Final Report
Fishery Resource Grants Program

Project Title: Validation of an Individual Quick Freezing (IQF) Process to Reduce *Vibrio vulnificus* Numbers to <30MPN/g in Raw Oysters

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Executive Summary

This validation study was conducted during the months of July, August and Sept., 2003. The Food and Drug Administration and the Interstate Shellfish Sanitation Conference require that oysters used for a validation study are collected during the same processing day, are from the same lot of shellfish, and have an adjusted geometric mean (AGM) MPN of at least 100,000/g of *Vibrio vulnificus* as an initial load. The oysters were removed from refrigerated storage and held at 71°F (21°C) for 40-45 hours prior to freezing in order to achieve a minimum AGM of 100,000/g *Vibrio vulnificus*. After quick freezing the oysters at -70°F (-57°C) for nine minutes, approximately 8-10 weeks of frozen storage at 0°F (-18°C) were required to reduce *V. vulnificus* levels from 10^5 MPN/g to less than 30MPN/g. Initial *V. vulnificus* numbers in excess of 1,000,000/g, required prolonged frozen storage (>20 weeks) of the oysters at 0°F (-18°C) to reduce *V. vulnificus* numbers to less than 30MPN/g.

Little difference was found in the reduction of *V. vulnificus* numbers between frozen storage at 0°F (-18°C) or -15°F (-26°C). Thus, for cost effectiveness, 0°F (-18°C) is the recommended frozen storage temperature for the half shell oysters.

Two additional processing trials will be scheduled for early and mid-July, 2004 to confirm these findings. These two additional trials will be conducted without any further cost to the Fishery Resource Grants Program.

Background

The majority of seafood related illnesses in the U.S. can be attributed to consumption of raw or under cooked molluscan shellfish. Oysters are filter feeders that can concentrate pathogens. *Vibrio vulnificus (Vv)* is a naturally occurring estuarine bacterium that can accumulate in oysters and cause illness in consumers. *V. vulnificus* have caused illness and death in people following consumption of raw or under cooked oysters. In the U.S.A. since 1998, cases of *V. vulnificus* infections averaged approximately 40 with about 20 deaths per year. *V. vulnificus* are also found in oysters harvested from the Chesapeake Bay. With recent disease outbreaks and increased pressure from consumer advocacy groups, it is imperative to validate post harvest treatments (PHT) for eliminating pathogens in raw molluscan shellfish.

Low temperature quick freezing (IQF) of oysters using liquid CO_2 or nitrogen followed by frozen storage at 0°F (-18°C) can reduce levels of these pathogens. Recently, the State of California implemented regulations requiring all oysters from the Gulf of Mexico region sold in California to be certified *V. vulnificus* free. Other states may adopt similar regulations. If oyster populations in the Chesapeake Bay are eventually restored, using resistant native and/or non-native oysters, it is certainly possible that oysters from the Mid-Atlantic region will also require some type of PHT to ensure product safety. All PHT require validation before they are accepted by the Food and Drug Administration (FDA).

Objective

To validate a freezing process for raw oysters that can reduce *V. vulnificus* numbers from 10^5 MPN/g to <30MPN/g.

Materials and Methods

The freezing process validation project was conducted in cooperation with the Virginia Department of Shellfish Sanitation. The analyses for *V. vulnificus* were conducted at the Virginia Tech VSAREC, Hampton, VA, at the Virginia Tech Food Science Department, Blacksburg, VA, and at the National Marine Fisheries Service, National Seafood Inspection Laboratory, Pascagoula, MS.

The IQF freezing and frozen storage profiles were collected at the processing plant during each validation run using wire thermocouples (Appendix I). Approximately 2,000-3,000 (10,000-15,000 total) oysters were run on each of five processing dates (i.e., July 7, July 21, Aug. 11, Aug. 18, and Sept. 9). Analyses for *V. vulnificus,* moisture and pH were conducted on each batch of oysters (Appendix II). The requirements for validating a post harvest treatment process were determined by the FDA and the Interstate Shellfish Sanitation Conference (ISSC) (Appendix III). Procedures and protocols were developed after extensive discussions with Dr. Robert Wittman (Associate Director, Virginia Department of Shellfish Sanitation). Dr. Robert Wittman serves on the validation subcommittee of the ISSC. In addition, a copy of the proposed protocol was sent to Mr. Don Kraemer, Associate Director, FDA Office of Seafood, to ensure that all procedures meet with the FDA requirements.

The validation study was conducted during the months of July, August and Sept., 2003. Raw oysters on the half shell were frozen at -57°C (-70° F) for nine minutes and then stored at -0°F (-18°C).

ISSC Procedures

1). The ISSC preliminary procedures for post harvest method validation studies were followed. Data on ten processed samples on each of five processing days were collected and analyzed. The samples used on a processing day came from the same lot of shellfish with an adjusted geometric mean (AGM) MPN of 100,000/g or greater as an initial load.

Preliminary studies were conducted to determine the requirements needed to ensure a geometric mean (AGM) MPN of 100,000/g or greater as an initial load.

A). Ten oyster composites (ten oysters per composite) were collected immediately prior to IQF freezing. Ten additional samples were also collected immediately after IQF freezing. These 20 samples were analyzed in duplicate for *V. vulnificus* numbers. This procedure was repeated on five separate processing days.

B). Ten composites (10 oysters per composite) from each trial were removed periodically from frozen storage and analyzed in duplicate for *V. vulnificus* numbers.

ISSC Validation Protocol

The Interstate Shellfish Sanitation Conference (ISSC) and the Food and Drug Administration (FDA) established the following guidelines for validating Post Harvest Treatments (PHT) for oysters (Appendix III). For the process to be validated, no more than three samples out of 30 may fail. Failure is indicated by more than two out of five MPN tubes in any sample being positive for *V. vulnificus*. If any one sample has all five MPN tubes positive, the validation process will fail.

For Validation Testing
30 samples (ten from each of three processing days)
5 = the number of tubes in a single dilution series
0.1= the amount (g) of sample inoculated into each tube
2 = the maximum number of positive tubes for each sample to pass; if 3 or 4 tubes are positive, the sample fails
3 = the maximum number of SAMPLES out of 30 that can fail and the PROCESS is validated
If all five tubes in any one sample are positive, the PROCESS validation fails
A positive tube is defined as a tube which is confirmed to contain *V. vulnificus*.

True concentration (*Vibrio*/g)	Probability (%) of Process being validated
1	>95
2	95
3	54
4	11
5	1

NOTE: For each sample
If 2 out of 5 tubes are +, the MPN/g is 5
If 4 out of 5 tubes are +, the MPN/g is 16
If all 5 tubes are +, the PROCESS is not validated

Microbiological Analyses

FDA/ BAM Methodology was used for the study (FDA/BAM 2001). The microbiological protocol followed the recommendations of the ISSC work group on validation of PHT.

Results and Discussion

The attached tables contain all the results for the validation study (Appendix II). Approximately 2,000-3,000 oysters were run on each of five processing dates (i.e., July 7, July 21, Aug. 11, Aug. 18, and Sept. 9).

The tables for July 7, July 21, Aug. 11, and Sept. 9 contain data for samples stored at 0°F (-18°C).

The two tables for Aug. 11, and Aug, 18, 2003, contain data for oysters stored at -15 F (-26°C) [(initially the storage temperature was -23°F (-31°C), but the hurricane forced us to switch to -15 F (-26°C)].

The oysters were analyzed at the 0.01 level (<30MPN/g), and some were also analyzed at the 0.1 level (<3 MPN/g).

Table 1 below, is a summary of the data during frozen storage at 0°F (-18°C). The samples frozen on Aug. 11 and Sept. 9, 2003, had initial numbers of *V. vulnificus* in excess of 1,000,000/g. These high starting levels resulted in the need for prolonged storage of the samples in order to reduce the *V. vulnificus* numbers to <30MPN/g. The required frozen storage period (18 weeks to more than 20 weeks) is not economically viable for use by oyster processing companies.

However, the samples frozen on July 7 and July 21 had initial *V. vulnificus* numbers between 100,000/g and 1,000,000/g, and can be used to validate the process. Based on the data of July 7 and Aug. 18, it will require approximately 8-10 weeks of storage at 0°F to achieve the required reduction of *V. vulnificus* numbers from 100,000/g to less than 30MPN/g.

The samples frozen on Aug.18, on the other hand, had initial *V. vulnificus* numbers less than the required 100,000/g, and cannot be used for validation of the process. These data are included for information purposes only.

