

Proposal for Consideration at the Interstate Shellfish Sanitation Conference 2011 Biennial Meeting		<input checked="" type="checkbox"/> Growing Area <input type="checkbox"/> Harvesting/Handling/Distribution <input type="checkbox"/> Administrative
Name of Submitter:	Mercuria Cumbo	
Affiliation:	Northeast Laboratory Evaluation Officers and Managers (NELEOM)	
Address:	MEDMR Lamoine WQ Laboratory 22 Coaling Station Rd. Lamoine, ME 04605	
Phone:	207 667-5654	
Fax:	207 664-0592	
Email:	mercuria.cumbo@maine.gov	
Proposal Subject:	Update Microbiology Laboratory Evaluation Checklist	
Specific NSSP Guide Reference:	2009 NSSP Section IV. Guidance Documents Chapter II. Growing Areas .11 Evaluation of Laboratories By State Shellfish Laboratory Evaluation Officers Including Laboratory Evaluation Checklists Laboratory Evaluation Checklist - Microbiology	
Text of Proposal/ Requested Action	<p>Update Microbiology Laboratory Evaluation Checklist. Please find the updated Microbiology Laboratory Checklist attached - word document titled "Revised Microbiology Checklist 11-08-2010.doc".</p> <p>A summary of the changes is:</p> <ul style="list-style-type: none"> • Renumbered checklist items to accommodate proposed additions and deletions and to better identify each checklist item. • Added, deleted or changed language for checklist items to be consistent with the PSP laboratory evaluation checklist. • Deleted the requirement for metals testing on reagent water and the inhibitory residue test for washed labware and increased the requirements for the bromothymol blue test. • Clarified and defined requirements for laboratory equipment, reagents including the bacterial quality control requirements for media productivity and method process control testing. • Update thermometer requirements to accommodate state bans on the use of mercury thermometers. • Updated the sterility check requirements for both in lab sterilized items and purchased pre-sterilized items. 	
Public Health Significance:	<p>The current microbiology laboratory checklist was last revised in 2009 when the male specific coliphage method was approved and added to the checklist. Deficiencies have been identified while using the microbiology checklist in evaluation of laboratories and the microbiology checklist is inconsistent with some requirements in the PSP checklist. It is important that the checklist items and quality assurance requirements are clear and understandable. It is important that quality assurance requirements among the different laboratory evaluation checklists remain as consistent as possible since many monitoring laboratories perform multiple types of tests and are evaluated using multiple NSSP checklists; inconsistencies among the checklist cause confusion, extra expense and work for the laboratories.</p>	
Cost Information (if available):	None	

PUBLIC HEALTH SERVICE
U.S. FOOD AND DRUG ADMINISTRATION
~~SHELLFISH PROGRAM IMPLEMENTATION BRANCH~~ OFFICE OF FOOD SAFETY
~~SHELLFISH SAFETY TEAM~~ SHELLFISH AND AQUACULTURE POLICY BRANCH
5100 PAINT BRANCH PARKWAY
COLLEGE PARK, MD 20740-3835
TEL. ~~301240-436 402-2151/21472055~~ FAX ~~301240-436 402-26012672~~

SHELLFISH LABORATORY EVALUATION CHECKLIST

LABORATORY:

ADDRESS:

TELEPHONE:

FAX:

EMAIL:

DATE OF EVALUATION:

DATE OF REPORT:

LAST EVALUATION:

LABORATORY REPRESENTED BY:

TITLE:

LABORATORY EVALUATION OFFICER:

SHELLFISH SPECIALIST:

REGION:

OTHER OFFICIALS PRESENT:

TITLE:

Items which do not conform are noted by:

C- Critical K - Key O - Other NA- Not Applicable Conformity is noted by a "√"

Check the applicable analytical methods:

- Multiple Tube Fermentation Technique for Seawater (APHA)[PART II]
- Multiple Tube Fermentation Technique for Seawater using MA-1 [PART II]
- Membrane Filtration Technique for Seawater using mTEC [PART II]
- Multiple Tube Fermentation Technique for Shellfish Meats (APHA)[PART III]
- Standard Plate Count for Shellfish Meats [PART III]
- Elevated Temperature Coliform Plate Method for Shellfish Meats [PART III]
- Male Specific Coliphage for Soft-shelled Clams and American Oysters [PART III]

PART 1 - QUALITY ASSURANCE

CODE	REF.	ITEM
K	8, 11	<u>L1</u> Quality Assurance (QA) Plan

		<input type="checkbox"/>	4- 1.1.1 Written Plan (Check those items which apply.)
		<input type="checkbox"/>	a. Organization of the laboratory.
		<input type="checkbox"/>	b. Staff training requirements.
		<input type="checkbox"/>	c. Standard operating procedures.
		<input type="checkbox"/>	d. Internal quality control measures for equipment, <u>their calibration, maintenance, repair, and for performance checks and rejection criteria established</u>
		<input type="checkbox"/>	e. Laboratory safety.
		<input type="checkbox"/>	f. Internal performance assessment.
		<input type="checkbox"/>	g. External performance assessment.
C	8	<input type="checkbox"/>	6- 1.1.2 QA Plan Implemented
K	11	<input type="checkbox"/>	7- 1.1.3 The Laboratory participates in a proficiency testing program annually. Specify Program(s) _____
1.2 Educational/Experience Requirements			
C	State's Human Resources Department	<input type="checkbox"/>	2- 1.2.1 In state/ <u>county</u> laboratories, the supervisor meets the state/ <u>county</u> educational and experience requirements for managing a public health laboratory
K	State's Human Resources Department	<input type="checkbox"/>	3- 1.2.2 In state/ <u>county</u> laboratories, the analyst(s) meets the state/ <u>county</u> educational and experience requirements for processing samples in a public health laboratory.
C	USDA Microbiology & EELAP	<input type="checkbox"/>	4- 1.2.3 In <u>private commercial</u> laboratories, the supervisor must have at least a bachelor's degree in microbiology, biology, or equivalent discipline with at least two years of laboratory experience.
K	USDA Microbiology & EELAP	<input type="checkbox"/>	5- 1.2.4 In <u>private commercial</u> laboratories, the analyst(s) must have at least a high school diploma and shall have at least three months of experience in laboratory sciences.
1.3 Work Area			
O	8,11	<input type="checkbox"/>	1- 1.3.1 Adequate for workload and storage.
K	11	<input type="checkbox"/>	2- 1.3.2 Clean, well lighted.
K	11	<input type="checkbox"/>	3- 1.3.3 Adequate temperature control.
O	11	<input type="checkbox"/>	4- 1.3.4 All work surfaces are nonporous, easily cleaned and disinfected.
K	11	<input type="checkbox"/>	5- Microbiological quality and density of air is < 15 colonies/plate in a 15 minute exposure determined monthly and results recorded. 1.3.5 Microbiological quality of the air contains fewer than 15 colonies for a 15 minute exposure and determined monthly. The results are recorded and records maintained.
O	11	<input type="checkbox"/>	6- Pipette aid used, mouth pipetting not permitted. -Moved to equipment 1.4.25
1.4 Laboratory Equipment			
O	9	<input type="checkbox"/>	1- 1.4.1 To determine the pH of prepared media, the pH meter has a standard accuracy of 0.1 units.
O	14	<input type="checkbox"/>	2- 1.4.2 pH electrodes consisting of pH half cell and reference half cell or equivalent combination electrode/ <u>triode</u> (free from <u>silver/silver chloride</u> (Ag/AgCl) or contains an ion exchange barrier <u>preventing passage of Ag ions into the medium which may effect the accuracy of the pH reading</u>) <u>to prevent the passage of silver (Ag) ions into the substance being measured.</u>
K	11	<input type="checkbox"/>	3- 1.4.3 The effect of temperature on the pH is compensated for by an ATC probe or by manual adjustment.
K	8	<input type="checkbox"/>	4- 1.4.4 pH meter is calibrated daily or with each use and records are maintained. <u>Results are recorded and records maintained.</u>
K	11	<input type="checkbox"/>	5- 1.4.5 A minimum of two standard buffer solutions is used to calibrate the pH meter.

