Proposal for Consideration at the Interstate Shellfish Sanitation Conference 2011 Biennial Meeting	☒ **Growing Area** ☐ **Harvesting/Handling/Distribution** ☐ **Administrative**
Name of Submitter:	Mercuria Cumbo
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Proposal Subject:	Total Coliform Method for Shellfish Dealer Process Water using the membrane filtration techniques with mEndo LES agar
Specific NSSP Guide Reference:	2009 NSSP Section IV Guidance Documents Chapter II Growing Areas .10 Approved NSSP Laboratory Tests - Type I and Type II Microbiological Methods, UV treated Seawater
Text of Proposal/ Requested Action	Accept Total Coliform Method for Shellfish Dealer Process Water using the membrane filtration techniques with mEndo LES agar as an alternative method to the APHA MPN method for the presence/absence of total coliforms in UV treated seawater. Single Laboratory Validation Study Results and Method approval application attached.
Public Health Significance:	This method produces results in 24 hours and is a less labor intensive method for laboratories. This more rapid test method would allow operators of facilities who provide disinfected process water for shellfish in wet storage and depuration operations the ability to know they have a problem and take the required remediation action on a more timely basis. It would reduce the workload for the laboratory performing the testing.
Cost Information (if available):	This alternative test should be less costly to the laboratories.

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

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

Name of the New Method	Total Coliform Method for Shellfish Dealer Process Water using the membrane filtration techniques with mEndo LES agar
Name of the Method Developer	Mercuria Cumbo and Cathy L. Vining
Developer Contact Information	Maine Department of Marine Resources Lamoine Water Quality Laboratory 22 Coaling Station Rd. Lamoine, ME 04605 207-667-5654 Mercuria.cumbo@maine.gov Cathy.l.vining@maine.gov

Checklist	Y/N	Submitter Comments
A. Need for the New Method		
1. Clearly define the need for which the method has been developed.	Y	Alternative method which is more rapid than current NSSP approved method. Less labor intensive for laboratory
2. What is the intended purpose of the method?	Y	Shellfish dealer disinfected process water
3. Is there an acknowledged need for this method in the NSSP?	Y	NSSP requires testing of disinfected process water
4. What type of method? i.e. chemical, molecular, culture, etc.	Y	Microbiological culture
B. Method Documentation		
1. Method documentation includes the following information:		
Method Title	Y	Total Coliform Method for Shellfish Dealer Process Water using the membrane filtration technique with mEndo LES agar
Method Scope	Y	Presence/absence of total coliform in disinfected shellfish process water
References	Y	See attached document
Principle	Y	See attached document
Any Proprietary Aspects	Y	none
Equipment Required	Y	Membrane filtration apparatus
Reagents Required	Y	
Sample Collection, Preservation and Storage Requirements	Y	
Safety Requirements	Y	
Clear and Easy to Follow Step-by-Step Procedure	Y	

Quality Control Steps Specific for this Method	Y	
C. Validation Criteria		
1 Accuracy / Trueness	NA	Performance criteria previously established for this method.
2 Measurement Uncertainty	NA	Performance criteria previously established for this method.
3 Precision Characteristics (repeatability and reproducibility)	NA	Performance criteria previously established for this method.
4 Recovery	NA	Performance criteria previously established for this method.
5. Specificity	NA	Performance criteria previously established for this method.
6. Working and Linear Ranges	NA	Performance criteria previously established for this method.
7 Limit of Detection	NA	Performance criteria previously established for this method.
8 Limit of Quantitation / Sensitivity	NA	Performance criteria previously established for this method.
9. Ruggedness	NA	Performance criteria previously established for this method.
10. Matrix Effects	NA	Performance criteria previously established for this method.
11. Comparability (if intended as a substitute for an established method accepted by the NSSP)	Y	See attached document. Method is comparable for presence/absence with the NSSP approved APHA MPN Lactose Broth/Brilliant green Bile Broth Total Coliform method.
D. Other Information		
1. Cost of the Method		Comparable to approved method
2. Special Technical Skills Required to Perform the Method		Comparable to approved method
3. Special Equipment Required and Associated Cost		Membrane filtration apparatus
4. Abbreviations and Acronyms Defined		
5. Details of Turn Around Times (time involved to complete the method)		22 - 24 hours
6. Provide Brief Overview of the Quality Systems Used in the Lab		Study performed in two Maine State Shellfish Sanitation program laboratories which have quality assurance plans, have been evaluated and found to conform with the requirements of the NSSP for microbiological laboratories.
Submitters Signature **Mercuria Cumbo**	Date: **June 3, 2011**	
Submission of Validation Data and Draft Method to Committee	Date:	
Reviewing Members	Date:	
Accepted	Date:	