Summary Table 1
Number of Weeks in Frozen Storage at 0°F (-18°C) Required to Reduce *V. vulnificus* Numbers in Oysters to <30MPN/g

Date Frozen	Weeks at 0° F	Vv Levels
■ Frozen July 7	8-10 Wks	<30MPN/g
■ Frozen July 21	10 Wks	<30MPN/g
■ Frozen Aug 11	18 Wks	<30MPN/g
■ Frozen Aug 18	12 Wks	<30MPN/g
■ Frozen Sept 9	>20 Wks	<30MPN/g

Conclusions

Initial *V. vulnificus* Numbers

In order to achieve initial required *V. vulnificus* levels of 100,000/g, the oysters must be removed from refrigerated storage and exposed to ambient temperature. In this validation study, oysters were removed from refrigerated storage and held at 71°F (21°C) for 40-45 hours prior to freezing. However, it is important that the initial *V. vulnificus* numbers do not exceed 1,000,000/g for the validation study. Higher initial numbers of *V. vulnificus* result in the need for prolonged frozen storage (> 20 weeks in some instances) to reduce *V. vulnificus* numbers in oysters from 10^5 MPN/g to <30MPN/g.

Frozen Storage at 0°F (-18°C) Versus -15°F (-23°C)

Little difference was found in the reduction of *V. vulnificus* numbers at frozen storage temperatures of either 0°F (-18°C) or -15°F (-26°C). Thus, for cost effectiveness, 0°F is the recommended frozen storage temperature for the half shell oysters.

Frozen Storage Time Required to Reduce *V. vulnificus* Numbers to <30MPN/g

The results indicate that after quick freezing oysters at -70°F (-57°C) for nine minutes, it then requires approximately 8-10 weeks of frozen storage at 0°F (-18°C) to reduce *V. vulnificus* levels to less than 30MPN/g.

Additional Studies Needed

Two additional processing trials will be scheduled for early and mid-July, 2004 to confirm these findings. These two additional trials will be conducted without any further cost to the Fishery Resource Grants Program.

Benefits to Industry

Validation of a freezing procedure that is accepted by the FDA, and is effective in reducing or eliminating *V. vulnificus* in raw oysters, will result in increased sales for oyster processing companies.

The majority of seafood related illnesses in the U.S. can be attributed to consumption of raw or under cooked molluscan shellfish. Oysters are filter feeders that can concentrate pathogens. *V. vulnificus* is a naturally occurring estuarine bacterium that can accumulate in oysters and cause illness in consumers. *V. vulnificus* has caused illness and death in people following consumption of raw or under cooked oysters. In the U.S.A. since 1998, cases of *V. vulnificus* infections averaged approximately 40 with about 20 deaths per year. *V. vulnificus* are also found in oysters harvested from the Chesapeake Bay.

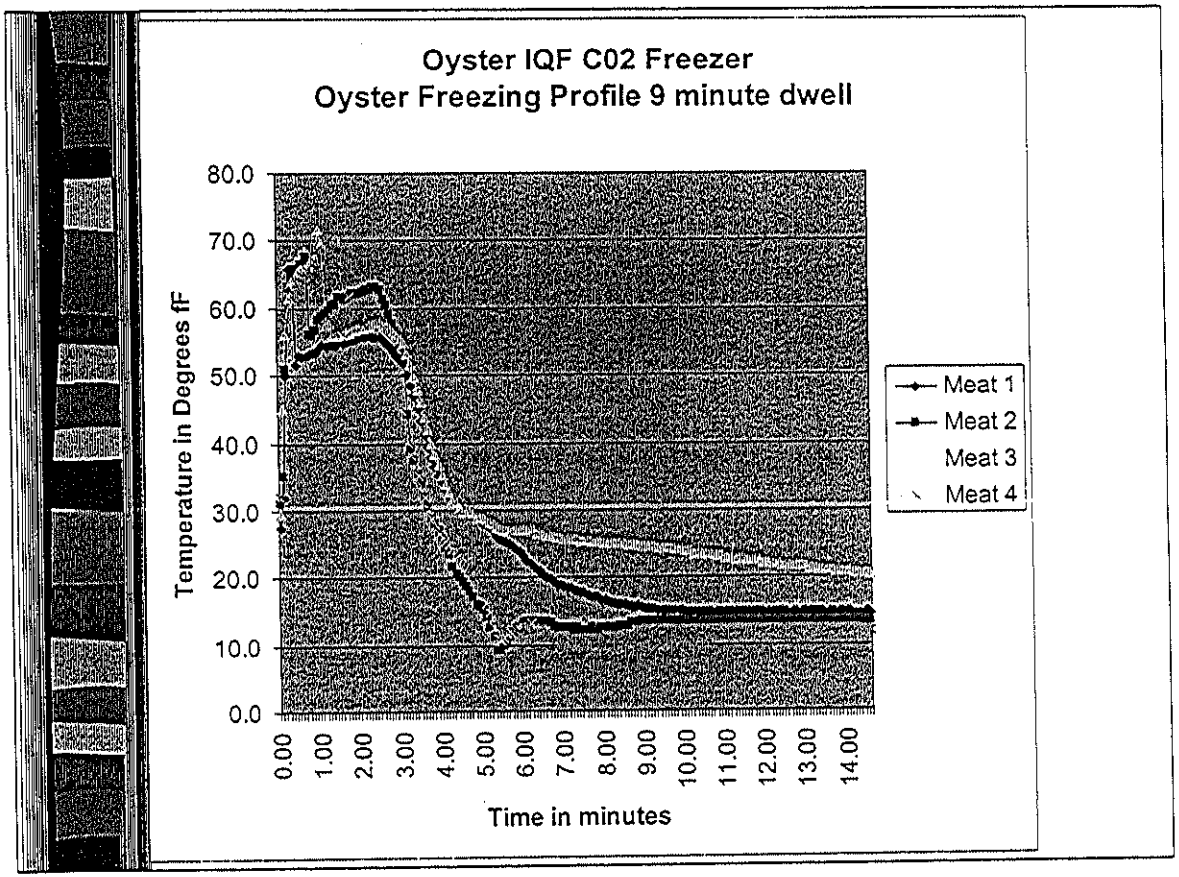
Recently, the State of California implemented regulations requiring all oysters from the Gulf of Mexico region sold in California to be certified *V. vulnificus* free. This regulation has resulted in losses in excess of $20 million for the Gulf Coast Industry. Oyster processors in Virginia and other Mid-Atlantic states have also had negative impacts on their business. Other states may adopt similar regulations. The Food and Drug Administration (FDA) is also encouraging the use of Post Harvest Treatments (PHT) to eliminate pathogens in raw molluscan shellfish. Freezing and subsequent frozen storage of raw oysters has been identified as a PHT that can be effective to reduce and/or eliminate *V. vulnificus*. However, all post harvest treatments require validation before they are accepted by the FDA. This validation study will provide data to the FDA so that the frozen oysters can be labeled as *V. vulnificus* free. In addition, if oyster numbers in the Chesapeake Bay are eventually restored, using resistant native and/or non-native oysters, it is certainly possible that oysters from the Mid-Atlantic will also require some type of PHT to ensure product safety.