			The first must be near the electrode isopotential point (pH 7). The second near the expected sample pH (i.e., pH 4 or pH 10). Standard buffer solutions are used once daily and discarded.
O	8,15	<input type="checkbox"/>	6. Electrode effectiveness is determined daily or with each use. Method of determination _____. <u>1.4.6 Electrode acceptability is determined daily or with each use by the millivolt procedure or through determination of the slope. (Circle the method used.)</u>
K	9	<input type="checkbox"/>	7. 1.4.7 Balance provides a sensitivity of at least 0.1 g at a load of 150 g weights of use.
K	11,13	<input type="checkbox"/>	8. Balance checked monthly using NIST Class S or ASTM Class 1 or 2 weights or equivalent and records are maintained. <u>1.4.8 Balance calibrations are checked monthly according to manufacturer's specifications using NIST Class S or ASTM Class 1 or 2 weights or equivalent. The accuracy of the balance is verified at the weight range of use. Results are recorded and records maintained.</u>
K	11	<input type="checkbox"/>	9. 1.4.9. Refrigerator temperature (s) monitored at least once daily <u>on workdays</u> and recorded. Results are recorded and records maintained
K	1	<input type="checkbox"/>	10. 1.4.10 Refrigerator temperature maintained at 0° to 4°C.
C	9	<input type="checkbox"/>	11. 1.4.11 The temperature of the incubator is maintained at 35 ± 0.5°C.
C	11	<input type="checkbox"/>	12. 1.4.12 Thermometers used in the air incubator(s) are graduated at no greater than 0.5° 0.1° C increments.
K	9	<input type="checkbox"/>	13. 1.4.13 Working thermometers are located on top and bottom shelves of use in the air incubator(s).
C	11	<input type="checkbox"/>	14. 1.4.14 Temperature of the waterbath is maintained at 44.5 ± 0.2°C under any all loading capacity conditions.
C	9	<input type="checkbox"/>	15. 1.4.15 The thermometers used in the waterbath are graduated in 0.1°C increments.
O-C	13	<input type="checkbox"/>	16. 1.4.16 The waterbath has adequate capacity for workload.
K	9	<input type="checkbox"/>	17. 1.4.17 The level of water in the waterbath covers the level of liquid in the incubating tubes.
K	8, 11	<input type="checkbox"/>	18. 1.4.18 Air incubator/waterbath temperatures are taken twice daily and recorded on <u>workdays.</u> <u>The results are recorded and records maintained.</u>
K	13	<input type="checkbox"/>	19. Working thermometers are tagged with identification, date of calibration, calibrated temperature and correction factor.
K-C	4	<input type="checkbox"/>	20. 1.4.19 All working thermometers are appropriately immersed.
<u>C</u>	<u>29</u>	<input type="checkbox"/>	<u>1.4.20 Either mercury-in-glass thermometers or non-mercury-in-glass thermometers having the accuracy (uncertainty), tolerance and response time of mercury are used as working thermometers. In the case of the waterbath, low drift electronic resistance thermometers with an accuracy of +0.05°C may also be used.</u>
K-C	11	<input type="checkbox"/>	21. A standards thermometer has been calibrated by NIST or one of equivalent accuracy at the points 0°, 35° and 44.5° C (45.5° C for ETCP). Calibration records maintained. <u>1.4.21 A standards thermometer has been calibrated by a qualified calibration laboratory using a primary standard traceable to NIST or an equivalent authority at the points 0°, 35° and 44.5°C (45.5°C for ETCP). These calibration records are maintained.</u>
K	9	<input type="checkbox"/>	22. 1.4.22 Standards thermometers is are checked annually for accuracy by ice point determination. Results recorded and maintained. Date of most recent determination _____.
<u>C</u>	<u>29</u>	<input type="checkbox"/>	<u>1.4.23 Either mercury-in-glass thermometers, non-mercury-in-glass thermometers having the accuracy (uncertainty), tolerance and response time of mercury or</u>

			<u>low drift electronic resistance thermometers with an accuracy of $\leq \pm 0.05^\circ\text{C}$ are used as the laboratory standards thermometer. (Circle the thermometer type used.)</u>
K	13	<input type="checkbox"/>	23- <u>1.4.24</u> Incubator and waterbath working thermometers are checked annually against the standards thermometer at the temperatures at which they are used. <u>Results are recorded and records maintained.</u>
<u>O</u>	<u>11</u>	<input type="checkbox"/>	<u>1.4.25</u> Appropriate pipet aids are available and used to inoculate samples. <u>Mouth pipetting is not permitted.</u>
<u>1.5 Labware and Glassware Washing</u>			
O	9	<input type="checkbox"/>	1- <u>1.5.1</u> Utensils and containers are clean borosilicate glass, stainless steel or other noncorroding materials
K	9	<input type="checkbox"/>	2- <u>1.5.2</u> Culture tubes are of a suitable size to accommodate the volume for nutritive ingredients and samples
K	9	<input type="checkbox"/>	3- <u>1.5.3</u> Sample containers are made of glass or some other inert material (ie polypropylene).
O	9	<input type="checkbox"/>	4- <u>1.5.4</u> Dilution bottles and tubes are made of borosilicate glass or plastic and closed with rubber stoppers, caps or screw caps with nontoxic liners.
K	9	<input type="checkbox"/>	5- <u>1.5.5</u> Graduations are indelibly marked on dilution bottles and tubes or an acceptable alternative method is used to ensure appropriate volumes.
K <u>C</u>	9	<input type="checkbox"/>	6- <u>1.5.6</u> Pipettes used to inoculate the sample deliver accurate aliquots, have unbroken tips and are appropriately graduated. Pipettes larger than 10 mL are not used to deliver 1mL <u>aliquots</u>; nor, are pipets larger than 1mL used to deliver 0.1 mL <u>aliquots</u>.
K	9	<input type="checkbox"/>	7- <u>1.5.7</u> Reusable sample containers are capable of being properly washed and sterilized.
K	9	<input type="checkbox"/>	8- <u>1.5.8</u> In washing reusable pipits, a succession of at least three fresh water rinses plus a final rinse of distilled/deionized water is used to thoroughly rinse off all the detergent.
<u>C</u>	9	<input type="checkbox"/>	9- <u>In washing reusable sample containers, glassware and plasticware, the effectiveness of the rinsing procedure is established annually and when detergent (brand or lot) is changed by the Inhibitory Residue Test as described in the current edition of Standard Methods for the Examination of Water and Wastewater. Records are kept.</u> Date of most recent testing _____ Average difference between Groups A and B _____ Average difference between Groups B and D _____ Detergent Brand _____ Lot # _____
<u>C</u>	<u>2</u>	<input type="checkbox"/>	<u>1.5.9 An alkaline or acidic detergent is used for washing glassware/labware.</u>
K <u>C</u>	11	<input type="checkbox"/>	10- <u>Once during each day of washing several pieces of glassware (pipettes, sample bottles, etc.) from one batch are tested for residual acid or alkali w/aqueous 0.04% bromthymol blue. Records are maintained.</u> <u>1.5.10 With each load of labware/glassware washed the contact surface of several dry pieces from each load are tested for residual detergent (acid or alkali) with aqueous 0.04% bromthymol blue. Results are recorded and records maintained.</u>
<u>1.6 Sterilization and Decontamination</u>			
O <u>K</u>	9	<input type="checkbox"/>	1- <u>1.6.1</u> Autoclave(s) are of sufficient size to accommodate the workload.
O	8	<input type="checkbox"/>	2- <u>1.6.2</u> Routine autoclave maintenance performed (e.g. pressure relief valves, exhaust trap, chamber drain) and <u>the records maintained.</u>
<u>O</u>	8	<input type="checkbox"/>	3- <u>Autoclave(s) and/or steam generators serviced annually or as needed by qualified technician and records maintained.</u>

C	11, 30	<input type="checkbox"/>	4. Autoclave(s) provides a sterilizing temperature of 121° C (tolerance 121 ± 2° C) as determined weekly using a calibrated working maximum registering thermometer or equivalent (thermocouples, platinum resistance thermometers). <u>1.6.3 The autoclave provides a sterilizing temperature of 121°C (tolerance 121 + 2°C) as determined for each load using a working maximum registering thermometer concluded to be within temperature tolerance specifications. As an alternative, an appropriate temperature monitoring device is used in place of the maximum registering thermometer when these are unavailable due to the ban on mercury.</u>
K	11	<input type="checkbox"/>	5. An autoclave standards thermometer has been calibrated by the National Institute of Standards and Technology (NIST) or its equivalent at 121° C. <u>1.6.4 An autoclave standards thermometer has been calibrated by a qualified calibration laboratory using a primary standard traceable to NIST or an equivalent authority at 121°C. Calibration at 100°C, the steam point, is also recommended but not required.</u>
K	16	<input type="checkbox"/>	6. The autoclave standards thermometer is checked every five years for accuracy at either 121° C or at the steam point. <u>1.6.5 The autoclave standards thermometer is checked every five years for accuracy at either 121°C or at 100°C, the steam point, if the thermometer has been previously calibrated at this temperature.</u> Date of most recent determination _____
K	1	<input type="checkbox"/>	7. <u>1.6.6 Working autoclave thermometers are checked against the autoclave standards thermometer at 121°C yearly.</u> Date of last check _____ Method _____
K	11	<input type="checkbox"/>	8. <u>1.6.7 Spore strips/suspensions appropriate for use in an autoclave are used monthly according to manufacturer's instructions to evaluate the effectiveness of the autoclave sterilization process. Results are recorded and the records maintained.</u>
O	11	<input type="checkbox"/>	9. <u>1.6.8 Heat sensitive tape is used with each autoclave batch.</u>
K	11, 13	<input type="checkbox"/>	10. <u>1.6.9 Autoclave sterilization records including length of sterilization, total heat exposure time and chamber temperature are maintained.</u> Type of record: Autoclave log, computer printout or chart recorder tracings. (Circle appropriate type or types.)
K	11	<input type="checkbox"/>	11. <u>1.6.10 For dry heat sterilized material, the hot-air sterilizing oven provides heating and sterilizing temperatures in the range of 160° to 180°C.</u>
K	9	<input type="checkbox"/>	12. <u>1.6.11 A thermometer capable of determining temperatures accurately in the range of 160 to 180°C is used to monitor the operation of the hot-air sterilizing oven when in use.</u>
K	13	<input type="checkbox"/>	13. <u>1.6.12 Records of temperatures and exposure times are maintained for the operation of the hot-air sterilizing oven during use.</u>
K	11	<input type="checkbox"/>	14. <u>1.6.13 Spore strips/suspensions are used quarterly to evaluate the effectiveness of the sterilization process in the hot-air oven. Records are maintained.</u>
K	11	<input type="checkbox"/>	15. <u>1.6.14 Reusable sample containers are sterilized for 60 minutes at 170°C in a hot-air oven or autoclaved for 15 minutes at 121°C.</u>
<u>OC</u>	1	<input type="checkbox"/>	16. The sterility of reusable/disposable sample containers is determined for each batch/lot. <u>1.6.15 The sterility of reusable sample containers is determined for each load sterilized. The results are recorded and the records maintained.</u>
<u>C</u>	<u>1</u>	<input type="checkbox"/>	<u>1.6.16 The sterility of pre-sterilized disposable sample containers is determined for each lot received. Results are recorded and the records maintained.</u>
K	9	<input type="checkbox"/>	17. <u>1.6.17 Reusable pipettes are stored and sterilized in aluminum or stainless steel canisters or equivalent alternative.</u>

