whose health condition(s) increase their risk for *Vibrio vulnificus* infection. The Education Program's objectives will be 1) to increase the target audience's awareness that eating raw, untreated oysters can be life-threatening to them, and; 2) to change the at-risk group's oyster-eating behavior, i.e., to reduce or stop eating raw, untreated oysters. The ISSC *Vibrio* Management Committee and the *Vibrio vulnificus* Education Subcommittee will evaluate Year 2001 survey results and compare them with the Year 2003 or 2004 survey results to determine the effectiveness in meeting the two objectives of the Vv education effort: (1) Show 40% increase in awareness of risk from Vv; and (2) Show 15% increase in at-risk consumers no longer eating raw oysters while minimizing impacts to non-at-risk consumer raw oyster consumption.

(i) The Consumer Education Program will focus educational efforts in California, Florida, Louisiana and Texas. The Education Program will make educational materials available to additional states upon request.

(ii) Educational approaches will emphasize partnerships with health and advocacy organizations, and include dissemination of printed materials, posting materials on the

Internet, broadcast of television spots, press releases, and other measures deemed effective such as the USDA Physician Notification Program.

(iii) Survey assessments at the state level shall be used as a means of assessing the baseline knowledge and effectiveness of educational interventions.

(b) Administration of a survey to determine the current *Vibrio vulnificus* disease reporting and education in each state.

(c) Creation of a working group to work cooperatively with local, state, and federal agencies and programs to assist in the collection of environmental and epidemiological data to further expand on the current information available. A coordinator may be utilized to facilitate the activities of this working group to develop standardized collection of environmental and epidemiological information from harvest to consumer.

(d) The Voting Delegates at the 2007 Biennial Meeting in Albuquerque, New Mexico approved appointment of a committee that will consist of three (3) epidemiologists and advisors as appropriate. The Committee will use this form to screen cases for the purposes of determining if a case is attributable to a single source state as well as whether the case is includable in the Vv Illness Reduction Goals. In addition, to ensure uniformity, the form shall be used for screening 2007-2008 cases and that cases from the baseline will be screened using the same form.

Criteria FOR INCLUDING Vv CASES IN ILLNESS REDUCTION CALCULATIONS and determining source states

1. Each case that is considered must be reported on a Center for Disease Control and Prevention Cholera and Other *Vibrio* Illness Surveillance Report (COVIS) Form CDC 52.79.
2. Each case must also be listed be on the FDA database (NSSP Guide for the Control of Molluscan Shellfish Guidance Documents Chapter IV .02).
3. The ISSC committee to review reported Vv illnesses to determine the appropriateness of inclusion into the database used for illness reduction calculations must have access to the COVIS form for each case (patient names and other necessary information appropriately redacted). The ISSC addendum form is also provided, where available. This access to the COVIS form is critical for adequate interpretation of the data collected during the state epidemiological investigation.
4. The ISSC Vv Illness Review Committee will complete the following criteria table for each case. These tables serve as documentation.
5. For cases to be included in illness reduction calculations the following criteria must be met:
 - Item 1-4 and 5a must be answered yes.
 - Should the COVIS form include information that suggests other exposures that may be responsible for the Vv illness further investigation may occur. Consultation with State Shellfish Control Authorities and Epidemiologist from the state is encouraged to determine which exposure should be recorded as the cause of illness. Should oyster consumption not be determined to be the cause of illness the case will not be counted. Should there be disagreements with the inclusion of a case; the disagreeing party may request a review. The request must include a rationale for the review and should be addressed to the Executive Board Chairman.

- If 5b is no, other exposures should be considered. If no other exposures exist, the case will not be counted.
- Should the only exposure be consumption of cooked oysters or unknown 5b will be checked yes.