References

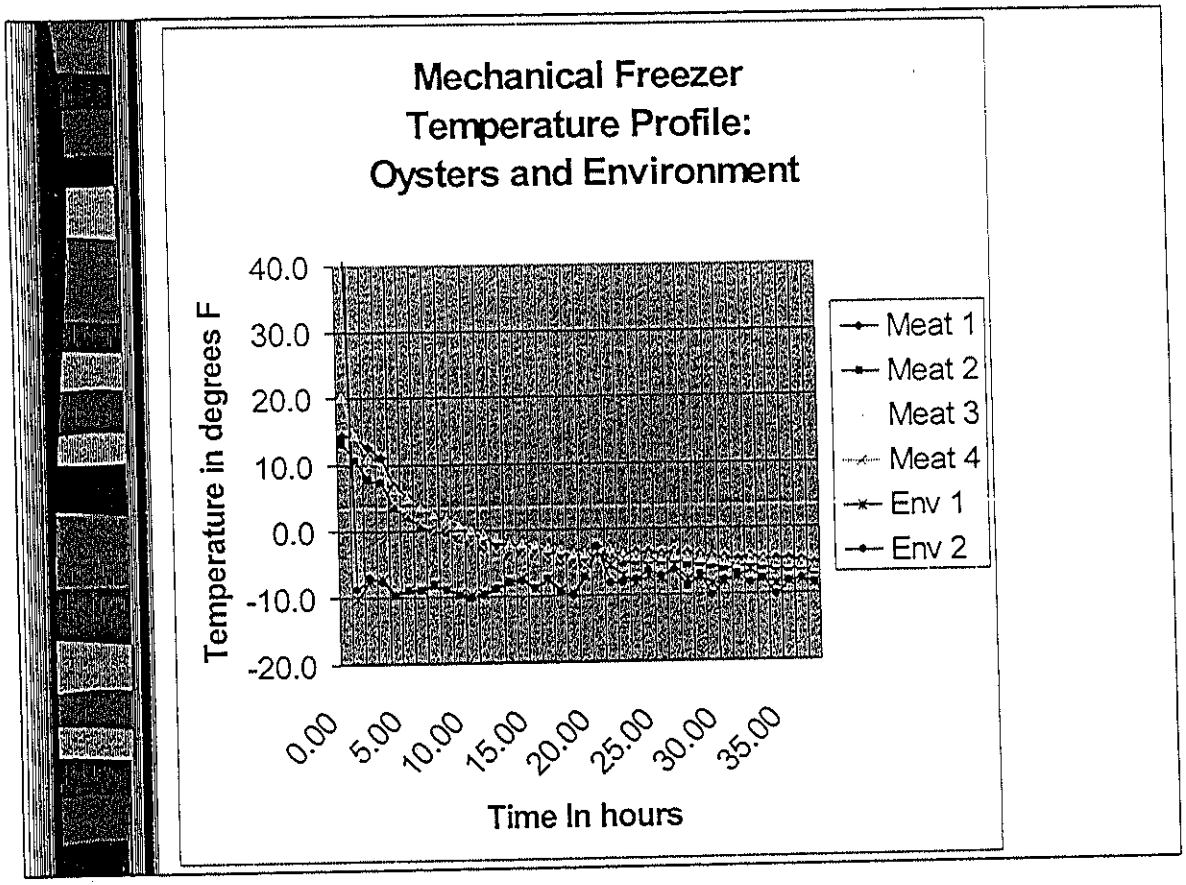
Anonymous. 2003. Some initial thoughts by the ISSC workgroup on validation of post harvest treatment methods for oysters. (Appendix III).

FDA/BAM. 2001. *Vibrio vulnificus*. Ch. 9 *In*: FDA Bacteriological Analytical Manual. Online, January 2001. Arlington, VA.

Freezing Times



Freezing Times



0° F

Trial I, frozen July 7, 2003

sample date: July 7, day zero (1-5 Hampton, 6-10 B'Burg)

	fresh samples								IQF frozen					
	A, # positive of 3				B, # positive of 3				A			B		
sample #	10-3 diln.	10-4 diln	10-5 diln.	MPN	10-3 diln.	10-4 diln	10-5 diln.	MPN	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	2	2	3	4.2 X 10⁴	1	2	1	1.5 X 10⁴	0.1 g	2	9	0.1 g	5	> 30
2	2	1	2	2.7 X 10⁴	1	3	3	1.1 X 10⁵	0.1 g	3	9	0.1 g	4	16
3	3	0	2	9.4 X 10⁴	0	3	3	1.1 X 10⁵	0.1 g	4	16	0.1 g	4	16
4	2	3	3	1.1 X 10⁵	2	3	2	1.1 X 10⁵	0.1 g	5	> 30	0.1 g	2	5
5	2	3	2	1.1 X 10⁵	1	3	3	1.1 X 10⁵	0.1 g	5	> 30	0.1 g	4	16
	10-4 diln.	10-5 diln.	10-6 diln	MPN	10-4 diln.	10-5 diln.	10-6 diln	MPN						
6	3	3	0	2.4 X 10⁵	3	3	0	2.4 X 10⁵	0.1 g	5	> 30	0.1 g	5	> 30
7	3	3	2	1.1 X 10⁶	3	3	1	4.6 X 10⁵	0.1 g	4	15	0.1 g	5	63
8	3	3	1	4.6 X 10⁵	3	3	2	1.1 X 10⁶	0.1 g	5	> 30	0.1 g	4	15
9	3	3	3	>1.1 X 10⁶	3	3	2	1.1 X 10⁶	0.1 g	5	> 30	0.1 g	5	> 30
10	3	3	1	4.6 X 10⁵	3	3	2	1.1 X 10⁶	0.1 g	5	> 30	0.1 g	5	> 30

sample date: July 21, 2 Weeks @0F (1-5, Hampton)

sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.1 g	2	5	0.1 g	2	5
2	0.1 g	3	9	0.1 g	0	< 2
3	0.1 g	3	9	0.1 g	3	9
4	0.1 g	0	< 2	0.1 g	0	< 2
5	0.1 g	2	5	0.1 g	0	< 2

(6-10, B'Burg)

sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
6	0.1 g	0	< 2	0.1 g	0	< 2
7	0.1 g	0	< 2	0.1 g	1	2
8	0.1 g	0	< 2	0.1 g	0	< 2
9	0.1 g	2	5	0.1 g	1	2
10	0.1 g	0	< 2	0.1 g	0	< 2

sample date: Aug. 4, 4 Weeks @0F (1-5, Hampton)

sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.1 g	2	5	0.1 g	2	5
2	0.1 g	5	> 30	0.1 g	3	9
3	0.1 g	5	> 30	0.1 g	5	> 30
4	0.1 g	5	> 30	0.1 g	5	> 30
5	0.1 g	4	16	0.1 g	4	16

(6-10, B'Burg)

sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
6	0.1 g	3	9	0.1 g	2	5
7	0.1 g	1	2	0.1 g	3	9
8	0.1 g	3	9	0.1 g	2	5
9	0.1 g	2	5	0.1 g	2	5
10	0.1 g	3	9	0.1 g	2	5

sample date: Aug. 19, 6 Weeks @0F (all samples to B'Burg)

sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.01 g	3	92	0.01 g	2	51
2	0.01 g	3	92	0.01 g	2	51
3	0.01 g	2	51	0.01 g	1	22
4	0.01 g	1	22	0.01 g	3	92
5	0.01 g	2	51	0.01 g	2	51

sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
6	0.01 g	2	51	0.01 g	1	22
7	0.01 g	1	22	0.01 g	2	51
8	0.01 g	2	51	0.01 g	0	< 22
9	0.01 g	1	22	0.01 g	0	< 22
10	0.01 g	2	51	0.01 g	0	< 22

sample date: Sept. 2, 8 Weeks @0F (all samples to B'Burg)

sample #	A			B			sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g		inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.01 g	0	< 22	0.01 g	2	51	6	0.01 g	1	22	0.01 g	0	< 22
2	0.01 g	1	22	0.01 g	2	51	7	0.01 g	1	22	0.01 g	0	< 22
3	0.01 g	1	22	0.01 g	1	22	8	0.01 g	0	< 22	0.01 g	0	< 22
4	0.01 g	1	22	0.01 g	2	51	9	0.01 g	3	92	0.01 g	1	34
5	0.01 g	1	22	0.01 g	1	22	10	0.01 g	0	< 22	0.01 g	1	34

sample date: Sept. 15, 10 Weeks @0F (all samples to Mississippi)

sample #	A			B			sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g		inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.01 g	2	51	0.01 g	1	22	6	0.01 g	1	22	0.01 g	1	34
2	0.01 g	3	92	0.01 g	2	51	7	0.01 g	0	< 22	0.01 g	0	< 22
3	0.01 g	0	< 34	0.01 g	0	< 22	8	0.01 g	0	< 22	0.01 g	0	< 22
4	0.01 g	0	< 22	0.01 g	1	22	9	0.01 g	1	22	0.01 g	0	22
5	0.01 g	2	51	0.01 g	0	< 22	10	0.01 g	0	< 22	0.01 g	0	< 22

sample date: Sept. 30, 12 Weeks @0F (all samples to Hampton)

sample #	A					B				
	inoculum	# positive	inoculum	# positive	MPN/g	inoculum	# positive	inoculum	# positive	MPN/g
1	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2
2	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2
3	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2
4	0.1 g	0	0.01 g	0	< 2	0.1 g	1	0.01 g	0	2
5	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2
6	0.1 g	0	0.01 g	0	< 2	0.1 g	1	0.01 g	0	2
7	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2
8	0.1 g	1	0.01 g	0	2	0.1 g	0	0.01 g	0	< 2
9	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2
10	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2