K	9	<input type="checkbox"/>	18- <u>1.6.18</u> Reusable pipettes (in canisters) are sterilized in a hot-air oven at 170°C for 2 hours.
Θ <u>C</u>	2	<input type="checkbox"/>	19- The sterility of reusable/disposable pipettes is determined with each batch/lot. Results are recorded and maintained. <u>1.6.19 The sterility of reusable pipettes is determined with each load sterilized. Results are recorded and records maintained.</u>
<u>C</u>	<u>2</u>	<input type="checkbox"/>	<u>1.6.20 The sterility of pre-sterilized disposable pipets is determined with each lot received. Results are recorded and the records maintained.</u>
K	18	<input type="checkbox"/>	20- <u>1.6.21</u> Hardwood applicator transfer sticks are properly sterilized. <u>Method of sterilization</u>
<u>C</u>	<u>2</u>	<input type="checkbox"/>	<u>1.6.22 The sterility of the hardwood transfer sticks is checked routinely. Results are recorded and the records maintained.</u>
O	13	<input type="checkbox"/>	21- Spent broth cultures and agar plates are decontaminated by autoclaving for at least 30 minutes before conventional disposal. <u>1.6.23 Spent broth cultures and agar plates are decontaminated before disposal.</u> <u>Method</u>
<u>1.7 Media Preparation</u>			
K	3, 5	<input type="checkbox"/>	1- <u>1.7.1</u> Media is commercially dehydrated except in the case of medium A-1 which is <u>must</u> be prepared from the individual components and modified MacConkey agar which may be prepared from its components.
O	11	<input type="checkbox"/>	2- <u>1.7.2</u> Dehydrated media and media components properly stored in cool, clean, dry place.
O	11	<input type="checkbox"/>	3- <u>1.7.3</u> Dehydrated media are labeled with <u>the analyst's initials</u> date of receipt and date opened.
C	12	<input type="checkbox"/>	4- <u>1.7.4 Caked or expired media or media components are discarded.</u>
C	11	<input type="checkbox"/>	5- Make up water is distilled or deionized (<i>circle one</i>) and exceeds 0.5 megohm resistance or is less than 2μ Siemens/cm conductivity at 25° C to be tested and recorded monthly for resistance or conductivity (<i>circle the appropriate</i>) <u>1.7.5 Reagent water is distilled or deionized (circle appropriate choice), tested monthly and exceeds 0.5 megohm-cm resistance (2 megohms-cm in-line) or is less than 2.0 μSiemens/cm conductivity at 25°C. (Circle the appropriate water quality descriptor determined.) Results are recorded and the records maintained.</u>
C	11	<input type="checkbox"/>	6- <u>1.7.6 Makeup Reagent water is analyzed for residual chlorine monthly and is at a non-detectable level (< 0.1 ppm). Results are recorded and the records are maintained.</u> <u>Specify method of determination</u>
K	11	<input type="checkbox"/>	7- Make up water is free from trace (<0.05mg/L) dissolved metals, specifically Cd, Cr, Cu, Ni, Pb, and Zn as determined annually with total heavy metal content < or equal to 1.0mg/L and records are maintained.
K	11	<input type="checkbox"/>	8- <u>1.7.7</u> Make up <u>Reagent</u> water contains <1000 <u>≤100</u> CFU/mL as determined monthly using the heterotrophic plate count method. <u>Results are recorded and the records maintained.</u>
K	11	<input type="checkbox"/>	9- <u>1.7.8</u> <u>Commercially prepared dehydrated</u> media are sterilized according to the manufacturer's instructions.
K	9	<input type="checkbox"/>	10- <u>1.7.9</u> The volume and concentration of media in the tube are suitable for the amount of sample inoculated.
C	11	<input type="checkbox"/>	11- <u>1.7.10 Total time of exposure of sugar broths to autoclave temperatures does not exceed 45 minutes.</u>
C	1	<input type="checkbox"/>	12- Media sterility and positive and negative controls are run with each lot of commercially prepared media or are run with each batch of media prepared from its components as a check of media productivity. Results recorded and records

			maintained. <u>1.7.11 Media sterility is determined for each load sterilized. Results are recorded and the records maintained.</u>
<u>C</u>	<u>1</u>	<input type="checkbox"/>	<u>1.7.12 Media productivity is determined using media appropriate, properly diluted positive and negative control cultures for each lot of dehydrated media received or with each batch of media prepared from the individual components. When an alternative visual temperature monitoring device is used in place of the maximum registering autoclave thermometer, media productivity is determined using media appropriate, properly diluted positive and negative control cultures with each batch of media prepared.</u>
O	9	<input type="checkbox"/>	13- <u>1.7.13</u> Sterile phosphate buffered dilution water is used as the sample diluent.
K	11	<input type="checkbox"/>	14- <u>1.7.14</u> The pH of the prepared media is determined after sterilization to ensure that it is consistent with manufacturer's requirements. <u>Results are recorded and records are maintained.</u>
<u>1.8 Storage of Prepared Culture Media</u>			
Θ <u>K</u>	9	<input type="checkbox"/>	1- <u>1.8.1</u> Prepared culture media are stored in a cool, clean, dry space where excessive evaporation and the danger of contamination are minimized.
K	5,11	<input type="checkbox"/>	2- <u>1.8.2</u> Brilliant green bile 2% broth and A-1 media are stored in the dark.
K	13	<input type="checkbox"/>	3- <u>1.8.3</u> Stored media are labeled with the <u>storage</u> expiration date or <u>the</u> sterilization date.
O	9	<input type="checkbox"/>	4- <u>1.8.4</u> Storage of prepared culture media at room temperature does not exceed 7 days.
O	2	<input type="checkbox"/>	5- <u>1.8.5</u> Storage under refrigeration of prepared <u>broth</u> media with loose fitting closures shall not exceed 1 month.
O	11	<input type="checkbox"/>	6- <u>1.8.6</u> Storage under refrigeration of prepared <u>culture</u> media with screw-cap closures does not exceed 3 months.
K	17	<input type="checkbox"/>	7- <u>1.8.7</u> All prepared <u>media</u> <u>MPN broth</u> stored under refrigeration are held at room temperature overnight prior to use. Culture tubes containing any type of precipitate or Durham tubes containing air bubbles are discarded.
PART II - SEAWATER SAMPLES			
<u>2.1 Collection and Transportation of Samples</u>			
C	11	<input type="checkbox"/>	1- <u>2.1.1</u> <u>Sample</u> containers are of a suitable size to contain at least 100 <u>110</u> mL of <u>sample</u> and to allow <u>adequate</u> headspace for <u>proper</u> shaking. Seawater samples are collected in clean, sterile, watertight, properly labeled sample containers.
K	1	<input type="checkbox"/>	2- <u>2.1.2</u> Samples are identified with collectors name, harvest area, <u>sampling station</u> , time and date of collection.
C	9	<input type="checkbox"/>	3- After collection, seawater samples shall be kept at a temperature between 0 and 10°C until examined. <u>2.1.3 Immediately after collection, seawater samples are placed in dry storage (ice chest or equivalent) which is maintained between 0° and 10°C with ice or cold packs for transport to the laboratory. Once received, the samples are placed in the refrigerator unless processed immediately.</u>
K <u>O</u>	1	<input type="checkbox"/>	4- <u>2.1.4</u> A temperature blank is used to determine the temperature of samples upon receipt at the laboratory. Results are recorded and maintained.
C	9	<input type="checkbox"/>	5- Examination of the sample is initiated as soon as possible after collection. However, seawater samples are not tested if they are held beyond 30 hours of refrigeration. <u>2.1.5 Analysis of the sample is initiated as soon as possible after collection. Seawater samples are not tested if they have been held for more than 30 hours from the time of collection.</u>
<u>2.2 Bacteriological Examination of Seawater by the APHA MPN</u>			
C	9	<input type="checkbox"/>	1- <u>2.2.1</u> Lactose broth or lauryl tryptose broth is used as the presumptive medium. <i>(Circle appropriate one.)</i>