<i>Vibrio vulnificus</i> Criteria Table				
Case Identifier / Number		Criteria Status Determination		
Criteria		Yes	No	Unknown
1.	Etiologically Confirmed	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2.	Septicemia Illness	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3.	Reporting State (CA, FL, LA, TX)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4.	Commercial Harvest from US Production	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5.	Exposures			
a.	Onset Consistent with Consumption of Oysters	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b.	Raw or undercooked oysters	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
6.	Traceback Information			
a.	Were shipping tags available or was other traceback information reported	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b.	State of harvest and harvest area (s)			<input type="checkbox"/>
c.	Harvest date (s)			<input type="checkbox"/>
7.	Case Determination			
a.	Is case included in Vv illness reduction Calculations	<input type="checkbox"/>	<input type="checkbox"/>	
b.	Is case attributed to a single source state	<input type="checkbox"/>	<input type="checkbox"/>	
<p>Instructions for completing Criteria Table:</p> <ul style="list-style-type: none"> ○ Check YES if Criterion is confirmed from the COVIS form or addendum. ○ Check NO if Criterion is not confirmed from the COVIS form or addendum. ○ Check UNKNOWN if Criterion is not clear or absent from the COVIS form or addendum. ○ No Criterion can have more than one check entered. ○ Each Criterion must have one check entered (YES, NO, or UNKNOWN). <p>These criteria tables will be used to review reported Vv illnesses to determine the appropriateness of inclusion into the database used for illness reduction calculations and will also be used for identifying other source states.</p>				

(e) Industry-implemented post-harvest controls to reduce *Vibrio vulnificus* levels in oyster shellstock which may include: time-temperature, post harvest treatment (i.e. hydrostatic pressure, cool pasteurization, IQF, and irradiation--pending approval), rapid chilling and other emerging technologies.

(f) Pursuit of ISSC options such as industry education and communication; FDA label incentives; PHT specific growing area classifications; targeted time/temperature assessment by FDA during annual shellfish program evaluations; assistance, as necessary, for the further study and possible implementation of dockside icing to investigate its effects on shelf life and variations in the effectiveness of the method as a result of seasonal and regional differences and incentives to add refrigeration capacity to harvest vessels. The goal will be to provide incentives necessary to post-harvest treat 25 percent of all oysters intended for the raw, half-shell market during the months of

May through September harvested from a Source State by the end of the third year (December 31, 2004). The assessment will include the capacity of all operational plants and the capacity of plants under construction. Should the 25 percent goal not be accomplished, the VMC will investigate and report their findings as to why the goal was not reached.

(g) Development by the VMC of a list of issues relating to public health, various technologies including Post-harvest treatments; marketability; shelf -life and similar matters that lend themselves to investigation. The VMC will work with FDA, NOAA, CDC, EPA, the shellfish industry and other entities as appropriate to obtain or facilitate the investigation of the issues listed and take the results into account as it develops plans or recommended Issues for the ISSC.

(h) Provision for VMC compilation and review of the data on rates of illness, which will be made available to the ISSC at the ISSC Biennial meeting following the year in which the data was gathered. In the event that the data is not available at the time of the meeting, the VMC shall meet and review the data when it becomes available and issue a compilation report, which will be made available to the entire ISSC membership. In the event there is no Biennial meeting scheduled for a certain year, the VMC shall meet and review the data when it becomes available and issue a compilation report which will be made available to the entire membership.

(i) Provision for a VMC evaluation of the effectiveness of reduction efforts, which will be conducted at the end of the fifth year (December 31, 2006). The evaluation will determine whether the 40 percent, 5-year goal to reduce the rate of illness or education/consumer intervention or post harvest controls performance measures set forth in prior work plans have been achieved. Should the VMC evaluation indicate the 40 percent, 5 year goal has not been accomplished, the committee will identify additional harvest controls in the 2007 - 2008 work plan to assure achievement of the 60 percent reduction in the rate of illness goal by the close of the seventh year. In addition, the VMC will evaluate the requirements in Section 04.C. with the possibility of changing the controls to achieve remaining illness reduction goals.

(j) Should a disagreement arise between FDA and the Authority on the equivalency of a control as described in .04(C), the V.v. Subcommittee will be requested to provide guidance.

(k) In 2006 the Executive Board directed the elimination of the Vv & Vp subcommittees. The VMC assumed all responsibilities of the subcommittees as outlined in the *Vibrio vulnificus* Management Guidance Document. Representation on the VMC Committee will be consistent with all guidance (VMC and Vv subcommittee) outlined in the *Vibrio vulnificus* Management Guidance Document.

(l) Shellstock Harvested in Source States Harvesters must include on the tag of all product harvested for restricted use the statement “for shucking by a certified dealer” and/or “For PHP Only.” Harvesting controls must be provided by the Authority to ensure that restricted use shellstock is not diverted to retail or food service. Dealers must establish a restricted use shellstock Critical Limit as part of their HACCP Plan for receiving. A shipping Critical Control Point must include a restricted use shellstock disposition step. Restricted use shellstock is not intended for retail or food service.