Trial II, frozen July 21, 2003

sample date: July 21, day zero (1-5 Hampton, 6-10 B'Burg) fresh samples pH = 6.11 Moisture = 84.7%

	A, # positive of 3				B, # positive of 3			
sample #	10-3 diln.	10-4 diln	10-5 diln.	MPN	10-3 diln.	10-4 dilN	10-5 dilN.	MPN
1	3	2	3	2.8 X 10⁴	2	2	2	3.6 X 10³
2	2	3	3	1.1 X 10⁵	3	3	2	1.1 X 10⁵
3	3	3	3	1.1 X 10⁵	3	2	2	2.1 X 10⁴
4	3	3	2	1.1 X 10⁵	2	2	3	4.2 X 10⁴
5	3	3	2	1.1 X 10⁵	3	3	2	1.1 X 10⁵
	10-4 dilN.	10-5 dilN.	10-6 dilN	MPN	10-4 dilN.	10-5 dilN.	10-6 dilN	MPN
6	3	3	3	>1.1 X 10⁶	3	3	1	4.6 X 10⁵
7	3	3	3	>1.1 X 10⁶	3	3	3	>1.1 X 10⁶
8	3	3	3	>1.1 X 10⁶	3	3	1	4.6 X 10⁵
9	3	3	1	4.6 X 10⁵	3	3	3	>1.1 X 10⁶
10	3	3	2	1.1 X 10⁶	3	3	1	4.6 X 10⁵

IQF Frozen pH = 6.43 moisture = 86.5%

	A			B		
sample #	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.1 g	5	> 30	0.1 g	5	> 30
2	0.1 g	5	> 30	0.1 g	5	> 30
3	0.1 g	5	> 30	0.1 g	5	> 30
4	0.1 g	5	> 30	0.1 g	5	> 30
5	0.1 g	5	> 30	0.1 g	5	> 30
6	0.1 g	4	16	0.1 g	4	16
7	0.1 g	5	> 30	0.1 g	5	> 30
8	0.1 g	5	> 30	0.1 g	5	> 30
9	0.1 g	5	> 30	0.1 g	5	> 30
10	0.1 g	5	> 30	0.1 g	5	> 30

sample date: Aug.4, 2 Weeks @0F pH = 6.40 moisture = 87.0% (1-5, Hampton) (6-10, B'Burg)

	A			B		
sample #	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.1 g	5	> 30	0.1 g	5	> 30
2	0.1 g	2	5.1	0.1 g	5	> 30
3	0.1 g	5	> 30	0.1 g	5	> 30
4	0.1 g	4	16	0.1 g	4	16
5	0.1 g	5	> 30	0.1 g	5	> 30
6	0.1 g	0	< 2.2	0.1 g	1	2.2
7	0.1 g	2	5.1	0.1 g	1	2.2
8	0.1 g	2	5.1	0.1 g	2	5.1
9	0.1 g	1	2.2	0.1 g	1	2.2
10	0.1 g	1	2.2	0.1 g	2	5.1

sample date: Aug. 18, 4 Weeks @0F pH = 6.24 moisture =87.9% (all samples to Hampton)

	A			B		
sample #	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.01g	0	< 22	0.01g	1	22
2	0.01g	0	< 22	0.01g	0	< 22
3	0.01g	0	< 22	0.01g	0	< 22
4	0.01g	0	< 22	0.01g	1	22
5	0.01g	1	22	0.01g	1	22
6	0.01g	0	< 22	0.01g	3	92
7	0.01g	0	< 22	0.01g	1	22
8	0.01g	1	22	0.01g	2	51
9	0.01g	2	51	0.01g	2	51
10	0.01g	3	92	0.01g	2	51

sample date: Sept. 3, 6 Weeks @0F pH = 6.27 moisture = 89.2% (all samples to Hampton)

	A			B		
sample #	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.01g	5	> 160	0.01g	5	> 160
2	0.01g	3	92	0.01g	4	160
3	0.01g	4	160	0.01g	3	92
4	0.01g	3	92	0.01g	4	160
5	0.01g	5	> 160	0.01g	5	> 160
6	0.01g	2	51	0.01g	2	51
7	0.01g	2	51	0.01g	2	51
8	0.01g	1	22	0.01g	2	51
9	0.01g	2	51	0.01g	2	51
10	0.01g	1	22	0.01g	1	22

sample date: Sept. 16, 8 Weeks @0F (all samples to B'Burg)

sample #	A			B			sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g		inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.01g	2	51	0.01g	3	92	6	0.01g	2	51	0.01g	3	92
2	0.01g	3	92	0.01g	3	92	7	0.01g	4	160	0.01g	3	92
3	0.01g	4	160	0.01g	3	92	8	0.01g	4	160	0.01g	3	92
4	0.01g	3	92	0.01g	4	160	9	0.01g	5	> 160	0.01g	4	160
5	0.01g	5	> 160	0.01g	4	160	10	0.01g	2	51	0.01g	2	51

sample date: Sept. 30, 10 Weeks @0F (all samples to B'Burg)

sample #	A			B			sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g		inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.01g	0	< 22	0.01g	1	22	6	0.01g	1	22	0.01g	0	< 22
2	0.01g	3	92	0.01g	0	< 22	7	0.01g	2	51	0.01g	2	51
3	0.01g	1	22	0.01g	0	< 22	8	0.01g	1	22	0.01g	1	22
4	0.01g	4	160	0.01g	2	51	9	0.01g	1	22	0.01g	1	22
5	0.01g	0	< 22	0.01g	0	< 22	10	0.01g	2	51	0.01g	1	22

sample date: Oct. 13, 12 Weeks @0F pH = 6.57 moisture = 86.9% (all samples to Hampton)

sample #	A					B				
	inoculum	# positive	inoculum	# positive	MPN/g	inoculum	# positive	inoculum	# positive	MPN/g
1	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2
2	0.1 g	1	0.01 g	0	2	0.1 g	2	0.01 g	0	5
3	0.1 g	1	0.01 g	0	2	0.1 g	1	0.01 g	0	2
4	0.1 g	1	0.01 g	2	6	0.1 g	2	0.01 g	1	7
5	0.1 g	4	0.01 g	2	22	0.1 g	3	0.01 g	0	8
6	0.1 g	1	0.01 g	0	2	0.1 g	1	0.01 g	0	2
7	0.1 g	2	0.01 g	0	5	0.1 g	2	0.01 g	1	7
8	0.1 g	2	0.01 g	0	5	0.1 g	0	0.01 g	0	< 2
9	0.1 g	2	0.01 g	0	5	0.1 g	1	0.01 g	1	4
10	0.1 g	1	0.01 g	0	2	0.1 g	0	0.01 g	0	< 2

sample date: Oct. 27, 14 Weeks @0F (all samples to Mississippi)

sample #	A					B				
	inoculum	# positive	inoculum	# positive	MPN/g	inoculum	# positive	inoculum	# positive	MPN/g
1	0.1 g	3			9.2	0.1 g	0			< 2.2
2	0.1 g	3			9.2	0.1 g	3			9.2
3	0.1 g	3			9.2	0.1 g	3			9.2
4	0.1 g	0			< 2.2	0.1 g	0			< 2.2
5	0.1 g	3			9.2	0.1 g	2			5.1
6	0.1 g	1			2.2	0.1 g	0			< 2.2
7	0.1 g	1			2.2	0.1 g	1			2.2
8	0.1 g	0			< 2.2	0.1 g	1			2.2
9	0.1 g	0			< 2.2	0.1 g	0			< 2.2
10	0.1 g	0			< 2.2	0.1 g	0			< 2.2

sample date: Nov. 10, 16 Weeks @0F (all samples to Hampton)

sample #	A					B				
	inoculum	# positive	inoculum	# positive	MPN/g	inoculum	# positive	inoculum	# positive	MPN/g
1	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2
2	0.1 g	1	0.01 g	0	2	0.1 g	1	0.01 g	0	2
3	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2
4	0.1 g	2	0.01 g	0	5	0.1 g	1	0.01 g	0	2
5	0.1 g	1	0.01 g	0	2	0.1 g	1	0.01 g	0	2
6	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2
7	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2
8	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2
9	0.1 g	1	0.01 g	0	2	0.1 g	0	0.01 g	0	< 2
10	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2