Recommendations for Further Work	Date:
Comments:	

DEFINITIONS

1. Accuracy/Trueness - Closeness of agreement between a test result and the accepted reference value.
2. Analyte/measurand - The specific organism or chemical substance sought or determined in a sample.
3. Blank - Sample material containing no detectable level of the analyte or measurand of interest that is subjected to the analytical process and monitors contamination during analysis.
4. Comparability - The acceptability of a new or modified method as a substitute for an established method in the NSSP. Comparability must be demonstrated for each substrate or tissue type by season and geographic area if applicable.
5. Fit for purpose - The analytical method is appropriate to the purpose for which the results are likely to be used.
6. HORRAT value - HORRAT values give a measure of the acceptability of the precision characteristics of a method.[4]
7. Limit of Detection - the minimum concentration at which the analyte or measurand can be identified. Limit of detection is matrix and analyte/measurand dependent.[4]
8. Limit of Quantitation/Sensitivity - the minimum concentration of the analyte or measurand that can be quantified with an acceptable level of precision and accuracy under the conditions of the test.
9. Linear Range - the range within the working range where the results are proportional to the concentration of the analyte or measurand present in the sample.
10. Measurement Uncertainty - A single parameter (usually a standard deviation or confidence interval) expressing the possible range of values around the measured result within which the true value is expected to be with a stated degree of probability. It takes into account all recognized effects operating on the result including: overall precision of the complete method, the method and laboratory bias and matrix effects.
11. Matrix - The component or substrate of a test sample.
12. Method Validation - The process of verifying that a method is fit for purpose.[1]
13. Precision - the closeness of agreement between independent test results obtained under stipulated conditions.[1, 2] There are two components of precision:
    a. Repeatability - the measure of agreement of replicate tests carried out on the same sample in the same laboratory by the same analyst within short intervals of time.
    b. Reproducibility - the measure of agreement between tests carried out in different laboratories. In single laboratory validation studies reproducibility is the closeness of agreement between results obtained with the same method on replicate analytical portions with different analysts or with the same analyst on different days.
14. Quality System - The laboratory's quality system is the process by which the laboratory conducts its activities so as to provide data of known and documented quality with which to demonstrate regulatory compliance and for other decision-making purposes. This system includes a process by which appropriate analytical methods are selected, their capability is evaluated, and their performance is documented. The quality system shall be documented in the laboratory's quality manual.
15. Recovery - The fraction or percentage of an analyte or measurand recovered following sample analysis.

16. Ruggedness - the ability of a particular method to withstand relatively minor changes in analytical technique, reagents, or environmental factors likely to arise in different test environments.[4]
17. Specificity - the ability of a method to measure only what it is intended to measure.[1]
18. Working Range - the range of analyte or measurand concentration over which the method is applied.

REFERENCES:

1. Eurachem Guide, 1998. The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics. LGC Ltd. Teddington, Middlesex, United Kingdom.
2. IUPAC Technical Report, 2002. Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis, Pure Appl. Chem., Vol. 74, (5): 835-855.
3. Joint FAO/IAEA Expert Consultation, 1999. Guidelines for Single-Laboratory Validation of Anilytical Methods for Trace-Level Concentrations of Organic Chemicals.
4. MAF Food Assurance Authority, 2002. A Guide for the Validation and Approval of New Marine Biotoxin Test Methods. Wellington, New Zealand.
5. National Environmental Laboratory Accreditation. , 2003. Standards. June 5.
6. EPA. 2004. EPA Microbiological Alternate Procedure Test Procedure (ATP) Protocol for Drinking Water, Ambient Water, and Wastewater Monitoring Methods: Guidance. U.S. Environmental Protection Agency (EPA), Office of Water Engineering and Analysis Division, 1200 Pennsylvania Avenue, NW, (4303T), Washington, DC 20460. April.

Single Laboratory Validation (SLV) Protocol
For Submission to the Interstate Shellfish Sanitation Conference (ISSC)
For Method Approval

Application from Maine State Department of Marine Resources Single Laboratory Validation Study in support of acceptance of an alternative method for determining the presence/absence of total coliforms in disinfected shellfish process water.