<u>C</u>	<u>2</u>	<input type="checkbox"/>	<u>2.2.2 The media productivity controls utilized are properly diluted and appropriate for the presumptive medium being used. The results are recorded and the records maintained.</u> <u>Positive productivity control</u> _____ <u>Negative productivity control</u> _____
C	9	<input type="checkbox"/>	2. <u>2.2.3</u> Sample and dilutions of sample are shaken <u>mixed</u> vigorously (25 times in a 12" arc in 7 seconds) before inoculation.
C	9	<input type="checkbox"/>	3. <u>2.2.4</u> In a multiple dilution series not less than 3 tubes per dilution are used (5 tubes are recommended).
C	6	<input type="checkbox"/>	4. <u>2.2.5</u> In a single dilution series not less than 12 tubes are used (for depuration at least 5 tubes are used).
K <u>C</u>	6	<input type="checkbox"/>	5. <u>2.2.6</u> In a single dilution series, the volumes analyzed <u>examined</u> are adequate to meet the needs of routine monitoring. Sample volume inoculated _____ Range of MPN _____ Strength of media used _____
K	9	<input type="checkbox"/>	6. <u>2.2.7</u> Inoculated media tubes are placed in an air incubator incubated in air at $35 \pm 0.5^\circ\text{C}$ for up to 48 ± 3 hours.
K <u>C</u>	2	<input type="checkbox"/>	7. Positive and negative control cultures accompany samples throughout the procedure. Records are maintained. <u>2.2.8</u> <u>Appropriately diluted process control cultures accompany the samples throughout both the presumptive and confirmed phases of incubation. Results are recorded and the records maintained.</u> <u>Positive process control</u> _____ <u>Negative process control</u> _____
K	9	<input type="checkbox"/>	8. Inoculated media are read after 24 ± 2 hours and 48 ± 3 hours of incubation and transferred at both intervals if positive for gas. 2.2.9 <u>2.2.9</u> Inoculated tubes are read after 24 ± 2 hours and 48 ± 3 hours of incubation and transferred at both time intervals if positive for growth (the presence of turbidity) and gas or effervescence in the culture tube. These tubes are considered presumptive positive requiring further confirmatory testing
			<u>2.3 Confirmed Test for Seawater by APHA MPN</u>
C	9	<input type="checkbox"/>	1. <u>2.3.1</u> Brilliant green bile 2% broth (BGB) is used as the confirmatory medium for total coliforms.
C	9	<input type="checkbox"/>	2. <u>2.3.2</u> EC medium is used as the confirmatory medium for fecal coliforms.
<u>C</u>	<u>2</u>	<input type="checkbox"/>	<u>2.3.3 The media productivity controls utilized are properly diluted and appropriate for the confirmed medium being used. The results are recorded and the records maintained.</u> <u>Positive productivity control</u> _____ <u>Negative productivity control</u> _____
K	9, 11	<input type="checkbox"/>	3. <u>2.3.4</u> Transfers are made to BGB/EC by either sterile loop or sterile hardwood transfer stick from positive presumptives tubes incubated for 24 and 48 hours <u>as appropriate</u> . (Circle the method of transfer.)
K	2	<input type="checkbox"/>	4. When the inoculation of both EC and BGB broths is performed using the same loop or transfer stick, the order of inoculation is EC first, followed by BGB.
C	9	<input type="checkbox"/>	5. <u>2.3.5</u> BGB tubes are incubated at $35 \pm 0.5^\circ\text{C}$.
K	9	<input type="checkbox"/>	6. <u>2.3.6</u> BGB tubes are read after 48 ± 3 hours of incubation.
C	9	<input type="checkbox"/>	7. <u>2.3.7</u> EC tubes are incubated in a circulating waterbath <u>maintained</u> at $44.5 \pm 0.2^\circ\text{C}$ for 24 ± 2 hours.
<u>C</u>	<u>9</u>	<input type="checkbox"/>	<u>2.3.8</u> <u>EC tubes are read after 24 ± 2 hours of incubation.</u>
C	9	<input type="checkbox"/>	8. <u>2.3.9</u> The presence of <u>turbidity and</u> any amount of gas or effervescence in the culture tube constitutes a positive test.

			<u>2.4 Computation of Results – APHA MPN</u>
K	9	<input type="checkbox"/>	1- <u>2.4.1</u> Results of multiple dilution tests are read from tables in <i>Recommended Procedures for the Examination of Sea Water and Shellfish</i> , Fourth 4 th Edition.
K	7	<input type="checkbox"/>	2- <u>2.4.2</u> Results from single dilution series are calculated from Hoskins' equation or interpolated from Figure 1, Public Health Report 1621 entitled "Most Probable Numbers for Evaluation of Coli aerogenes Tests by Fermentation Tube Method".
K C	7, 9	<input type="checkbox"/>	3- <u>2.4.3</u> Results are reported as MPN/100 mL of sample.
			<u>2.5 Bacteriological Examination of Seawater by the MA-1 Method</u>
C	<u>5</u>	<input type="checkbox"/>	<u>2.5.1</u> A-1 medium complete is used in the analysis.
C	<u>2, 31</u>	<input type="checkbox"/>	<u>2.5.2</u> A-1 medium without salicin is used in the analysis. <u>Comparability testing with medium A-1 complete has been undertaken and the results justify exclusion of the salicin from the formulation of medium A-1.</u>
C	5	<input type="checkbox"/>	1- <u>2.5.3</u> A-1 medium sterilized for 10 minutes at 121°C.
C	<u>2</u>	<input type="checkbox"/>	<u>2.5.4</u> <u>The media productivity controls used are properly diluted and appropriate for use with A-1 medium. The results are recorded and the results maintained.</u> Positive productivity control _____ Negative productivity control _____
C	9	<input type="checkbox"/>	2- <u>2.5.5</u> Sample and dilutions of sample are shaken mixed vigorously (25 times in a 12" arc in 7 seconds) before inoculation.
C	9	<input type="checkbox"/>	3- <u>2.5.6</u> In a multiple dilution series not less than 3 tubes per dilution are used (5 tubes are recommended).
C	6	<input type="checkbox"/>	4- <u>2.5.7</u> In a single dilution series at least 12 tubes are used.
K C	6	<input type="checkbox"/>	5- <u>2.5.8</u> In a single dilution series, the volumes <u>analyzed examined</u> are adequate to meet the needs of routine monitoring. Sample volume inoculated _____ Range of MPN _____ Strength of media used _____
K C	2	<input type="checkbox"/>	6- Positive and negative control cultures accompany samples throughout the procedure. Records are maintained. <u>2.5.9</u> <u>Appropriately diluted process control cultures accompany the samples throughout both resuscitation and waterbath incubation Results are recorded and the records maintained.</u> Positive <u>process</u> control _____ Negative <u>process</u> control _____
C	2,5	<input type="checkbox"/>	7- <u>2.5.10</u> Inoculated media tubes are placed in an air incubator at 35 ± 0.5°C for 3 ± 0.5 hours of resuscitation.
C	5	<input type="checkbox"/>	8- <u>2.5.11</u> After 3 ± 0.5 hours resuscitation at 35°C, inoculated tubes media are incubated at 44.5 ± 0.2° C in a circulating waterbath for the remainder of the 24 ± 2 hours.
C	5	<input type="checkbox"/>	9- <u>2.5.12</u> The presence of <u>turbidity and</u> any amount of gas or effervescence in the culture tube constitutes a positive test.
			<u>2.6 Computation of Results - MPN</u>
K	9	<input type="checkbox"/>	1- <u>2.6.1</u> Results of multiple dilution tests are read from tables in <i>Recommended Procedures for the Examination of Sea Water and Shellfish</i> , 4 th Edition.
K	7	<input type="checkbox"/>	2- <u>2.6.2</u> Results from single dilution series are calculated from Hoskins' equation or interpolated from Figure 1, Public Health Report 1621 entitled "Most Probable Numbers for Evaluation of Coli aerogenes Tests by Fermentation Tube Method".
K C	7, 9	<input type="checkbox"/>	3- <u>2.6.3</u> Results are reported as MPN/100 mL of sample.
			<u>2.7 Bacteriological Examination Analysis of Seawater by Membrane Filtration</u>