Trial III, frozen Aug. 11, 2003

sample date: Aug. 11, day zero (1-5 Hampton, 6-10 B'Burg)

	fresh samples				pH = 6.39	Moisture = 82.5%		
		A, # positive of 3				B, # positive of 3		
sample #	10-4 diln.	10-5 diln	10-6 diln.	MPN	10-4 diln.	10-5 diln	10-6 diln.	MPN
1	3	3	3	1.1 X 10^7	3	2	3	2.9 X 10^6
2	3	1	1	7.5 X 10^5	3	2	2	2.1 X 10^6
3	3	3	3	1.1 X 10^7	3	3	2	1.1 X 10^7
4	3	3	1	4.6 X 10^6	3	3	1	4.6 X 10^6
5	3	2	1	1.5 X 10^6	3	2	1	1.5 X 10^6

	IQF frozen			pH = 6.49	moisture = 87.2%	
	A			B		
sample #	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.1 g	3	9	0.1 g	3	9
2	0.1 g	1	2	0.1 g	2	5
3	0.1 g	2	5	0.1 g	2	5
4	0.1 g	3	9	0.1 g	4	16
5	0.1 g	3	9	0.1 g	3	9
6	0.1 g	4	16	0.1 g	4	16
7	0.1 g	5	> 30	0.1 g	5	> 30
8	0.1 g	5	> 30	0.1 g	5	> 30
9	0.1 g	5	> 30	0.1 g	5	> 30
10	0.1 g	5	> 30	0.1 g	5	> 30

sample date: Oct. 7, 8 Weeks @-0 F (all samples to B'Burg)

sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.01 g	3	92	0.01 g	4	160
2	0.01 g	3	92	0.01 g	4	160
3	0.01 g	2	51	0.01 g	2	51
4	0.01 g	1	22	0.01 g	4	160
5	0.01 g	0	< 22	0.01 g	1	22
6	0.01 g	2	51	0.01 g	4	160
7	0.01 g	4	160	0.01 g	2	51
8	0.01 g	2	51	0.01 g	3	92
9	0.01 g	2	51	0.01 g	3	92
10	0.01 g	3	92	0.01 g	2	51

sample date: Oct. 21, 10 Weeks @-0 F (all samples to B'Burg)

sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.01 g	2	51	0.01 g	3	92
2	0.01 g	1	22	0.01 g	2	51
3	0.01 g	2	51	0.01 g	3	92
4	0.01 g	2	51	0.01 g	1	22
5	0.01 g	1	22	0.01 g	2	51
6	0.01 g	3	92	0.01 g	3	92
7	0.01 g	3	92	0.01 g	2	51
8	0.01 g	2	51	0.01 g	3	92
9	0.01 g	2	51	0.01 g	2	51
10	0.01 g	2	51	0.01 g	3	92

Trial III, frozen Aug. 11, 2003

sample date: Aug. 11, day zero (1-5 Hampton, 6-10 B'Burg)

	fresh samples				pH = 6.39	Moisture = 82.5%		
	A, # positive of 3				B, # positive of 3			
sample #	10-4 diln.	10-5 diln	10-6 diln.	MPN	10-4 diln.	10-5 dilN	10-6 dilN.	MPN
1	3	3	3	1.1 X 107	3	2	3	2.9 X 106
2	3	1	1	7.5 X 105	3	2	2	2.1 X 106
3	3	3	3	1.1 X 107	3	3	2	1.1 X 107
4	3	3	1	4.6 X 106	3	3	1	4.6 X 106
5	3	2	1	1.5 X 106	3	2	1	1.5 X 106

	IQF frozen			pH = 6.49	moisture = 87.2%	
	A			B		
sample #	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.1 g	3	9	0.1 g	3	9
2	0.1 g	1	2	0.1 g	2	5
3	0.1 g	2	5	0.1 g	2	5
4	0.1 g	3	9	0.1 g	4	16
5	0.1 g	3	9	0.1 g	3	9
6	0.1 g	4	16	0.1 g	4	16
7	0.1 g	5	> 30	0.1 g	5	> 30
8	0.1 g	5	> 30	0.1 g	5	> 30
9	0.1 g	5	> 30	0.1 g	5	> 30
10	0.1 g	5	> 30	0.1 g	5	> 30

sample date: Sept. 8, 4 Weeks @-23 F (all samples to Mississippi)

	A			B				A			B		
sample #	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g	sample #	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.01 g	4	160	0.01 g	5	> 160	6	0.01 g	5	> 160	0.01 g	4	160
2	0.01 g	5	> 160	0.01 g	4	160	7	0.01 g	5	> 160	0.01 g	4	160
3	0.01 g	5	> 160	0.01 g	5	> 160	8	0.01 g	5	> 160	0.01 g	5	> 160
4	0.01 g	5	> 160	0.01 g	5	> 160	9	0.01 g	5	> 160	0.01 g	5	> 160
5	0.01 g	5	> 160	0.01 g	5	> 160	10	0.01 g	5	> 160	0.01 g	5	> 160

sample date: Sept. 23, 6 Weeks @-15 F (all samples to Mississippi)

	A			B				A			B		
sample #	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g	sample #	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.01 g	1	22	0.01 g	0	< 22	6	0.01 g	3	92	0.01 g	4	160
2	0.01 g	5	> 160	0.01 g	3	92	7	0.01 g	4	160	0.01 g	5	> 160
3	0.01 g	5	> 160	0.01 g	5	> 160	8	0.01 g	5	> 160	0.01 g	5	> 160
4	0.01 g	5	> 160	0.01 g	5	> 160	9	0.01 g	5	> 160	0.01 g	5	> 160
5	0.01 g	4	160	0.01 g	5	> 160	10	0.01 g	4	160	0.01 g	5	> 160

sample date: Oct. 7, 8 Weeks @-15 F pH = 6.3 moisture = 86.0% (all samples to Hampton)

	A					B				
sample #	inoculum	# positive	inoculum	# positive	MPN/g	inoculum	# positive	inoculum	# positive	MPN/g
1	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2
2	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2
3	0.1 g	0	0.01 g	1	2	0.1 g	0	0.01 g	0	< 2
4	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2
5	0.1 g	1	0.01 g	2	6	0.1 g	0	0.01 g	1	2
6	0.1 g	0	0.01 g	3	6	0.1 g	0	0.01 g	4	8
7	0.1 g	1	0.01 g	2	6	0.1 g	0	0.01 g	2	4
8	0.1 g	1	0.01 g	0	2	0.1 g	1	0.01 g	0	2
9	0.1 g	2	0.01 g	1	7	0.1 g	3	0.01 g	1	11
10	0.1 g	0	0.01 g	1	2	0.1 g	0	0.01 g	1	2

sample date: Oct. 21, 10 Weeks @-15 F (all samples to Hampton)

sample #	A					B				
	inoculum	# positive	inoculum	# positive	MPN/g	inoculum	# positive	inoculum	# positive	MPN/g
1	0.1 g	0	0.01 g	4	8	0.1 g	0	0.01 g	4	8
2	0.1 g	0	0.01 g	5	10	0.1 g	0	0.01 g	4	8
3	0.1 g	0	0.01 g	5	10	0.1 g	0	0.01 g	2	4
4	0.1 g	0	0.01 g	4	8	0.1 g	0	0.01 g	5	10
5	0.1 g	0	0.01 g	5	10	0.1 g	0	0.01 g	4	8
6	0.1 g	0	0.01 g	3	6	0.1 g	0	0.01 g	4	8
7	0.1 g	0	0.01 g	3	6	0.1 g	0	0.01 g	3	6
8	0.1 g	0	0.01 g	3	6	0.1 g	0	0.01 g	4	8
9	0.1 g	1	0.01 g	3	8	0.1 g	1	0.01 g	4	10
10	0.1 g	2	0.01 g	5	17	0.1 g	1	0.01 g	4	10

sample date: Nov. 3, 12 Weeks@-15 F pH = 6.39 moisture = 87.2% (all samples to Hampton)