Section A. Justification for New Method

Name of the New Method:	Total Coliform Method for Shellfish Dealer Process Water using the membrane filtration technique with mEndo LES agar
Specify the Type of Method:	microbiological, membrane filtration
Name of Method Developer:	Mercuria Cumbo and Cathy L. Vining
Developer Contact Information	Maine Department of Marine Resources Lamoine Water Quality Lab 22 Coaling Station Rd. Lamoine, ME 04605 Mercuria.Cumbo@maine.gov Cathy.L.Vining@maine.gov
Date of Submission:	June 3, 2011

Introduction:

This single laboratory validation study was conducted at both of the Maine Department of Marine Resources (MEDMR) Water Quality Laboratories, the laboratories that support the MEDMR growing area classification program. The Laboratories are referred to as Lamoine and Boothbay in this report. The study was carried out in each laboratory separately using disinfected recirculating wet storage process water from five facilities who submit samples on a weekly basis to the MEDMR laboratories for testing. The Lamoine study analyzed three of the facilities and the Boothbay study analyzed two of the facilities. The study was conducted over a one year period and represents all seasons.

The results of the study indicate that the MF method using mEndo LES agar is a viable alternative procedure for the APHA MPN as a presence/absence test for total coliforms in disinfected shellfish process water.

Purpose and Intended Use of the Method:

Where disinfection is applied to process waters in wet storage and depuration facilities, the NSSP Program Guide for the Control of Molluscan Shellfish requires that the disinfection system produces process water with no detectable coliforms using an NSSP approved method. We are proposing the use of the Total coliform membrane filtration method using mEndo LES agar for the presence or absence of total coliforms in disinfected shellfish process water as an alternative to the APHA multiple tube fermentation MPN total coliform method.

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

Currently there is one NSSP approved method; APHA multiple tube fermentation MPN method for total coliforms. This method requires two media and up to five days to complete. On the contrary the membrane filtration (MF) method is read in 22 to 24 hours. This method is comparable to the MPN method but has the advantage of providing sample results more quickly. When there are problems with the disinfection or process water system causing the presence of coliforms, the quicker analysis turnaround would allow the operator to take action on a timelier basis. The MF method requires less media and generally is less labor intensive for the laboratory.

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

There are two limitations with membrane filtration methods. Turbidity in the samples can hinder filtration and the presence of high levels of non coliform bacteria can suppress the growth of coliforms. Neither of these limitations should be applicable to disinfected process water. Successful disinfection of process water requires that turbidity be eliminated. Shellfish process water is generally filtered before it goes to the disinfection process. The disinfection process should reduce the levels of all bacteria in the process water. Neither turbid process water samples nor non-coliform bacteria were encountered in any of the process waters analyzed for this validation study.

Other Comments:

This method has been in use for more than 40 years and is published in Standard Methods for the Examination of Water and Wastewater. It has been approved by EPA for use with potable water (for compliance with the US Drinking water program) and environmental waters including marine waters. The purpose of this validation study was to determine its comparability with the NSSP approved method for determining the presence/absence of total coliforms in disinfected shellfish process water. The membrane filtration technique as a microbiological tool for bacterial identification has a precedent in the NSSP with the MF method for fecal coliforms.

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Section B. Method Documentation

Total Coliform Method in Shellfish Dealer Process Water using the Membrane Filtration Techniques with mEndo LES Agar

Method Scope.

This method is a standard widely used method with a long history. It first appeared in the Twelfth Edition of Standard Methods for the Examination of Water and Wastewater in 1965 for fresh water applications. Since this time its uses have widened. It is an EPA approved method for the Safe Drinking Water Program and EPA approved for analyzing environmental waters including marine waters for Total Coliforms. The FDA has adopted this method for testing bottled water as an indication of insanitation or possible contamination. Within the US Safe Drinking Water Program the total coliform standard is no detectable coliforms and generally uses a presence/absence reporting format. Disinfected shellfish process waters must meet this standard. The purpose of this validation study was to determine the method's applicability to determine the presence/absence of total coliforms in disinfected shellfish process water. Specifically, the study was conducted on five different recirculating wet storage facilities in Maine. Each facility has a different process water system providing filtration followed by UV disinfection.

References:

American Public Health Association (APHA). 1992. *Standard Methods for the Examination of Water and Wastewater*, 20th Edition. APHA/AWWA/WEF, Washington, D.C. 9222B.

U.S. Environmental Protection Agency. 1978. *Microbiological Methods for Monitoring the Environment, Water and Wastes*. EPA/600/8/78/017. EPA, Washington, D.C. Part III Section B.

U.S. Food and Drug Administration (FDA).1995.*Bacteriological Analytical Manual*. U.S. FDA, 8th Edition, AOAC, Arlington,VA. Chapter 4 Section III.

Principle:

The membrane filtration method using mEndo LES agar provides a direct count of bacteria in processed water based on the development of colonies on the surface of the membrane filter. A quantity of water is filtered using a vacuum pump through the membrane which retains the bacteria. After filtration the membrane containing the bacterial cells is placed on mEndo agar, a selective and differential medium, incubated at 35°C for 24 hours. Following incubation, red colonies with or without metallic sheen are counted with the aid of a fluorescent lamp and stereo dissecting microscope. One volume of 100ml sample is used. Counting of colonies is not necessary; any presence of total coliforms is unacceptable.