			<u>(MF) using mTEC Agar -Materials and Equipment</u>
C	23, 24	<input type="checkbox"/>	1- <u>2.7.1</u> When used for elevated temperature incubation <u>in conjunction with ethafoam resuscitation</u> , the temperature of the hot air incubator is maintained at 44.5 ± 0.5°C under any loading capacity.
C	23	<input type="checkbox"/>	2- <u>2.7.2</u> When using a waterbath for elevated temperature incubation, the level of the water completely covers the plates.
C	23	<input type="checkbox"/>	3- <u>2.7.3</u> Pre-sterilized plastic or sterile glass culture plates that are clear, flat bottomed, free of bubbles and scratches <u>with tight fitting lids</u> are used.
<u>C</u>	<u>2</u>	<input type="checkbox"/>	<u>2.7.4</u> <u>The sterility of pre-sterilized culture plates is determined for each lot received. Results are recorded and the records maintained.</u>
K	11	<input type="checkbox"/>	4- <u>2.7.5</u> Colonies are counted with the aid of magnification.
C	11, 23	<input type="checkbox"/>	5- <u>2.7.6</u> Membrane filters are made from cellulose ester material, white, grid marked, 47 mm in diameter with a pore size of 0.45 µm and certified by the manufacturer for fecal coliform analyses.
<u>Ø C</u>	<u>2</u>	<input type="checkbox"/>	6- <u>2.7.7</u> Lot number, date of receipt and if provided the expiration date of the membrane filters are recorded <u>and records maintained.</u>
<u>C</u>	<u>2</u>	<input type="checkbox"/>	<u>2.7.8</u> <u>When initiating monitoring by mTEC or switching brands or types of membrane filters used and no previous lots of filters are available for comparing acceptable performance, an appropriate method for determining the suitability of the lot is developed and the comparison testing implemented. The results are recorded and this record is maintained.</u>
K	2, 11	<input type="checkbox"/>	7- <u>2.7.9</u> New lots of membrane filters are checked by comparing recovery of fecal coliform organisms against membrane filters from previously acceptable lots.
C	2	<input type="checkbox"/>	8- <u>2.7.10</u> The sterility of each lot or autoclave batch of membrane filters are checked before use.
K	2	<input type="checkbox"/>	9- <u>2.7.11</u> Membrane filters which are beyond their expiration date are not used.
O	11	<input type="checkbox"/>	10- <u>2.7.12</u> Forceps tips are clean.
O	11	<input type="checkbox"/>	11- <u>2.7.13</u> Forceps tips are smooth without pitting or corrugations to damage the filters being manipulated.
K	11	<input type="checkbox"/>	12- <u>2.7.14</u> Forceps are dipped in alcohol and flame sterilized between sample filters.
K	11	<input type="checkbox"/>	13- <u>2.7.15</u> If indelible graduation marks are used on clear glass or plastic funnels to measure sample volumes, their accuracy is checked <u>gravimetrically or</u> with a Class A graduated cylinder before use and periodically rechecked. Funnels having a tolerance greater than 2.5% are not used. Checks are recorded and records maintained
K	11	<input type="checkbox"/>	14- <u>2.7.16</u> Membrane filtration units are made of stainless steel, glass or autoclavable plastic free of scratches, corrosion and leaks.
C	11	<input type="checkbox"/>	15- <u>2.7.17</u> <u>Membrane filter assemblies are autoclave sterilized for 15 minutes at 121°C prior to the start of a filtration series.</u>
O	11, 23, 26	<input type="checkbox"/>	16- <u>2.7.18</u> A UV sterilization unit is used to disinfect filter assemblies between sample and filtration runs.
K	11	<input type="checkbox"/>	17- <u>2.7.19</u> If used , The effectiveness of the UV sterilization unit is determined by biological testing monthly. Results are recorded and records maintained.
<u>K</u>	<u>2</u>	<input type="checkbox"/>	<u>2.7.20</u> <u>Maintenance of the UV sterilization unit is performed as needed. This maintenance is documented and the records maintained.</u>
			<u>2.8 Media Preparation and Storage– MF using mTEC Agar</u>
K	11	<input type="checkbox"/>	1- <u>2.8.1</u> Phosphate buffered saline is used as the sample diluent <u>and filter funnel rinse</u> .
C	11	<input type="checkbox"/>	2- <u>2.8.2</u> <u>The phosphate buffered saline is properly sterilized.</u>
K	23	<input type="checkbox"/>	3- <u>2.8.3</u> A sufficient amount of medium (4-5 mL) is used in each plate.
O	11	<input type="checkbox"/>	4- <u>2.8.4</u> Refrigerated prepared plates are stored for no more than 2 weeks in sealed plastic bags or containers to minimize evaporation.
			<u>2.9 Sample Analyses –MF using mTEC Agar</u>
C	24	<input type="checkbox"/>	1- <u>2.9.1</u> mTEC agar is used.

<u>C</u>	<u>2</u>	<input type="checkbox"/>	<u>2.9.2 The media productivity controls used are properly diluted and appropriate for use with mTEC medium. The results are recorded and the results maintained.</u> <u>Positive productivity control</u> _____ <u>Negative productivity control</u> _____
C	23	<input type="checkbox"/>	2. <u>2.9.3</u> The sample is mixed <u>shaken</u> vigorously (25 times in a 12" arc in 7 seconds) before filtration.
C	23	<input type="checkbox"/>	3. <u>2.9.4</u> The membrane is placed grid side up within the sterile filter apparatus.
C	23, 25	<input type="checkbox"/>	4. <u>2.9.5</u> Sample volumes tested are consistent with the sampling regime employed (i.e., half log or other appropriate dilutions are used with systematic random sampling).
C	23	<input type="checkbox"/>	5. <u>2.9.6</u> Sample volumes are filtered under vacuum.
K	26	<input type="checkbox"/>	6. <u>2.9.7</u> The pressure of the vacuum pump does not exceed 15 psi.
C	23, 26	<input type="checkbox"/>	7. <u>2.9.8</u> The sides of the filter funnel are rinsed at least twice with 20-30 mL of sterile phosphate buffered saline after sample filtration.
C	23	<input type="checkbox"/>	8. <u>2.9.9</u> The membrane filter is removed from the filtering apparatus with sterile forceps and rolled onto mTEC agar so that no bubbles form between the filter and the agar.
C	11	<input type="checkbox"/>	9. <u>2.9.10</u> Blanks are run at the beginning of filtration, after every 10 th aliquot and at the end of the filtration run to check the sterility of the testing system (phosphate buffered saline, filter funnel, forceps, membrane filter, media and culture plate).
<u>KC</u>	2, 11	<input type="checkbox"/>	10. Positive and negative control cultures accompany samples throughout the procedure. Records are maintained. <u>2.9.11 Appropriately diluted process control cultures accompany the samples throughout both resuscitation and elevated temperature incubation. Results are recorded and the records maintained.</u> <u>Positive process control</u> _____ <u>Negative process control</u> _____
C	11, 23, 24	<input type="checkbox"/>	11. <u>2.9.12 Inoculated plates are placed inverted wither directly in an air incubator or in a watertight, tightly sealed container at 35 + 0.5°C for 2 hours of resuscitation prior to waterbath incubation or in Ethyfoam for incubation in air at 44.5 + 0.5°C.</u> <u>Inoculated plates are placed inverted into a watertight, tightly sealed container prior to being placed in the air incubator and incubated at 35 + 0.5°C for 2 hours of resuscitation. Alternatively inoculated plates may be placed in ethafoam prior to air incubation at 44.5 + 0.5°C for 24 + 2 hours.</u>
C	11, 23, 24	<input type="checkbox"/>	12. <u>2.9.13</u> After 2 hours of resuscitation at 35°C, <u>the watertight, tightly sealed containers are transferred to a circulating waterbath at 44.5 + 0.2°C, submerged completely and incubated for 22-24 hours. Individual plates are transferred inverted to a watertight container, tightly sealed and submerged completely in a circulating waterbath at 44.5 + 0.2°C for 22-24 hours of incubation.</u>
			<u>2.10 Computation of Results- MF using mTEC Agar</u>
C	23	<input type="checkbox"/>	1. <u>2.10.1</u> All yellow, yellow-green or yellow-brown colonies are counted.
C	23	<input type="checkbox"/>	2. <u>2.10.2</u> Only plates having 80 or fewer colonies are counted. If it is <u>unavoidable necessary</u> to use plates having more than 80 colonies, counts are given as >80 x 100/the volume of <u>sample</u> filtered.
<u>C</u>	<u>2, 11, 23</u>	<input type="checkbox"/>	<u>2.10.3 When multiple dilutions are filtered, the laboratory has developed a procedure for assessing the contribution of all positive dilutions to the final count.</u>
K	23, 11	<input type="checkbox"/>	3. <u>2.10.4</u> The number of fecal coliforms is calculated by the following equation: Number of fecal coliforms per 100 mL = [number of colonies counted <u>per plate</u>