sample #	A					B				
	inoculum	# positive	inoculum	# positive	MPN/g	inoculum	# positive	inoculum	# positive	MPN/g
1	0.1 g	3	0.01 g	5	24	0.1 g	3	0.01 g	4	21
2	0.1 g	3	0.01 g	4	21	0.1 g	2	0.01 g	3	12
3	0.1 g	4	0.01 g	5	38	0.1 g	3	0.01 g	2	14
4	0.1 g	2	0.01 g	5	17	0.1 g	0	0.01 g	3	6
5	0.1 g	4	0.01 g	5	38	0.1 g	0	0.01 g	4	8
6	0.1 g	3	0.01 g	4	21	0.1 g	0	0.01 g	5	10
7	0.1 g	4	0.01 g	4	32	0.1 g	2	0.01 g	4	14
8	0.1 g	2	0.01 g	3	12	0.1 g	4	0.01 g	2	22
9	0.1 g	1	0.01 g	2	6	0.1 g	2	0.01 g	3	12
10	0.1 g	2	0.01 g	4	14	0.1 g	4	0.01 g	2	22

sample date: Nov. 17, 14 Weeks @-15 F (all samples to Mississippi)

sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.01 g	5	> 160	0.01 g	3	92
2	0.01 g	4	160	0.01 g	3	92
3	0.01 g	4	160	0.01 g	5	> 160
4	0.01 g	4	160	0.01 g	3	92
5	0.01 g	5	> 160	0.01 g	5	> 160
6	0.01 g	5	> 160	0.01 g	5	> 160
7	0.01 g	3	92	0.01 g	4	160
8	0.01 g	5	> 160	0.01 g	5	> 160
9	0.01 g	5	> 160	0.01 g	5	> 160
10	0.01 g	2	51	0.01 g	2	51

sample date: Dec. 1, 16 Weeks @-15 F (all samples to Mississippi)

sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.01 g	5	> 160	0.01 g	5	> 160
2	0.01 g	1	22	0.01 g	2	51
3	0.01 g	4	160	0.01 g	5	> 160
4	0.01 g	3	92	0.01 g	3	92
5	0.01 g	3	92	0.01 g	2	51
6	0.01 g	3	92	0.01 g	5	> 160
7	0.01 g	3	92	0.01 g	4	160
8	0.01 g	3	92	0.01 g	5	> 160
9	0.01 g	5	> 160	0.01 g	5	> 160
10	0.01 g	4	160	0.01 g	3	92

sample date: Nov. 3, 12 Weeks @-0 F (all samples to B'Burg)

sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.01 g	4	160	0.01 g	3	92
2	0.01 g	2	51	0.01 g	2	51
3	0.01 g	3	92	0.01 g	3	92
4	0.01 g	5	> 160	0.01 g	4	160
5	0.01 g	3	92	0.01 g	3	92
6	0.01 g	3	92	0.01 g	2	51
7	0.01 g	2	51	0.01 g	2	51
8	0.01 g	1	22	0.01 g	2	51
9	0.01 g	1	22	0.01 g	0	< 22
10	0.01 g	1	22	0.01 g	1	22

sample date: Nov. 17, 14 Weeks @-0 F (all samples to B'Burg)

sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.01 g	4	160	0.01 g	3	92
2	0.01 g	4	160	0.01 g	5	> 160
3	0.01 g	4	160	0.01 g	5	> 160
4	0.01 g	1	22	0.01 g	3	92
5	0.01 g	3	92	0.01 g	3	92
6	0.01 g	1	22	0.01 g	1	22
7	0.01 g	3	92	0.01 g	4	160
8	0.01 g	5	> 160	0.01 g	4	160
9	0.01 g	4	160	0.01 g	3	92
10	0.01 g	4	160	0.01 g	4	160

sample date: Dec. 1, 16 Weeks@0 F pH = 6.09 moisture = 86.9% (all samples to Hampton)

sample #			A					B		
	inoculum	# positive	inoculum	# positive	MPN/g	inoculum	# positive	inoculum	# positive	MPN/g
1	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	1	2
2	0.1 g	0	0.01 g	1	2	0.1 g	1	0.01 g	1	4
3	0.1 g	1	0.01 g	1	4	0.1 g	3	0.01 g	1	11
4	0.1 g	3	0.01 g	1	11	0.1 g	2	0.01 g	0	5
5	0.1 g	0	0.01 g	1	2	0.1 g	0	0.01 g	1	2
6	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2
7	0.1 g	2	0.01 g	0	5	0.1 g	1	0.01 g	0	2
8	0.1 g	1	0.01 g	1	4	0.1 g	1	0.01 g	1	4
9	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2
10	0.1 g	0	0.01 g	0	< 2	0.1 g	0	0.01 g	0	< 2

Trial IV, frozen Aug. 18, 2003

Aug. 18

sample date: Aug. 18, day zero (1-5 at Hampton, 6-10 B'burg)

	fresh samples				pH = 6.40	Moisture = 85.8%				IQF Frozen			pH = 6.35	moisture = 90.5%	
	A, # positive of 3				B, # positive of 3					A			B		
sample #	10-4 dlln.	10-5 dlln	10-6 dlln.	MPN	10-4 dlln.	10-5 dlln	10-6 dlln.	MPN	sample #	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	3	3	0	2.4 X 10^5	3	3	2	1.1 X 10^6	1	0.01 g	3	92	0.01 g	4	160
2	3	3	0	2.4 X 10^5	2	2	1	3.6 X 10^4	2	0.01 g	4	160	0.01 g	4	160
3	2	3	1	3.6 X 10^4	2	3	1	3.6 X 10^4	3	0.01 g	5	> 160	0.01 g	4	160
4	2	0	0	9.3 X 10^3	2	2	0	2.1 X 10^4	4	0.01 g	2	51	0.01 g	3	92
5	2	2	2	3.5 X 10^4	3	3	0	2.4 X 10^5	5	0.01 g	4	160	0.01 g	4	160
6	3	2	0	9.3 X 10^4	3	2	1	1.5 X 10^5	6	0.1 g	4	16	0.1 g	4	16
7	2	0	0	9.3 X 10^3	3	0	0	2.3 X 10^4	7	0.1 g	5	> 30	0.1 g	5	> 30
8	3	3	0	2.4 X 10^5	3	2	1	1.5 X 10^5	8	0.1 g	5	> 30	0.1 g	5	> 30
9	3	2	1	1.5 X 10^5	3	1	0	4.3 X 10^4	9	0.1 g	5	> 30	0.1 g	5	> 30
10	3	1	0	4.3 X 10^4	2	0	0	9.3 X 10^3	10	0.1 g	5	> 30	0.1 g	5	> 30

sample date: Sept. 29, 6 Weeks @-15 F (all samples to Mississippi)

sample #	A			B			sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g		inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.01 g	2	51	0.01 g	3	92	6	0.01 g	0	< 22	0.01 g	0	< 22
2	0.01 g	1	22	0.01 g	2	51	7	0.01 g	0	< 22	0.01 g	1	22
3	0.01 g	1	22	0.01 g	2	51	8	0.01 g	1	22	0.01 g	0	< 22
4	0.01 g	0	< 22	0.01 g	1	22	9	0.01 g	0	< 22	0.01 g	0	< 22
5	0.01 g	0	< 22	0.01 g	0	< 22	10	0.01 g	0	< 22	0.01 g	1	22

sample date: Oct.13, 8 Weeks @-15 F (1-5 to Mississippi) (6-10 to B'Burg)

sample #	A			B			sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g		inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.01 g	0	< 22	0.01 g	1	22	6	0.01 g	4	160	0.01 g	2	51
2	0.01 g	2	51	0.01 g	2	51	7	0.01 g	3	92	0.01 g	3	92
3	0.01 g	0	< 22	0.01 g	0	< 22	8	0.01 g	3	92	0.01 g	4	160
4	0.01 g	1	22	0.01 g	2	51	9	0.01 g	4	160	0.01 g	4	160
5	0.01 g	0	< 22	0.01 g	0	< 22	10	0.01 g	2	51	0.01 g	1	22

sample date: Oct.27, 10 Weeks @-15 F (1-5 to Mississippi) (6-10 to B'Burg)

sample #	A			B			sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g		inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1							6	0.01 g	2	51	0.01 g	0	< 22
2							7	0.01 g	1	22	0.01 g	0	< 22
3							8	0.01 g	2	51	0.01 g	0	< 22
4							9	0.01 g	2	51	0.01 g	2	51
5							10	0.01 g	1	22	0.01 g	2	51

sample date: Nov. 10, 12 Weeks @-15 F (all samples to Mississippi) (6-10 to B'Burg)

sample #	A			B			sample #	A			B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g		inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.01 g	0	< 22	0.01 g	0	< 22	6	0.01 g	0	< 22	0.01 g	0	< 22
2	0.01 g	0	< 22	0.01 g	0	< 22	7	0.01 g	0	< 22	0.01 g	0	< 22
3	0.01 g	0	< 22	0.01 g	0	< 22	8	0.01 g	0	< 22	0.01 g	0	< 22
4	0.01 g	0	< 22	0.01 g	0	< 22	9	0.01 g	0	< 22	0.01 g	0	< 22
5	0.01 g	0	< 22	0.01 g	0	< 22	10	0.01 g	0	< 22	0.01 g	0	< 22