Should a disagreement arise between FDA and the Authority on the equivalency of a control as described in .04(C), the V.v. Subcommittee will be requested to provide guidance.

In 2006 the Executive Board directed the elimination of the Vv & Vp subcommittees. The VMC assumed all responsibilities of the subcommittees as outlined in the *Vibrio vulnificus* Management Guidance

Document. Representation on the VMC Committee will be consistent with all guidance (VMC and Vv subcommittee) outlined in the *Vibrio vulnificus* Management Guidance Document.

.03 *Vibrio parahaemolyticus* Control Plan Guidance

A. Risk Evaluation

The determination of Reasonably Likely to Occur should be conducted as follows:

1. A risk evaluation as described in Proposal 07-202 (with the understanding that ISSC has not adopted nor endorsed the FDA *Vp* Risk Assessment); or
2. The risk factor decision tree under development by the VMC using the risk factors included in Proposal 07-202; or
3. Other approaches approved by the State Authority that provide at least an equivalent level of protection and reduce the risk so that it no longer constitutes an annual occurrence.

B. *Vibrio parahaemolyticus* Control Plan

1. Triggers

A plan for an area(s) or a state must include control measures for the month(s) in which:

- a. The total number of *Vp* illnesses is two or more in a three (3) year period; or
- b. The area was epidemiologically linked to an outbreak within the prior five (5) years and the plan must also apply to the period 30 days prior to the first day of harvest of the outbreak and 30 days after the last day of harvest associated with the outbreak; or
- c. The average water temperatures representative of harvesting conditions exceed 60 °F for states bordering the Pacific Ocean and 81 °F for states bordering the Gulf of Mexico and Atlantic Ocean (New Jersey and south). See exemption in the NSSP Model Ordinance Chapter II.@.05.B.2.; or

The regulatory authority to administer this plan is [To be filled in by the Authority].

2. Control Measures

- a. Post Harvest Processing (PHP).
- b. Closing the area to oyster harvest.
- c. Restrict oyster harvest to product labeled for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing.

The authority must notify harvesters and dealers of those areas restricted to harvest for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing.or "For PHP Only." Harvesters must include on the tag of all product harvested in these areas the statement for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing.or "For PHP Only." Harvesting controls must be provided by the Authority to ensure that restricted use shellstock is not diverted to retail or food service. Dealers must establish a for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing.or "For PHP Only" labeling Critical Limit as part of their HACCP plan for receiving. A shipping Critical Control Point must include a for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing.or "For PHP Only" labeling requirement. Restricted Use Shellstock is not intended for retail or food service.

- d. Limit time from harvest to refrigeration to no more than five (5) hours or other times based on modeling and sampling in consultation with FDA.
- e. Limit time from harvest to refrigeration such that levels of total *Vp* after completion of cooling to 60 °F do not increase more than 0.75 log from levels at harvest. Calculations for 0.75 log increase can be based on the table as shown below or based on validation studies. The authority may use the FDA Risk Assessment to determine the initial "at harvest" levels.
- f. The term refrigeration is storage in a container that is capable of dropping and maintaining ambient air temperature of 45 °F (7.5 °C).
- g. Other control measures based on appropriate scientific studies

3. Plan Effectiveness as Demonstrated by:

a. Post Harvest Processing.

Conduct end product testing consistent with PHP verification protocol as provided in the NSSP Guide for the Control of Molluscan Shellfish. Test results shall demonstrate the level of total *Vp* in the final product does not exceed the average levels found in the area at times of the year the state had determined *Vp* illness is not reasonably likely to occur.

Data may be shared between states or other entities as may be appropriate considering the characteristics of the harvest area(s), such as temperature, hydrological patterns, etc. In the absence of such state data, use 100/gm for the Pacific and 1000/gm for the Atlantic/Gulf as provided in the FDA Risk Assessment.

Note: These levels are significantly higher than those allowed in validation/verification to non-detectable. Labeling "for added safety" would not be permitted unless the lower levels were reached.

b. Closing the area to oyster harvest.