			used in the count / volume (s) of sample filtered in ml] x 100.
K C	23, 11	<input type="checkbox"/>	4. <u>2.10.5</u> Results are reported as CFU/100 mL of sample.
PART III - SHELLFISH SAMPLES			
<u>3.1</u> Collection and Transportation of Samples			
C	9	<input type="checkbox"/>	1. <u>3.1.1</u> A representative sample of shellstock is collected.
K	9	<input type="checkbox"/>	2. <u>3.1.2</u> Shellstock samples are collected in clean, waterproof, puncture resistant containers loosely sealed.
K	9	<input type="checkbox"/>	3. <u>3.1.3</u> Shellstock samples are labeled with collector's name, type of shellstock, the source or harvest area, sampling station, time, date and place (if applicable market sample) of collection.
C	9	<input type="checkbox"/>	4. Shellstock samples are maintained in dry storage between 0 and 10° C until examined. <u>3.1.4 Immediately after collection, shellfish samples are placed in dry storage (ice chest or equivalent) which is maintained between 0° and 10°C with ice or cold packs for transport to the laboratory. Once received, the samples are placed under refrigeration unless processed immediately.</u>
C	1	<input type="checkbox"/>	5. <u>3.1.5 Examination/Analysis</u> of the samples is initiated as soon as possible after collection. However, Shellfish samples are not tested examined if the time interval between collection and analysis examination exceeds 24 hours.
<u>3.2</u> Preparation of Shellfish for Examination			
K	2,11	<input type="checkbox"/>	1. <u>3.2.1</u> Shucking knives, scrub brushes and blender jars are (autoclave) sterilized for 15 minutes prior to use.
O	2	<input type="checkbox"/>	2. <u>3.2.2</u> Blades of shucking knives are not corroded.
O	9	<input type="checkbox"/>	3. Prior to scrubbing and rinsing debris off shellstock, the hands of the analyst are thoroughly washed with soap and water. <u>3.2.3 The hands of the analyst are thoroughly washed with soap and water immediately prior to cleaning the shells of debris.</u>
O	2	<input type="checkbox"/>	4. <u>3.2.4</u> The faucet used to provide the potable water for rinsing the shellstock does not contain an aerator.
K	9	<input type="checkbox"/>	5. <u>3.2.5</u> Shellstock are scrubbed with a stiff, sterile brush and rinsed under tap water of drinking water quality.
C	<u>2</u>	<input type="checkbox"/>	<u>3.2.6 If a water supply is a non-chlorinated private well, the water is tested every six months for total coliforms. Results are recorded and maintained.</u>
O	9	<input type="checkbox"/>	6. <u>3.2.7</u> Shellstock are allowed to drain in a clean container or on clean towels prior to opening.
K	9	<input type="checkbox"/>	7. <u>3.2.8</u> Immediately prior to opening shucking, the hands (or gloved hands) of the analyst are thoroughly washed with soap and water and rinsed in 70% alcohol.
K C	9	<input type="checkbox"/>	8. <u>3.2.9</u> Shellstock are not shucked directly through the hinge.
C	9	<input type="checkbox"/>	9. <u>3.2.10</u> Contents of shellstock (liquor and meat) are shucked into a sterile, tared blender jar or other sterile container.
K	9	<input type="checkbox"/>	10. <u>3.2.11</u> At least 200 grams of shellfish meat or a quantity of meat sufficient to cover the blender blades is used for the analysis.
K	<u>9</u>	<input type="checkbox"/>	<u>3.2.12 A representative sample of at least 12 shellfish is used for the analysis.</u>
K	2, 19	<input type="checkbox"/>	11. <u>3.2.13</u> The sample is weighed to the nearest 0.1 gram and an equal amount by weight of (tempered for ETCP) diluent is added.
O	9	<input type="checkbox"/>	12. <u>3.2.14</u> Sterile phosphate buffered dilution water is used as the sample diluent.
K	<u>3</u>	<input type="checkbox"/>	13. Sterile phosphate buffered saline is used as a sample diluent for the ETCP procedure. Moved to ETCP section
C	9	<input type="checkbox"/>	14. <u>3.2.15</u> Samples are blended at high speed for 60 to 120 seconds until homogenous.
K	9	<input type="checkbox"/>	15. For other shellstock, APHA Recommended Procedures are followed for the examination of freshly shucked and frozen shellfish meats.

			3.2.16 <u>APHA Recommended Procedures for the Examination of Sea Water And Shellfish</u> , Fourth Edition is followed for the analysis of previously shucked and frozen shellfish meats.
3.3 MPN Analysis for Fecal Coliform Organisms, Presumptive Test, APHA			
C	9	<input type="checkbox"/>	1- 3.3.1 Appropriate strength lactose or lauryl tryptose broth is used as presumptive media in the analysis. (circle appropriate choice) (<u>Circle the medium used.</u>)
<u>C</u>	<u>2</u>	<input type="checkbox"/>	3.3.2 <u>The media productivity controls utilized are properly diluted and appropriate for the presumptive medium being used. The results are recorded and the records maintained.</u> <u>Positive productivity control</u> _____ <u>Negative productivity control</u> _____
K	9	<input type="checkbox"/>	2- 3.3.3 Immediately (within 2 minutes) after blending, the ground sample is diluted and inoculated into tubes of presumptive media.
C	9	<input type="checkbox"/>	3- 3.3.4 No fewer than 5 tubes per dilution are used in a multiple dilution MPN series.
C	9	<input type="checkbox"/>	4- 3.3.5 Allowing for the initial 1:1 dilution of the sample, appropriate portions are inoculated (i.e., 2 ml of original 1:1 dilution for the 1 g portion) and diluted for subsequent inoculation (i.e., 22 ml of 1:1 diluted sample to 88 ml of diluent or the equivalent for 0.1 g portion). <u>All successive dilutions are prepared conventionally.</u>
K	6	<input type="checkbox"/>	5- 3.3.6 In a single dilution series, the volumes examined are adequate to meet the needs of routine monitoring. Sample volume inoculated _____ Range of MPN _____ Strength of media used _____
C	2	<input type="checkbox"/>	6- Positive and negative control cultures accompany samples throughout the procedure. Records are maintained. 3.3.7 <u>Appropriately diluted process control cultures accompany the samples throughout both the presumptive and confirmed phases of incubation. Results are recorded and the records maintained.</u> <u>Positive Process control</u> _____ <u>Negative Process control</u> _____
K	9	<input type="checkbox"/>	7- 3.3.8 Inoculated media are incubated at 35 ± 0.5°C.
K	10	<input type="checkbox"/>	8- Presumptive tubes are read at 24 ± 2 hours of incubation and transferred if positive. 3.3.9 <u>Tubes are read after 24+2 hours of incubation and transferred if positive for growth (the presence of turbidity and gas or effervescence in the culture tube). These tubes are considered presumptive requiring further confirmatory testing.</u>
3.4 Confirmed Test for Fecal Coliforms - APHA			
C	9	<input type="checkbox"/>	1- 3.4.1 EC medium is used as the confirmatory medium.
<u>C</u>	<u>2</u>	<input type="checkbox"/>	3.4.2 <u>The media productivity controls utilized are properly diluted and appropriate for use with EC medium. The results are recorded and the records maintained.</u> <u>Positive productivity control</u> _____ <u>Negative productivity control</u> _____
K	9, 11	<input type="checkbox"/>	2- 3.4.3 Transfers are made to EC medium by either sterile loop or hardwood sterile applicator <u>transfer</u> sticks from positive presumptives incubated for 24 hours . (<u>Circle the method of transfer.</u>)
C	9	<input type="checkbox"/>	3- 3.4.4 EC tubes are incubated in a circulating waterbath at 44.5 ± 0.2°C for 24 ± 2 hours.
K	9	<input type="checkbox"/>	4- 3.4.5 EC tubes are read for gas production after 24 ± 2 hours of incubation.
C	9	<input type="checkbox"/>	5- 3.4.6 The presence of <u>turbidity and any amount of gas and/or effervescence in</u>