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Analytes/Measurands:

Total coliform

Proprietary Aspects:

None

Method:

Sample Collection and Preservation:

1. Process water shall be collected in a labeled sterile bottle or sample bag large enough to contain 110 ml of sample. Sample container must be filled to allow an air space for allow shaking of the sample.
2. Paperwork must accompany the sample which identifies the sample collector, sample location, date and time of collection.
3. Sample shall be placed in a cooler with ice or ice packs to maintain the cooler temperature between 0 and 10°C during transport.
4. Samples are placed in the refrigerator when received at the laboratory.
5. Samples are analyzed as soon as possible and not longer than 30 hours from time of collection.

Equipment:

Reagent grade water
Sterile 1 liter media bottle w/ magnetic stir bar
Top loading balance
Petri dishes, sterile, plastic, 15 x 60 mm w/ loose lids
Membrane filtration units (filter base and funnel), sterilized
Ultraviolet unit for sanitization of filter funnel between filtrations
Filter manifold or filtering funnel
Carboy (vacuum capable) or Erlenmeyer vacuum flask to collect filtered waste water
Vacuum pump
Nalgene Autoclavable Low Boy Carboy, 8 liter and dispensing tubing, or autoclavable squirt bottle
Incubator maintained at 35 ± 5°C
Refrigerator maintained at 0-4°C
Timer for timing UV sterilization
Membrane filters, sterile, white, grid marked, 47 mm diameter with 0.45 ± 0.02 µm pore size
Forceps, straight or curved, with smooth tips to handle filters without damage
Alcohol burner
95% ethanol, methanol or isopropanol in small wide-mouth container for flaming forceps
Hand tally or electronic counting device
Stereo dissecting microscope with a cool white fluorescent lamp
Inoculation loops, 10 ul, sterile, disposable

Micropipetter (20-200ul)
Sterile Micropipetter tips
Quality control bacteria
Escherichia coli culture 10^{-7} dilution for QC (EC) or *Enterobacter aerogenes* culture 10^{-7} dilution for QC (EA)
Staphylococcus aureus culture 10^{-2} dilution for QC (SA)

Media and Reagents:
mEndo Agar LES
95%ethanol (not denatured)
Phosphate Buffered Saline (PBS)

1. Determine how many plates will be needed for testing. Each sample requires 1 plate plus start, end and 3 bacteria QC plates.
2. Use commercially available mEndo LES agar. Agar is used at the rate of 51 grams of mEndo LES agar in 1 liter of reagent grade water containing 20 ml of 95% ethanol (not denatured). Weigh appropriate amount of agar for volume of agar needed.
3. To prepare the media, heat slowly while stirring; boil for 1 minute in a large flask or sterile 1 liter bottle with cap which will help ensure complete boil time without boiling over. Do not autoclave.
4. Pour 5 to 7 grams mEndo agar into 60 mm Petri dishes. Weigh plates and record. If any plates fall below 5 grams they must be discarded.
5. Maximum storage time for plates is two weeks - preferably plates should be prepared shortly before use. Store prepared plates in the refrigerator in the dark.
6. Prepare phosphate buffered saline either in carboy or squeeze bottle. Autoclave appropriate amount of time for the volume.

Procedure:

1. Check bulbs in the UV sterilizer to insure that lights are working. Use appropriate eye protection to view UV bulbs.
2. Place sterile filter base unit(s) on filtering flask or filter manifold.
3. Use sterile forceps to aseptically place a sterile membrane filter on the filter base, grid side up. Filter forceps are sterilized by dipping into the 95% alcohol and flaming in the alcohol burner.
4. Begin series with a blank QC plates first by adding 20-40 ml of sterile PBS to the funnel with filter. Start filtration. Rinse sides of funnel at least twice with 20-30 ml of sterile PBS.
5. Use sterile forceps to aseptically remove the membrane filter from the filter base and roll it onto the mEndo Agar to avoid the formation of bubbles between the membrane and the agar surface. Place the filter grid side up on the media. Reseat the membrane if bubbles occur. Close the dish, invert.
6. In between filtering place the funnel(s) and base(s) in the UV sterilization unit for 2 minutes.
7. Repeat the above process for samples.