Issue a legally binding closure order(s). Conduct Patrol and maintain Patrol records for the area(s) in accordance with the NSSP MO requirements.

c. Restrict oyster harvest to product labeled for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing or "For PHP Only."

The authority must notify harvesters and dealers of those areas restricted to harvest for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing or "For PHP Only." Harvesters must include on the tag of all product harvested in these areas the statement "for shucking by a certified dealer, or other means to allow the hazard to be addressed by further processing" or "For PHP Only". Dealers must establish a for shucking by a certified dealer, or other means to allow the harvest to be addressed by further processing or "For PHP Only" labeling Critical Limit as part of their HACCP Plan for receiving. A shipping Critical Control Point must include for shucking

by a certified dealer, or other means to allow the hazard to be addressed by further processing or “For PHP Only” labeling requirement.

- d. Limit time from harvest to refrigeration to no more than five (5) hours or other times based on modeling and sampling in consultation with FDA. Compliance may be documented by State restriction orders, harvester records, dealer records, field records, storage records, harvester education/inspections, records of capable and operating refrigeration.
- e. Limit time from harvest to refrigeration such that levels of total Vp after completion of cooling to 60 °F do not increase more than 0.75 log from levels at harvest. Calculations for 0.75 log increase can be based on the table as shown below or based on validation studies. The authority may use the FDA Risk Assessment to determine the initial "at harvest" levels.
- f. The term refrigeration is storage in a container that is capable of dropping and maintaining ambient air temperature of 45°F (7.5°C).
- g. Other control measures based on appropriate scientific studies

4. Plan Modification

5. Cost Benefit Analysis (Optional)

Temperature specific <i>Vp</i> Growth rates and Doubling times for calculating cumulative growth based on hourly temperature observations.					
Oyster Temperature (degree F)	Growth rate (logs/hr)	doubling time (hrs)	Oyster Temperature (degree F)	Growth rate (logs/hr)	doubling time (hrs)
50	0.008	35.8			
51	0.011	28.4	76	0.147	2.05
52	0.013	23.1	77	0.156	1.93
53	0.016	19.2	78	0.165	1.83
54	0.019	16.1	79	0.174	1.73
55	0.022	13.8	80	0.183	1.64
56	0.025	11.9	81	0.193	1.56
57	0.029	10.4	82	0.203	1.48
58	0.033	9.14	83	0.213	1.41
59	0.037	8.11	84	0.224	1.34
60	0.042	7.24	85	0.235	1.28
61	0.046	6.50	86	0.246	1.23
62	0.051	5.87	87	0.257	1.17
63	0.056	5.33	88	0.268	1.12
64	0.062	4.86	89	0.280	1.07
65	0.068	4.45	90	0.292	1.03
66	0.074	4.09	91	0.304	0.99
67	0.080	3.77	92	0.317	0.95
68	0.086	3.49	93	0.330	0.91
69	0.093	3.24	94	0.343	0.88
70	0.100	3.01	95	0.356	0.85
71	0.107	2.81	96	0.370	0.81
72	0.115	2.63	97	0.383	0.79
73	0.122	2.46	98	0.397	0.76
74	0.130	2.31	99	0.412	0.73
75	0.139	2.17	100	0.426	0.71

Note: Growth rate (in logs/hr) = $(0.01122 \times \text{Temp} - 0.4689)^2$

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.04 Post Harvest Processing (PHP) Validation/Verification Guidance for *Vibrio vulnificus* and *Vibrio parahaemolyticus*

A. Process Validation

Used for the initial validation of a process or when there has been a change to a previous validation process.

1. Data on ten processed samples obtained on each of three processing days (total of 30 samples) are required.
2. All samples used on a processing day must come from the same lot of shellfish and be determined to have an adjusted geometric mean (AGM) MPN of 10,000 per gram or greater as described below for initial load testing.
3. Samples should be distributed throughout the processing day. A sample will consist of a composite of 10 to 12 oysters processed at one time.
4. The zero hour level may be achieved through naturally occurring *Vibrio* levels in shellfish and, where not practical, by time/temperature abuse. (Inoculated pack samples may be used as appropriate.)
5. Analytical methodology to determine *Vibrio* levels should be the official methods previously endorsed by the ISSC as indicated in Model Ordinance Chapter XVI. Post Harvest Processing.
6. Microbiological testing for initial levels will be by a 3-tube MPN using appropriate dilutions (10^{-1} to 10^{-6}).
7. Microbiological testing for processed samples will be by a single dilution five-tube MPN, inoculating with either 0.01 g or 0.1 g of shellfish per tube based upon the table below.
8. The numerical value of the endpoint criteria should be less than 30 per gram and achieves a minimum 3.52 log reduction.
9. For the 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.