Trial V, frozen Sept. 9, 2003

sample date: Sept. 9, day zero (1-5, Hampton) (6-10, B'Burg)

fresh samples

	A, # positive of 3				B, # positive of 3			
sample #	10-4 diln.	10-5 diln	10-6 diln.	MPN	10-4 diln.	10-5 diln	10-6 diln.	MPN
1	3	3	3	>1.1 X 10^6	3	3	3	>1.1 X 10^6
2	3	3	3	>1.1 X 10^6	3	3	2	1.1 X 10^6
3	3	2	3	2.9 X 10^5	3	3	3	>1.1 X 10^6
4	3	3	3	>1.1 X 10^6	3	3	3	>1.1 X 10^6
5	3	3	3	>1.1 X 10^6	3	2	3	2.9 X 10^5
	A, # positive of 3				B, # positive of 3			
sample #	10-4 diln.	10-5 diln	10-6 diln.	MPN	10-4 diln.	10-5 diln	10-6 diln.	MPN
6	3	3	1	4.6 X 10^5	3	3	1	4.6 X 10^5
7	3	3	3	>1.1 X 10^6	3	3	3	>1.1 X 10^6
8	3	3	3	>1.1 X 10^6	3	3	3	>1.1 X 10^6
9	3	3	3	>1.1 X 10^6	3	3	2	1.1 X 10^6
10	3	3	0	2.4 X 10^5	3	3	2	1.1 X 10^6

sample date: Nov. 17, 10 Weeks @0 F pH = 6.24 moisture = 88.4% (all samples to Hampton)

sample #	A					B				
	inoculum	# positive	inoculum	# positive	MPN/g	inoculum	# positive	inoculum	# positive	MPN/g
1	0.1 g	4	0.01 g	3	27	0.1 g	3	0.01 g	3	17
2	0.1 g	5	0.01 g	3	76	0.1 g	3	0.01 g	4	21
3	0.1 g	4	0.01 g	4	32	0.1 g	4	0.01 g	2	22
4	0.1 g	3	0.01 g	4	21	0.1 g	4	0.01 g	5	38
5	0.1 g	3	0.01 g	5	24	0.1 g	3	0.01 g	4	21
6	0.1 g	4	0.01 g	5	38	0.1 g	5	0.01 g	5	> 160
7	0.1 g	5	0.01 g	3	76	0.1 g	4	0.01 g	2	22
8	0.1 g	4	0.01 g	2	22	0.1 g	3	0.01 g	2	14
9	0.1 g	4	0.01 g	5	38	0.1 g	3	0.01 g	1	11
10	0.1 g	4	0.01 g	3	27	0.1 g	4	0.01 g	3	27

sample date: Dec. 1, 12 Weeks @-0 F (all samples to B'Burg)

sample #	A		B		
	inoculum	# positive	MPN/g	inoculum	# positive	MPN/g
1	0.01 g	4	160	0.01 g	5	> 160
2	0.01 g	4	160	0.01 g	3	92
3	0.01 g	4	160	0.01 g	4	160
4	0.01 g	3	92	0.01 g	3	92
5	0.01 g	3	92	0.01 g	3	92
6	0.01 g	4	160	0.01 g	5	> 160
7	0.01 g	3	92	0.01 g	3	92
8	0.01 g	2	51	0.01 g	3	92
9	0.01 g	4	160	0.01 g	4	160
10	0.01 g	5	> 160	0.01 g	5	> 160

sample date: Dec. 15, 14 Weeks @0 F pH = 6.14 moisture = 87.5% (all samples to Hampton)

sample #	A					B				
	inoculum	# positive	inoculum	# positive	MPN/g	inoculum	# positive	inoculum	# positive	MPN/g
1	0.1 g	2	0.01 g	0	5	0.1 g	3	0.01 g	2	14
2	0.1 g	2	0.01 g	3	12	0.1 g	4	0.01 g	1	18
3	0.1 g	5	0.01 g	3	76	0.1 g	2	0.01 g	1	7
4	0.1 g	2	0.01 g	2	9	0.1 g	3	0.01 g	3	17
5	0.1 g	5	0.01 g	1	40	0.1 g	4	0.01 g	1	18
6	0.1 g	2	0.01 g	4	14	0.1 g	2	0.01 g	2	9
7	0.1 g	3	0.01 g	1	11	0.1 g	3	0.01 g	1	11
8	0.1 g	4	0.01 g	3	27	0.1 g	4	0.01 g	2	22
9	0.1 g	5	0.01 g	2	54	0.1 g	4	0.01 g	4	32
10	0.1 g	4	0.01 g	2	22	0.1 g	4	0.01 g	2	22

sample date: Jan. 6, 17 Weeks @0 F pH = 6.36 moisture = 88.3% (all samples to Hampton)

sample #	A					B				
	inoculum	# positive	inoculum	# positive	MPN/g	inoculum	# positive	inoculum	# positive	MPN/g
1	0.1 g	5	0.01 g	0	30	0.1 g	4	0.01 g	1	18
2	0.1 g	3	0.01 g	2	14	0.1 g	5	0.01 g	3	76
3	0.1 g	5	0.01 g	5	>121	0.1 g	3	0.01 g	3	17
4	0.1 g	5	0.01 g	4	121	0.1 g	5	0.01 g	4	121
5	0.1 g	2	0.01 g	3	12	0.1 g	1	0.01 g	3	8
6	0.1 g	4	0.01 g	4	32	0.1 g	4	0.01 g	4	32
7	0.1 g	3	0.01 g	4	21	0.1 g	3	0.01 g	0	8
8	0.1 g	2	0.01 g	1	7	0.1 g	3	0.01 g	0	8
9	0.1 g	4	0.01 g	2	22	0.1 g	4	0.01 g	2	22
10	0.1 g	2	0.01 g	2	9	0.1 g	0	0.01 g	3	6

sample date: Jan.28, 20 Weeks @0 F pH = 6.12 moisture = 88.8% (all samples to Hampton)

sample #	A					B				
	inoculum	# positive	inoculum	# positive	MPN/g	inoculum	# positive	inoculum	# positive	MPN/g
1	0.1 g	4	0.01 g	3	27	0.1 g	3	0.01 g	2	14
2	0.1 g	2	0.01 g	5	17	0.1 g	3	0.01 g	5	24
3	0.1 g	5	0.01 g	5	>121	0.1 g	3	0.01 g	5	24
4	0.1 g	4	0.01 g	4	32	0.1 g	4	0.01 g	4	32
5	0.1 g	1	0.01 g	3	8	0.1 g	0	0.01 g	4	8
6	0.1 g	4	0.01 g	4	32	0.1 g	5	0.01 g	4	121
7	0.1 g	3	0.01 g	3	17	0.1 g	3	0.01 g	5	24
8	0.1 g	3	0.01 g	3	17	0.1 g	4	0.01 g	2	22
9	0.1 g	0	0.01 g	2	4	0.1 g	0	0.01 g	1	2
10	0.1 g	3	0.01 g	4	20	0.1 g	3	0.01 g	3	17

Some Initial Thoughts by the ISSC Workgroup on Validation of Post Harvest Treatment Methods for Oysters

1. Each PHT process must define processing parameters (time at temperature, time at pressure, etc.) to serve as the critical limits for the process. Validation of the process must be done at the critical process limits.

2. For initial validation of a process, data on ten processed samples obtained on each of three processing days (total of 30 samples) are required. 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 100,000 per gram or greater as an initial load. Samples should be distributed throughout the processing day. A sample will consist of a composite of 10 to 12 oysters processed at one time.

3. Microbiological testing for processed samples will be by a single dilution five-tube MPN, inoculating with 0.1g of shellfish per tube.

4. For the process to be validated, no more than three samples out of 30 may fail. Failure is indicated by more than two out of five MPN tubes in any sample being positive for *V. vulnificus*. If any one sample has all five MPN tubes positive, the validation process will fail.