8. Process water samples must be shaken 25 times in a 12" arc in 7 seconds and 100 ml quantity is filtered.
9. Start filtration and rinse funnel sides with 20 to 30 ml of PBS twice. When sample is completely through the filter, turn off the vacuum pump and remove filter with sterile forceps (alcohol dip and flamed), then placing onto the appropriately labeled mEndo plate.
10. Remember to sterilize forceps between use by dipping in alcohol and flaming.
11. Finish with bacteria QC controls by adding 20-40 ml of PBS to a funnel for each of the controls. Aseptically add 100 µl of 10^{-7} EC or EA solution to one funnel. This should give a count of 10-20 bacteria per plate. Inoculate a second funnel with 10 µl loopful of SA containing 20-40 ml PBS for a negative control. Rinse, filter, then place filters on labeled mEndo plates.
12. Incubate all plates at 35 ± 0.5°C for 22 - 24 hours.
13. Read plates under a stereo dissecting microscope. All red colonies with or without a metallic sheen are counted.
14. Any number of colonies shall be reported out as Positive for total coliforms in 100 ml of sample.

Quality Control:

Quality control measures are all those required in the NSSP microbiology laboratory checklist for the lab in general and specifically for the membrane filtration method. A membrane filtration procedure currently is approved and quality control requirements are established in the checklist.

Validation Data:

This method, membrane filtration using mEndo LES agar, is a standard method that has been employed for testing coliforms for more than 40 years. Performance criteria have previously been established for use for drinking water and environmental fresh and marine waters. The purpose of this study was to determine whether it would be acceptable as an alternative to the current NSSP approved method for determining the presence or absence of total coliforms in disinfected shellfish process water.

Two studies were conducted, one in each of the MEDMR Water Quality Laboratories. In this report the Laboratories are referred to as Lamoine and Boothbay. The data was analyzed for each laboratory separately.

Disinfected recirculated shellfish process water from five Maine facilities was used for the two studies; three facilities submit samples on a weekly basis for total coliform testing to Lamoine and two facilities submit samples to Boothbay. The process waters are normally absent of total coliforms, so it was necessary to spike the samples. For each round of testing an unspiked sample was tested by both methods. All unspiked samples were negative for total coliforms. The study was conducted over a period of a year, so all seasons are represented. To determine comparability testing was performed by both methods. For each round of testing, process water from one facility was spiked and analyzed. The samples were either spiked with *Escherichia*

coli, ATTC 11775 or *Enterobacter aerogenes,* ATTC 13048. Five to six aliquots of each process water sample were spiked at levels to provide determinate numbers of bacteria. Any dilutions that produced indeterminate results for either method was deleted from the computations. The spiked samples were analyzed in triplicate by both the APHA MPN method and MF method using mEndo LES agar. Ten rounds of testing were performed in Lamoine with 156 data points for each method. Boothbay completed 11 rounds with 150 data points for each method. One hundred (100) ml of sample was analyzed for each replicate. MPN were divided between 20 tubes with 5 ml per tube. The range for the MPN is <1 to >60 MPN/100ml. The range for the MF test is <1 to >80 CFU/100 ml.

Comparability :

For each laboratory the replicate data for the individual dilutions was averaged. The two methods were plotted against each other and a linear regression computed. All computations were performed on the log 10 transformation. The result is provided in the Figure 1 and 2. All of the data is presented in Table 1 and 2.

The linear regression line for the Lamoine method comparison is $y = 0.9687x - 0.0195$. Since we are looking at a presence/absence condition, the area of concern when comparing the method is 0. The linear regression was computed with the MPN as the x value and MF as the y value. The y intercept of the equation for the Lamoine data is -0.0195, statistically less than 0. The linear regression for Boothbay is $y = 0.97x + 0.027$. The y intercept of the Boothbay data is 0.027, essentially 0.