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

For example, if the AGM equals 50,000, then use the second row because $37,174 \leq 50,000 < 59,994$. The second row tells to inoculate with .01 grams of the original oyster homogenate in each tube and the test fails if more than one of the five tubes is positive.

B. Equipment Validation

Used to ensure that each new or modified unit of equipment will deliver the validated process.
May be accomplished using the following:

1. A physical test of the equipment (e.g., thermal distribution study) that is designed to ensure that, when properly operated, it will consistently deliver the validated process.
2. The process needs to be verified according to section D. before labeling claims can be made.

C. Initial Load Testing

Initial level of vibrios in shellfish for each lot of shellfish used in validation shall be 10,000 MPN per gram or greater based on the adjusted geometric mean (AGM) of the MPNs/g of four samples where the AGM is given by:

AGM = the geometric mean of the 4 MPNs/g multiplied by an adjustment factor of 1.3

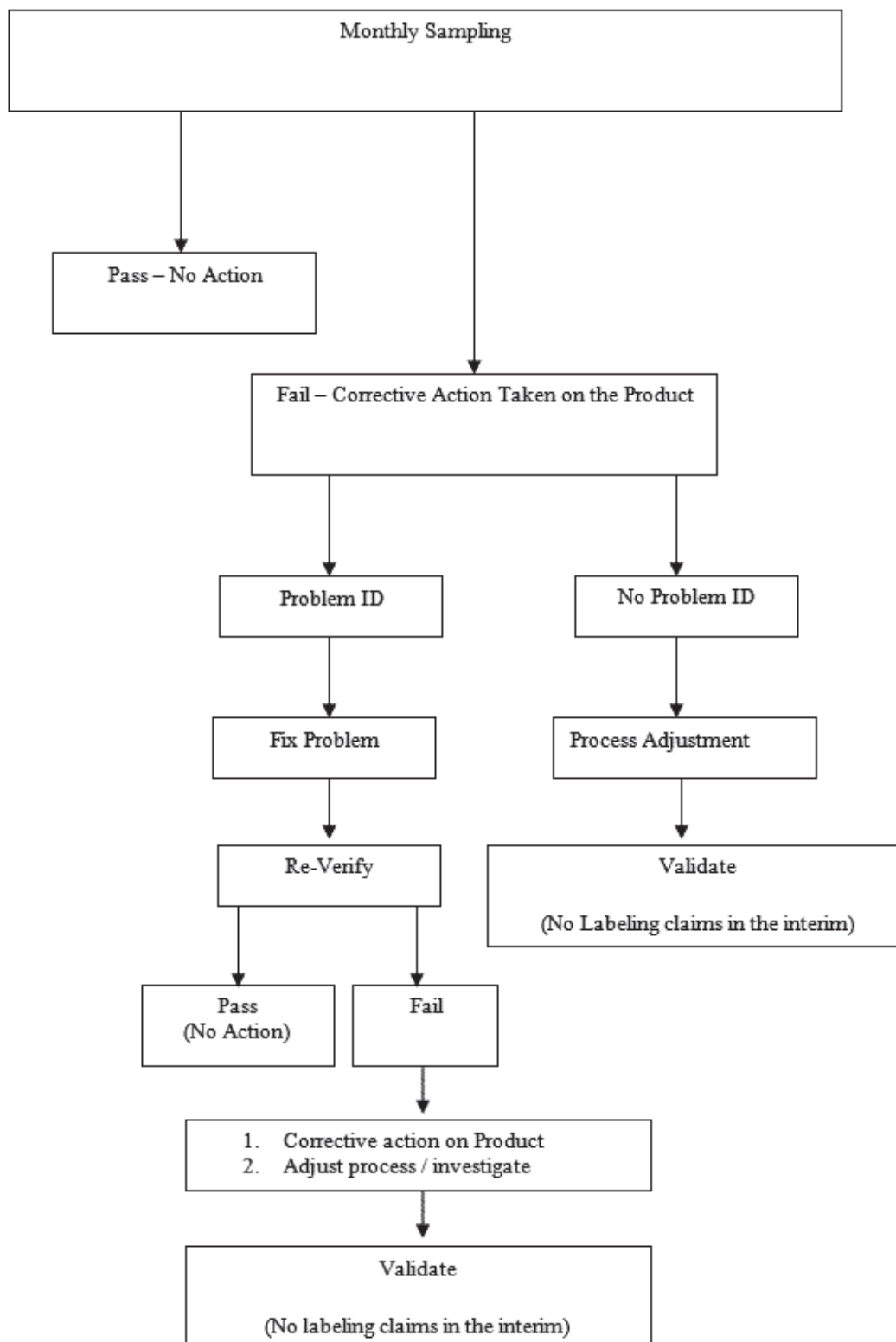
Note: If 4 samples from a lot of shellfish with a true density of 100,000 cells per gram are examined by the MPN procedure, the probability of the geometric mean of the MPNs showing 100,000 or greater is about 50%. In an attempt to improve the probability of samples being accepted when the true density is 100,000/g an adjustment factor of 1.3 was selected based upon statistical analysis.