			the Durham tube constitutes a positive test.
			<u>3.5 Computation of Results for MPN Analyses</u>
K	9	<input type="checkbox"/>	1. <u>3.5.1</u> Results of multiple dilution tests are read from tables in <i>Recommended Procedure for the Examination of Sea Water and Shellfish</i> , 4th Edition and multiplied by the appropriate dilution factor.
K	7	<input type="checkbox"/>	2. <u>3.5.2</u> Results from single dilution series are calculated from Hoskins' equation or interpolated from Figure 1, Public Health Report 1621 entitled "Most Probable Numbers for Evaluation of Coli aerogenes Tests by Fermentation Tube Method".
K <u>C</u>	9	<input type="checkbox"/>	3. <u>3.5.3</u> Results are reported as MPN/100 grams of sample.
			<u>3.6 Standard Plate Count Method</u>
O	20	<input type="checkbox"/>	1. <u>3.6.1</u> A standard plate count (SPC) analysis is <u>may be</u> performed in conjunction with the analysis for fecal coliform organisms.
K	9	<input type="checkbox"/>	2. <u>3.6.2</u> In the standard plate count procedure at least four plates <u>are used</u> , duplicates of two dilutions are used to provide 30 to 300 colonies per plate . <u>One of the dilutions should produce colonies of 30 to 300 per plate.</u>
K	2	<input type="checkbox"/>	3. <u>3.6.3</u> Fifteen to 20 mL of tempered sterile plate count agar is used <u>per plate</u> .
K <u>C</u>	9	<input type="checkbox"/>	4. <u>3.6.4</u> Agar tempering bath maintains the agar at 44- 46°C.
O <u>C</u>	9	<input type="checkbox"/>	5. Temperature control of the plate count agar is used in the tempering bath. <u>3.6.5 An agar based temperature control having a similar volume and shape as the tempering plate count agar is used in the tempering bath.</u>
K	9	<input type="checkbox"/>	6. <u>3.6.6</u> Not more than 1 mL nor less than 0.1 mL of sample or sample dilution is plated.
C	9	<input type="checkbox"/>	7. <u>3.6.7</u> Samples or sample dilutions to be plated are mixed <u>shaken</u> vigorously (25 times in a 12" arc in 7 seconds) before plating.
K	11	<input type="checkbox"/>	8. <u>3.6.8</u> Control plates are used to check <u>air quality and</u> the sterility of the air , agar and the diluent.
K	9,21	<input type="checkbox"/>	9. <u>3.6.9</u> Solidified plates are incubated at 35 ± 0.5°C for 48 ± 3 hours inverted and stacked no more than four high.
K	9	<input type="checkbox"/>	10. <u>3.6.10</u> Quebec Colony Counter or its equivalent is used to provide the necessary magnification and visibility for counting plates.
K	1	<input type="checkbox"/>	11. <u>3.6.11</u> A hand tally or its equivalent is used for accuracy in counting.
			<u>3.7 Computation of Results -SPC</u>
K	9	<input type="checkbox"/>	1. <u>3.7.1</u> Colony counts determined in accordance with Part III, A, Sections 4.31 through 4.33 in <i>Recommended Procedures for the Examination of Sea Water and Shellfish</i> , 4 th Fourth Edition.
C	19	<input type="checkbox"/>	2. <u>3.7.2</u> Colony counts are reported as APC/g of sample.
			<u>3.8 Bacteriological Examination Analysis of Shellfish Using the ETCP</u>
<u>C</u>	<u>2,3</u>	<input type="checkbox"/>	<u>3.8.1 Prepared modified MacConkey agar is used on the day that it is made.</u>
K	9	<input type="checkbox"/>	1. Sample homogenate is cultured within 2 minutes of blending.
K	3	<input type="checkbox"/>	2. <u>3.8.2</u> Double strength modified MacConkey agar is used.
C	3	<input type="checkbox"/>	3. Hydrated double strength Modified MacConkey Agar is heated to boiling, removed from the heat, and boiled again. This agar is never autoclaved. <u>3.8.3 Prepared double strength modified MacConkey agar is heated to boiling, removed from the heat, and boiled again. This agar is never autoclaved.</u>
K	2, 3	<input type="checkbox"/>	4. <u>3.8.4</u> Twice boiled, double strength modified MacConkey agar and sterile phosphate buffered saline are maintained in a tempering bath at 45 to 50°C until used. Prepared Modified MacConkey Agar is used on the day it is made.
<u>K</u>	<u>2, 3</u>	<input type="checkbox"/>	<u>3.8.5</u> Phosphate buffered saline is used as the sample diluent in the ETCP.
<u>C</u>	<u>2, 3</u>	<input type="checkbox"/>	<u>3.8.6 The phosphate buffered saline is tempered at 45 - 50°C to prevent premature solidification of the agar.</u>
<u>C</u>	<u>9</u>	<input type="checkbox"/>	<u>3.8.7 The sample homogenate is cultured within 2 minutes of blending.</u>

C	2,3	<input type="checkbox"/>	5. The equivalent of 6 grams of the homogenate is placed into a sterile container and the contents brought up to 60 ml with tempered, sterile phosphate buffered saline. <u>3.8.8 Six grams of shellfish (12 grams of homogenate if initially diluted 1:1) is placed into a sterile container and the contents brought up to 60 mL with sterile, tempered phosphate buffered saline.</u>
K	3	<input type="checkbox"/>	6. 3.8.9 Sixty (60) mL of tempered, twice boiled double strength Modified MacConkey Agar is added.
K	2,3, 22	<input type="checkbox"/>	7. 3.8.10 The container is gently swirled or rotated slowly inverted once to mix the contents, which are then subsequently distributed uniformly over 6 to 8 petri six plates.
C	1	<input type="checkbox"/>	8. <u>3.8.11 Media and diluent sterility are determined with each use. Results are recorded and the records maintained.</u>
C	1	<input type="checkbox"/>	9. To determine media productivity, positive and negative control cultures are pour plated in an appropriate concentration to accompany samples throughout the procedure. <u>3.8.12 Media productivity is determined using media appropriate properly diluted pour plated positive and negative control cultures for each batch of Modified MacConkey agar prepared.</u> Positive control culture _____ Negative control culture _____
C	3, 13	<input type="checkbox"/>	10. Plates are incubated inverted within 3 hours of plating in air at $45.5 \pm 0.5^\circ \text{C}$ for 18 to 30 hours. Plates are stacked not more than four high. <u>3.8.13 When solidified the plates are placed inverted into an air incubator at $45.5 \pm 0.5^\circ \text{C}$ for 18 to 30 hours of incubation.</u>
C	2	<input type="checkbox"/>	<u>3.8.14 Plates are stacked no more than three high in the incubator.</u>
C	2	<input type="checkbox"/>	<u>3.8.15 Appropriately diluted pour plated process control cultures accompany each set of samples throughout incubation. The results are recorded and the records maintained.</u> Positive process control _____ Negative process control _____
C	3		11. <u>3.8.16 Incubator temperature is maintained at $45.5 \pm 0.5^\circ \text{C}$.</u>
<u>3.9 Computation Expression of Results - ETCP</u>			
K	11	<input type="checkbox"/>	1. 3.9.1 Quebec Colony counter or its equivalent is used to provide the necessary magnification and visibility for counting.
O	1	<input type="checkbox"/>	2. 3.9.2 A hand tally or its equivalent is used to aid in counting.
C	3, 6	<input type="checkbox"/>	3. <u>3.9.3 All brick red colonies greater than 0.5 mm in diameter are totaled over all the plates and multiplied by a factor of 16.7 to report results as CFU/100 grams of sample.</u>
C	3	<input type="checkbox"/>	<u>3.9.4 Results are reported as CFU/100 grams of sample.</u>
Bacteriological Examination of Soft-shelled Clams and American Oysters for Male Specific Coliphage (MSC)			
<u>3.10 MSC Equipment and Supplies</u>			
K	30	<input type="checkbox"/>	1. 3.10.1 Sample containers used for the shucked sample are sterile, made of glass or some other inert material (i.e. polypropylene) and hold 100 – 125 mL.
C	27, 28	<input type="checkbox"/>	2. <u>3.10.2 The refrigerated centrifuge used must have the capacity to accommodate the amount of shellfish sample required for the procedure, perform at 9000 x g and maintain a temperature of 4°C.</u>
C	27, 28	<input type="checkbox"/>	3. <u>3.10.3 The tempering bath(s) must be able to maintain the temperature within 2°C of the set temperature.</u>
K	9	<input type="checkbox"/>	4. 3.10.4 The level of water in the tempering bath covers the level of liquid and agar in the container or culture tubes.
C	27, 28	<input type="checkbox"/>	5. <u>3.10.5 Sterile 0.22 µm pore size syringe filters and pre-sterilized plastic or sterile glass syringes are used to sterilize the antibiotic solutions.</u>