Figure 1 Lamoine Method Comparison

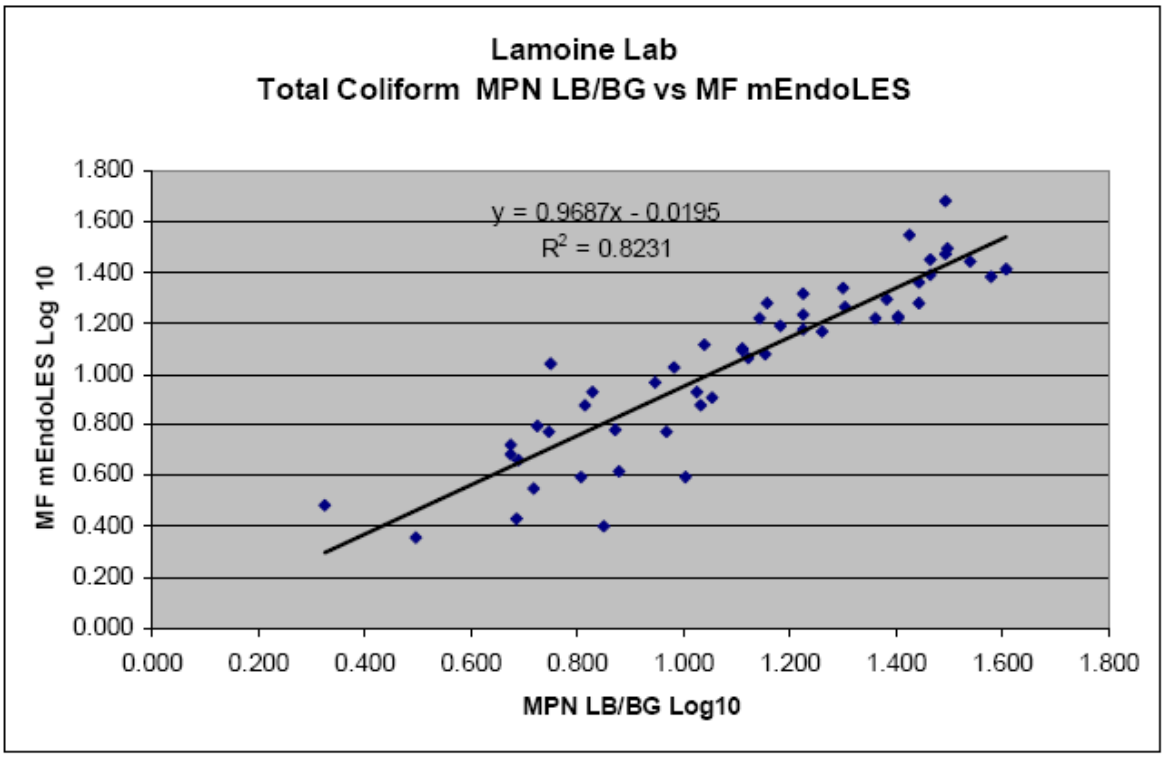

Figure 2. Boothbay Method Comparison

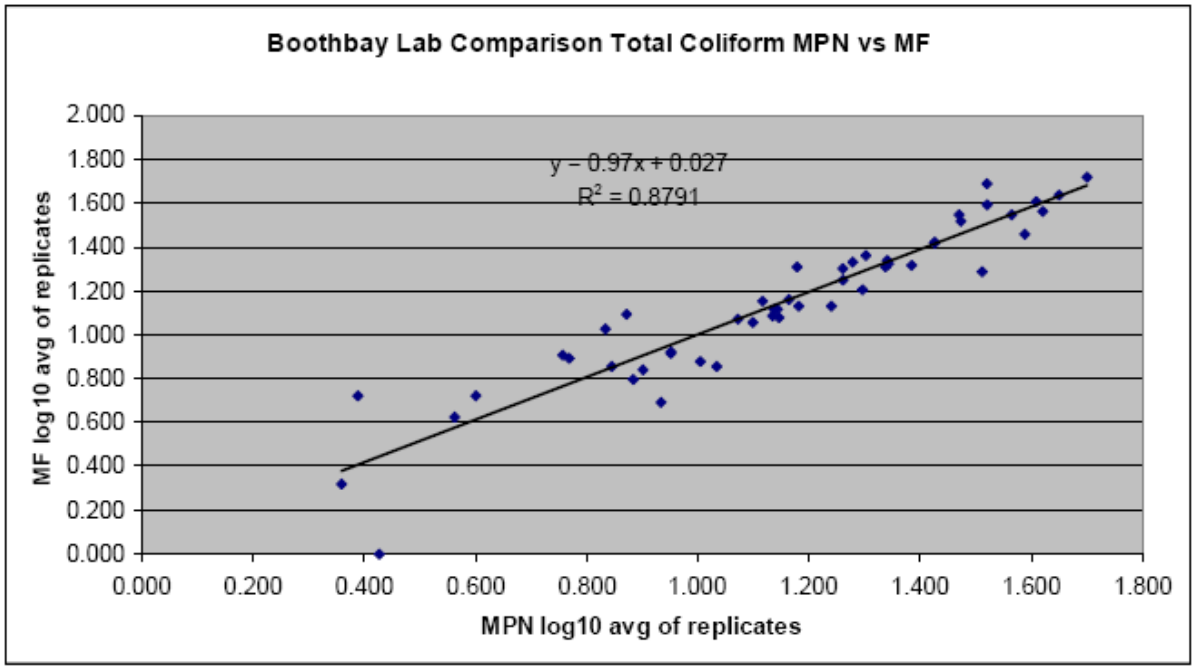

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Table 1 Lamoine method comparison data