D. Verification

Used to verify that a previously validated process is working properly.

1. Process verification by microbiological testing should be done monthly
2. The monthly sampling shall consist of 30 tubes from a minimum of three samples of 10 tubes each with an inoculum of 0.01 grams. Ideally, this would be done on three separate days of production, spread throughout the month, using a 10 tube MPN each day. If this is not feasible, the 30 tubes can consist of 3 samples from three consecutive days or 3 samples from a given day (from three separate lots if possible)
3. Each sample will consist of 10-12 oysters
4. If more than 11 tubes of the 30 most recent 3-10 tube samples within any calendar month are positive, then the process fails for that month. In this case, corrective actions as outlined in the Verification Sampling Plan Decision Tree must be taken and verification must be repeated within one week of the analysis indicating verification failure. Labeling claims may not be used during this time.
5. If all ten tubes are positive for any given sample, this is considered a verification failure and corrective actions must be taken immediately regardless of the result of the other samples for that month.
6. If verification fails twice during a twelve month period, revalidation is required and product should not be labeled until revalidation occurs.
7. The dealer in conjunction with the SSCA shall annually evaluate the previous 12 months of data and the HACCP plan.
8. The dealer may elect, with SSCA concurrence, to conduct quarterly sampling if the previous 12 verification samples pass.

Verification Sampling Protocol Decision Tree



Note: When a monthly verification fails, the verification must be reported within one week of failure

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Description: Flow chart showing the post harvest processing verification sampling protocol and decision making process.

Collect monthly shellfish meat samples for process verification.

If the monthly samples pass, no action is required.

If the monthly samples fail, take the following measures; (1) Identify the problem, (2) Fix the problem, (3) re-verify the process by sampling. If the re-verification samples pass, no further action is required. If the re-verification samples fail, then; (1) Corrective action must be taken on the product, (2) The process must be investigated, (3) Any problems identified must be adjusted, and (4) The process shall be revalidated. No labeling claims can be made during the interim revalidation process.

If the monthly samples fail and no problem can be identified then; (1) Adjustments shall be made to the process, and (2) The process shall be revalidated. No labeling claims can be made during the interim revalidation process.

.05 Guidance for Demonstrating the Effectiveness of Time to Temperature Reduction Criteria for *Vibrio vulnificus* and *Vibrio parahaemolyticus*

A. Time-to-Temperature Protocol

1. Identify the target time/temperature requirements for the specific cooling system/unit.
2. Demonstrate that each cooling method and unit is capable of meeting the target time/temperature by conducting a process study under worst case conditions for that unit. The following parameters should be considered and utilized in conducting the process study:
 - a. maximum load for the cooling unit
 - b. initial product temperature (studies have demonstrated that measurement of the external temperature and the internal meat temperatures are comparable and either can be used)
 - c. location of hot spot(s)
 - d. thermostat setting(s)
 - e. cooling methods(s) used
 - f. method of loading the cooling unit
3. Include a description of the process; a record of the process study conducted; and monitoring records in a HACCP Plan.
4. The protocol should be applied at the first point of refrigeration.