K	1	<input type="checkbox"/>	6- <u>3.10.6</u> The sterility of each lot of pre-sterilized syringes and syringe filters is determined. <u>Results are recorded and records maintained.</u>
K	1	<input type="checkbox"/>	7- <u>3.10.7</u> The sterility of each batch of reusable glass syringes is determined. <u>Results are recorded and records maintained.</u>
C	27, 28	<input type="checkbox"/>	8- <u>3.10.8</u> The balance used provides a sensitivity of at least 10 mg.
C	27, 28	<input type="checkbox"/>	9- <u>3.10.9</u> The temperature of the incubator used is maintained between 35 – 37°C.
C	28	<input type="checkbox"/>	10- <u>3.10.10</u> Sterile disposable 50 mL centrifuge tubes are used and their sterility is determined with each lot. <u>Results are recorded and records maintained.</u>
<u>3.11 MSC Media Preparation</u>			
K	28	<input type="checkbox"/>	1- <u>3.11.1</u> Media preparation and sterilization is according to the validated method.
K	27, 28	<input type="checkbox"/>	2- <u>3.11.2</u> Bottom agar, double strength soft agar and growth broth are prepared from their individual components.
K	27, 28	<input type="checkbox"/>	3- <u>3.11.3</u> Soft agar is prepared double strength in volumes of 2.5 mL.
C	27, 28	<input type="checkbox"/>	4- <u>3.11.4</u> The streptomycin and ampicillin solutions are added to tempered bottom agar.
O	27, 28	<input type="checkbox"/>	5- <u>3.11.5</u> Storage of the bottom agar under refrigeration does not exceed 1 month.
K	27, 28	<input type="checkbox"/>	6- <u>3.11.6</u> Unsterilized soft agar is stored at -20°C for up to 3 months.
K	27, 28	<input type="checkbox"/>	7- <u>3.11.7</u> The soft agar is removed from the freezer and sterilized for 15 minutes at 121°C before use.
K	27, 28	<input type="checkbox"/>	8- <u>3.11.8</u> Storage of growth broth in the refrigerator in loosely capped tubes/bottles does not exceed 1 month and in screw capped tubes/bottles does not exceed 3 months.
K	27, 28	<input type="checkbox"/>	9- <u>3.11.9</u> Bottom agar plates are allowed to reach room temperature before use.
<u>3.12 Preparation of the Soft-Shell Clams and American Oysters for MSC Analysis</u>			
K	2,11	<input type="checkbox"/>	1- <u>3.12.1</u> Shucking knives, scrub brushes and blender jars are autoclave sterilized for 15 minutes prior to use.
O	2	<input type="checkbox"/>	2- <u>3.12.2</u> The blades of shucking knives are not corroded.
O	9	<input type="checkbox"/>	3- <u>3.12.3</u> The hands of the analyst are thoroughly washed with soap and water <u>immediately prior to scrubbing and rinsing cleaning the shells of debris off the shellfish.</u>
O	2	<input type="checkbox"/>	4- <u>3.12.4</u> The faucet used for rinsing the shellfish does not contain an aerator.
K	9	<input type="checkbox"/>	5- <u>3.12.5</u> The shellfish are scrubbed with a stiff, sterile brush and rinsed under <u>tap</u> water of drinking water quality.
<u>C</u>	<u>2</u>	<input type="checkbox"/>	<u>3.12.6</u> If a water supply is a non-chlorinated private well, the water is tested every six months for total coliforms. Results are recorded and maintained.
O	9	<input type="checkbox"/>	6- <u>3.12.7</u> The shellfish are allowed to drain in a clean container or on clean towels unlayered prior to shucking.
K	9	<input type="checkbox"/>	7- <u>3.12.8</u> <u>Immediately</u> prior to shucking, the hands (or gloved hands) of the analyst are thoroughly washed with soap and water and rinsed in 70% alcohol.
C	9	<input type="checkbox"/>	8- <u>3.12.9</u> Shellfish are not shucked through the hinge.
C	9	<input type="checkbox"/>	9- <u>3.12.10</u> The contents of shellfish (liquor and meat) are shucked into a sterile, tared blender jar or other sterile container.
K	9	<input type="checkbox"/>	10- <u>3.12.11</u> <u>A representative sample of</u> at least 12 shellfish is used for the analysis.
<u>€ K</u>	2, 19	<input type="checkbox"/>	11- <u>3.12.12</u> The sample is weighed to the nearest 0.1 gram.
<u>3.13 MSC Sample Analysis</u>			
C	28	<input type="checkbox"/>	1- <u>3.13.1</u> <i>E.coli</i> Famp ATCC 700891 is the bacterial host strain used in this procedure.
K	27, 28	<input type="checkbox"/>	2- <u>3.13.2</u> Host cell growth broth is tempered at 35 – 37°C and vortexed (or shaken) to aerate prior to inoculation with host cells.
K	27, 28	<input type="checkbox"/>	3- <u>3.13.3</u> Several host cell colonies are transferred to a tube of tempered, aerated growth broth and incubated at 35 – 37°C to provide host cells in log phase growth for sample analysis.

C	27, 28	<input type="checkbox"/>	4. <u>3.13.4</u> Inoculated growth broth is incubated at 35 – 37°C for 4 to 6 hours to provide a host cell culture in log phase growth.
C	27, 28	<input type="checkbox"/>	5. <u>3.13.5</u> After inoculation, the host cell growth broth culture is not shaken.
C	28	<input type="checkbox"/>	6. <u>3.13.6</u> A 2:1 mixture of growth broth to shellfish tissue is used for eluting the MSC.
C	28	<input type="checkbox"/>	7. <u>3.13.7</u> The elution mixture is prepared w/v by weighing the sample and adding two equal portions of growth broth by volume to the shellfish tissue.
C	28	<input type="checkbox"/>	8. <u>3.13.8</u> The elution mixture is homogenized at high speed for 180 seconds.
C	28	<input type="checkbox"/>	9. <u>3.13.9</u> Immediately after blending, 33 grams of the homogenized elution mixture are weighed into centrifuge tubes.
C	28	<input type="checkbox"/>	10. <u>3.13.10</u> The homogenized elution mixture is centrifuged for 15 minutes at 9000 x g at 4°C.
C	27, 28	<input type="checkbox"/>	11. <u>3.13.11</u> The supernatant is pipetted off, weighed and the weight recorded.
C	27, 28	<input type="checkbox"/>	12. <u>3.13.12</u> The supernatant is allowed to warm to room temperature about 20 to 30 minutes.
K	27, 28	<input type="checkbox"/>	13. <u>3.13.13</u> The autoclaved soft agar is tempered and held at 50 – 52°C throughout the period of sample analysis.
K	27, 28	<input type="checkbox"/>	14. <u>3.13.14</u> Two hundred microliters (0.2 mL) of log phase host strain <i>E coli</i> is added to the tempering soft agar immediately prior to adding the sample supernatant.
K	27, 28	<input type="checkbox"/>	15. <u>3.13.15</u> The sample supernatant is shaken or vortexed before being added to the tempering soft agar.
C	27, 28	<input type="checkbox"/>	16. <u>3.13.16</u> 2.5 mL of sample supernatant is added to each tube of tempering soft agar.
C	27, 28	<input type="checkbox"/>	17. <u>3.13.17</u> The soft agar/sample supernatant/host cell mixture is gently rolled between the palms of the hands to mix.
C	27, 28	<input type="checkbox"/>	18. <u>3.13.18</u> The soft agar/sample supernatant/host cell mixture is overlaid onto bottom agar plates and swirled gently to distribute the mixture evenly over the plate.
C	28	<input type="checkbox"/>	19. <u>3.13.19</u> Ten (10) plates are used, 2.5 mL per plate for a total of 25 mL of supernatant analyzed per sample.
K	27, 28	<input type="checkbox"/>	20. <u>3.13.20</u> Negative and positive control plates are prepared and accompany each set of samples analyzed. <u>The results are recorded and records maintained.</u>
K	27, 28	<input type="checkbox"/>	21. <u>3.13.21</u> Growth broth is used as the negative control or blank.
K	27, 28	<input type="checkbox"/>	22. <u>3.13.22</u> Type strain MS2 (ATCC 15597) male specific bacteriophage <u>appropriately diluted to provide countable low levels of phage</u> is used as the positive control.
K		<input type="checkbox"/>	23. <u>3.13.23</u> A negative control plate is plated at the beginning and end of each set of samples analyzed.
K	27, 28	<input type="checkbox"/>	24. <u>3.13.24</u> The positive control is plated after all the samples are analyzed <u>inoculated</u> and immediately prior to the final negative control.
C	27, 28	<input type="checkbox"/>	25. <u>3.13.25</u> All plates are incubated at 35 – 37°C for 16 to 20 hours.
<u>3.14 Computation of Results - MSC</u>			
C	27	<input type="checkbox"/>	1. <u>3.14.1</u> Circular zones of clearing or plaques of any diameter in the lawn of host bacteria are counted.
C	28	<input type="checkbox"/>	2. <u>3.14.2</u> The working range of the method is 1 to 100 PFU per plate. When there are no plaques on all ten plates, the count is <6 PFU/100 grams for soft-shelled clams and <7 PFU/ 100 grams for American oysters. If the density exceeds 100 PFU per plate on all plates, the count is given as > 10,000 PFU/100 grams.
K	28	<input type="checkbox"/>	3. <u>3.14.3</u> The formula used for determining the density of MSC in PFU/100 grams is: (0.364)(N)(Ws), where N = total number of plaques counted on all 10 plates and Ws = weight of the supernatant used.
O	9	<input type="checkbox"/>	4. <u>3.14.4</u> The MSC count is rounded off conventionally to give a whole number.

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Total # of Critical, Key and Other (O)	
Nonconformities in Parts I-III	
B. Criteria for Determining Laboratory Status of the Microbiological Component:	
<p>1. Does Not Conform Status: The Microbiological component of this laboratory is not in conformity with NSSP requirements if:</p> <p>a. The total # of Critical nonconformities is ≥ 4 or</p> <p>b. The total # of Key nonconformities is ≥ 13 or</p> <p>c. The total # of Critical, Key and Other is ≥ 18</p> <p>2. Provisionally Conforms Status: The microbiological component of this laboratory is determined to be provisionally conforming to NSSP requirements if the number of critical nonconformities is ≥ 1 but ≤ 3</p>	
C. Laboratory Status (<i>circle appropriate</i>)	
<p>Does Not Conform Provisionally Conforms Conforms</p>	
<p>Acknowledgment by Laboratory Director/Supervisor:</p> <p>All corrective Action will be implemented and verifying substantiating documentation received by the Laboratory Evaluation Officer on or before _____</p> <p>Laboratory Signature: _____ Date: _____</p> <p>LEO Signature: _____ Date: _____</p>	

NSSP Form LAB-100 Microbiology Rev. 2010-11-08