DMR Water Quality Laboratory											
Lamoine, ME											
MF mENDO vs. MPN LB/BG Process Water Comparison											
				MPN LB/BG				MF mEndo LES			
Spiking Bacteria	Dealer	Date	Sample	Rep1	Rep2	Rep3	Geomean	Rep1	Rep2	Rep3	Geomean
Enterobacter aerogenes	TBR	9/21/2010	1	21	38	38	31	52	47	46	48
	TBR		2	21	38	38	31	24	28	39	30
	TBR		3	10	21	14	14	17	21	19	19
	TBR		4	12	16	12	13	12	13	10	12
	TBR		5	4.5	12	3.3	6	15	11	8	11
	TBR		6	8.6	8.6	5.8	8	9	4	2	4
	RDR	10/26/2010	1	28	32	24	28	25	25	19	23
	RDR		2	28	21	14	20	18	22	16	19
	RDR		3	21	8.6	12	13	13	11	13	12
	RDR		4	8.6	8.6	14	10	6	2	5	4
	RDR		5	2.1	5.8	8.6	5	3	7	7	5
	RDR		6	4.5	2.1	1	2	2	7	2	3
	MER	11/2/2010	1	28	28	18	24	24	19	17	20
	MER		2	21	24	12	18	16	13	15	15
	MER		3	12	10	10	11	10	12	5	8
	MER		4	5.8	7.1	10	7	7	4	8	6
	MER		5	10	4.5	5.8	6	4	5	3	4
	RDR	11/30/2010	2	60	32	28	38	31	23	20	24
	RDR		3	24	28	18	23	14	17	19	17
	RDR		4	14	21	12	15	18	17	12	15
	RDR		5	14	8.6	12	11	8	6	11	8
	RDR		6	5.8	4.5	4.5	5	4	3	8	5
	MER	12/7/2010	1	46	28	24	31	33	32	29	31
	MER		2	24	32	32	29	21	25	28	24
	MER		3	12	16	14	14	12	22	17	16
	MER		4	8.6	12	8.6	10	12	9	11	11
	MER		5	5.8	5.8	4.5	5	8	5	6	6
	MER		6	8.6	7.1	5.8	7	2	4	2	3
Escherichia coli	RDR	1/18/2011	4	38	38	46	40	23	24	32	26
	RDR		5	32	28	18	25	18	17	15	17
	RDR		6	14	24	14	17	14	21	17	17
	RDR	2/2/2011	2	21	28	32	27	34	34	38	35
	RDR		3	14	14	24	17	24	14	27	21
	RDR		4	12	10	18	13	14	18	8	13
	RDR		5	8.6	5.8	14	9	11	9	8	9
	RDR		6	7.1	8.6	4.5	7	6	8	9	8
	MER	2/8/2011	1	28	32	46	35	33	25	26	28
	MER		2	21	18	21	20	24	26	17	22

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DMR Water Quality Laboratory

Lamoine, ME

MF mENDO vs. MPN LB/BG Process Water Comparison

				MPN LB/BG				MF mEndo LES			
Spiking Bacteria	Dealer	Date	Sample	Rep1	Rep2	Rep3	Geomean	Rep1	Rep2	Rep3	Geomean
	MER		3	21	14	16	17	14	17	14	15
	MER		4	16	7.1	7.1	9	7	5	6	6
	MER		5	3.3	4.5	7.1	5	4	7	4	5
	MER		6	4.5	2.1	12	5	5	1	4	3
	RDR		2	32	24	32	29	21	34	32	28
	RDR		3	28	18	32	25	16	14	22	17
	RDR	3/8/2011	4	16	5.8	14	11	20	8	14	13
	RDR		5	12.0	12.0	8.6	11	8	6	9	8
	RDR		6	4.5	4.5	9	6	7	6	5	6
	MER		2	28	32	24	28	18	19	20	19
	MER		3	14	24	8.6	14	14	14	9	12
	MER	4/12/2011	4	12	12.0	2.1	7	10	5	12	8
	MER		5	4.5	4.5	7.1	5	3	5	3	4
	MER		6	3.3	2.1	4.5	3	3	2	2	2