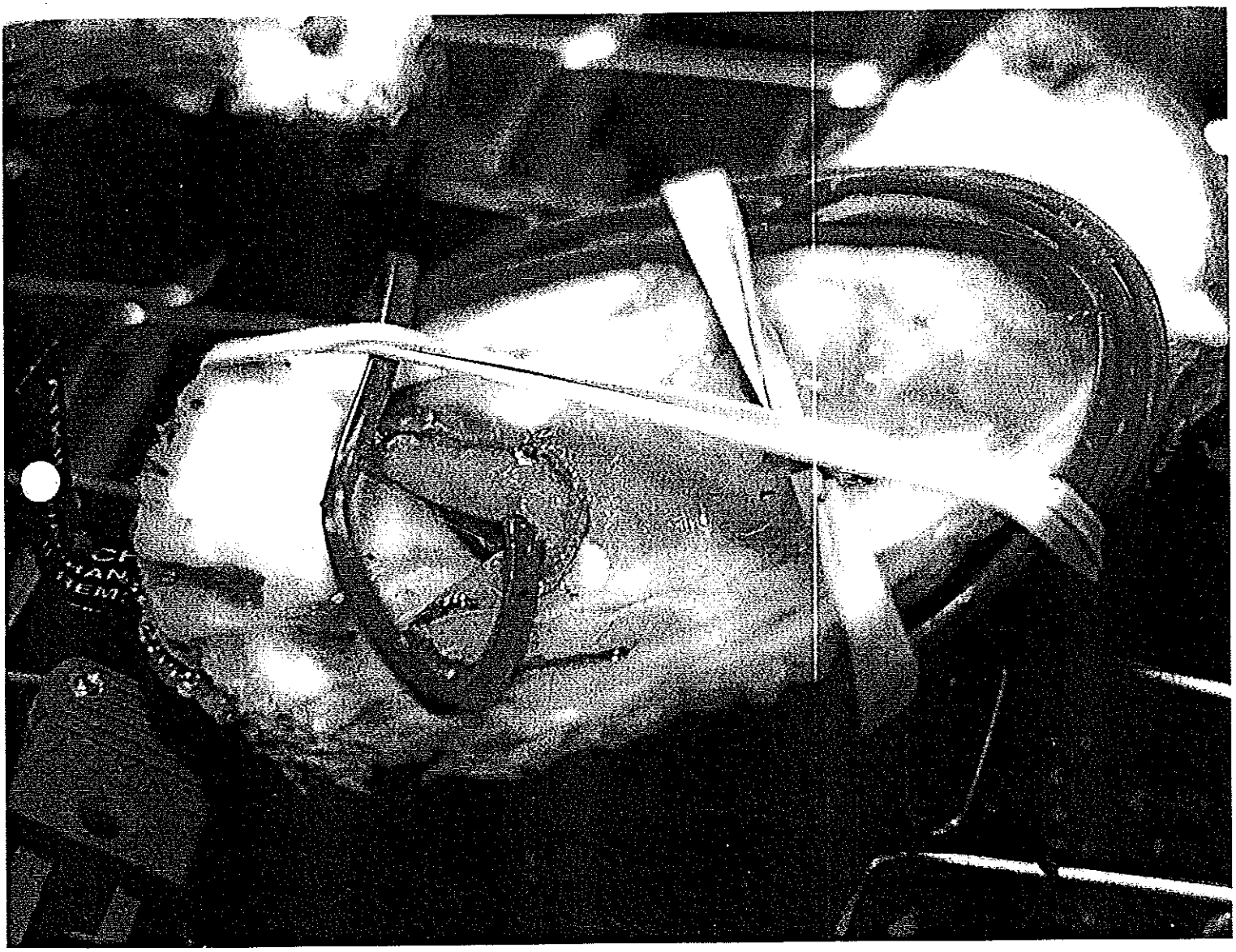
For Validation Testing:

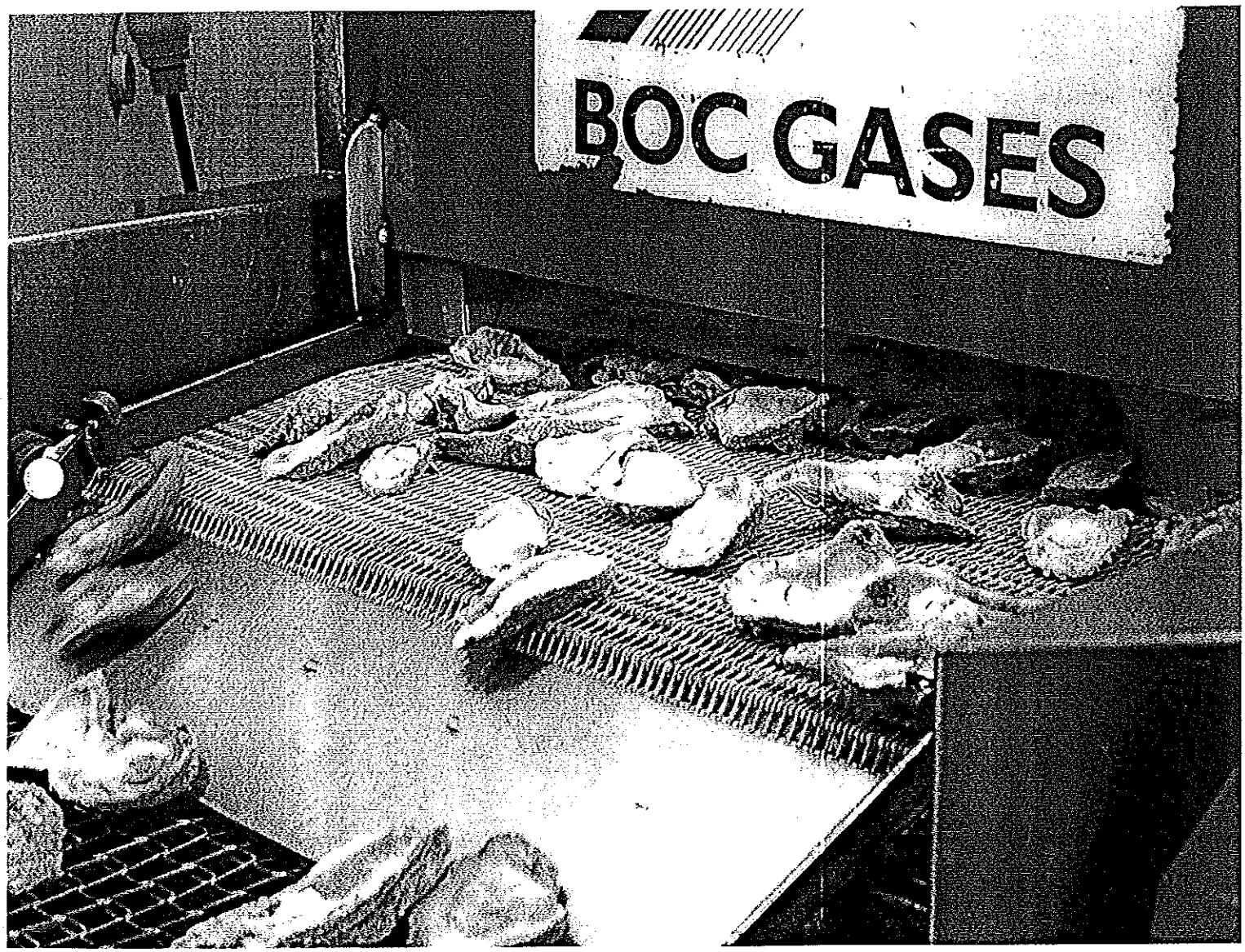
30 samples (ten from each of three processing days)
5 = the number of tubes in a single dilution series
0.1 = the amount (g) of sample inoculated into each tube
2 = the maximum number of positive tubes for each sample to pass; if 3 or 4 tubes are positive, the sample fails
3 = the maximum number of SAMPLES out of 30 that can fail and the PROCESS is validated
If all five tubes in any one sample are positive, the PROCESS validation fails
A positive tube is defined as a tube which is confirmed to contain *V. vulnificus*

The workgroup is still working on how the adjusted geometric mean for the initial load should be determined.

True concentration (*vibrios*/g)	Probability (%) of Process being validated
1	>95
2	95
3	54
4	11
5	1

NOTE: For each sample
If 2 out of 5 tubes are +, the MPN/g is 5
If 4 out of 5 tubes are +, the MPN/g is 16
If all 5 tubes are +, the PROCESS is not validated




BOC GASES