Table 2 Boothbay method comparison data

DMR Boothbay Water Quality Lab

MF mEndo LES vs. MPN LB/BG Process Water Comparison

				MPN LB/BG				MF mEndo LES counts			
Spiking Bacteria	Dealer	Date	Sample	Rep1	Rep2	Rep3	Geomean	Rep 1	Rep 2	Rep 3	Geomean
Escherichia coli	CHS		1	60	46	46	50.3	52	54	51	52.3
	CHS		2	24	14	32	22.1	20	24	19	20.9
	CHS	7/6/2010	3	14.01	14	13.99	14.0	14	12	10	11.9
	CHS		4	7.1	5.8	4.5	5.7	11	6	8	8.1
	CHS		1	38	38	46	40.5	36	44	42	40.5
	CHS		2	28	32	38	32.4	14	22	23	19.2
	CHS	7/19/2010	3	10	4.5	7.1	6.8	12	11	9	10.6
	CHS		4	3.3	3.3	5.8	4.0	6	2	12	5.2
	CHS		5	1	5.8	3.3	2.7	1.01	1	1	1.0
	SMF		1	38	46	21	33.2	54	49	44	48.8
	SMF	8/30/2010	2	24	24	18	21.8	26	20	16	20.3
	SMF		3	9	10	14	10.8	9	10	4	7.1
	SMF		4	2.1	3.3	7.1	3.7	5	3	5	4.2
	CHS	9/13/2010	1	28	38	46	36.6	32	46	30	35.3
	CHS		2	18	16	24	19.0	23	24	18	21.5

Single Laboratory Validation (SLV) Protocol
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DMR Boothbay Water Quality Lab

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				MPN LB/BG				MF mEndo LES counts			
Spiking Bacteria	Dealer	Date	Sample	Rep1	Rep2	Rep3	Geomean	Rep 1	Rep 2	Rep 3	Geomean
	CHS		3	16	12	16	14.5	20	11	14	14.5
	CHS		4	10	14	14	12.5	10	12	12	11.3
	CHS		5	4.5	10	10	7.7	6	10	4	6.2
	SMF		1	24	24	46	29.8	31	36	32	32.9
	SMF		2	21	32	21	24.2	22	14	29	20.7
	SMF	9/27/2010	3	10	12	21	13.6	16	14	8	12.1
	SMF		4	5.8	10	7.1	7.4	12	12	13	12.3
	SMF		5	10	4.5	4.5	5.9	12	5	8	7.8
	CHS		1	28	46	28	33.0	43	33	41	38.7
	CHS		2	18	28	21	22.0	29	15	24	21.9
	CHS	1/10/2011	3	18	14	14	15.2	14	12	15	13.6
	CHS		4	16	14	10	13.1	16	13	14	14.3
	CHS		5	7.1	7.1	10	8.0	12	4	7	7.0
	CHS		1	60	32	46	44.5	43	50	38	43.4
	CHS		2	24	28	28	26.6	30	27	23	26.5
	CHS	1/31/2011	3	24	18	18	19.8	21	11	18	16.1
	CHS		4	14	12	16	13.9	13	21	8	13.0
	CHS		5	12	7.1	12	10.1	5	12	7	7.5
Enterobacter Aerogenes	SMF		1	38	24	28	29.4	35	39	32	35.2
	SMF		2	16	21	18	18.2	25	19	17	20.1
	SMF	8/2/10	3	10	7.1	10	8.9	12	7	7	8.4
	SMF		4	2.1	3.3	2.1	2.4	6	8	3	5.2
	SMF		5	3	4	1	2.3	3	1	3	2.1
	CHS		2	32	18	14	20.1	21	21	28	23.1
	CHS	8/16/2010	3	10	10	7.1	8.9	6	9	10	8.1
	CHS		4	12	3.3	8.6	7.0	4	9	10	7.1
	SMF		2	38	60	32	41.8	38	34	38	36.6
	SMF	10/26/2010	3	28	24	28	26.6	29	29	22	26.4
	SMF		4	16	18	12	15.1	20	19	22	20.3
	SMF		5	12	12	18	13.7	13	14	12	13.0
	CHS	11/29/2010	1	60	21	46	38.7	18	33	39	28.5
	CHS		2	18	21	16	18.2	15	17	22	17.8
	CHS		3	21	18	14	17.4	12	19	11	13.6
	CHS		4	8.6	16	12	11.8	17	7	14	11.9

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				MPN LB/BG				MF mEndo LES counts			
Spiking Bacteria	Dealer	Date	Sample	Rep1	Rep2	Rep3	Geomean	Rep 1	Rep 2	Rep 3	Geomean
	CHS		5	8.6	8.59	8.61	8.6	8	3	5	4.9

Summary of Results:

The membrane filtration method using mEndo LES agar for total coliform is a published standard method in use for over forty years. It is approved for use with drinking water (potable) and environmental water, fresh and marine. The performance criteria for this test has been previously established, therefore there was no need to complete performance criteria for this single laboratory validation study. The purpose of the study was to determine its comparability with the NSSP approved method.

As a presence/absence test the area of concern is zero (0). From the linear regression when the MPN is 0, at Lamoine the MF method is less than 0 (statistically) and at Boothbay it is 0.027, essentially 0.

This study indicates that the MF method is a viable alternative procedure for the APHA MPN as a presence/absence test for total coliforms in disinfected shellfish process water.