

**PUBLIC HEALTH SERVICE**  
**U.S. FOOD AND DRUG ADMINISTRATION**  
**OFFICE OF FOOD SAFETY**  
**SHELLFISH AND AQUACULTURE POLICY BRANCH**  
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**COLLEGE PARK, MD 20740-3835**  
**TEL. 240- 402-2151/2055/4960 FAX 301-436-2601**

**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:**

**Conformity it noted by a “√”**

**C- Critical   K - Key   O - Other   NA- Not Applicable**

**Check the applicable analytical methods:**

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

## PART 1 - QUALITY ASSURANCE

CODE	REF.	ITEM	
K	8, 11	<b>1.1 Quality Assurance (QA) Plan</b>	
		<input type="checkbox"/>	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, their calibration, maintenance, repair, performance, and rejection criteria established.
		<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"/>	<b>1.1.2 QA Plan Implemented.</b>
K	11	<input type="checkbox"/>	1.1.3 The Laboratory participates in a proficiency testing program annually. Specify Program(s)_____
<b>1.2 Educational/Experience Requirements</b>			
C	State's Human Resources Department	<input type="checkbox"/>	<b>1.2.1 In state/county laboratories, the supervisor meets the state/county educational and experience requirements for managing a public health laboratory.</b>
K	State's Human Resources Department	<input type="checkbox"/>	1.2.2 In state/county laboratories, the analyst(s) meets the state/county educational and experience requirements for processing samples in a public health laboratory.
C	USDA Microbiology & EELAP	<input type="checkbox"/>	<b>1.2.3 In commercial laboratories, the supervisor must have at least a bachelor's degree or equivalent in microbiology, biology, or equivalent discipline with at least two years of laboratory experience.</b>
K	USDA Microbiology & EELAP	<input type="checkbox"/>	1.2.4 In commercial laboratories, the analyst(s) must have at least a high school diploma and shall have at least three months of experience in laboratory sciences.
<b>1.3 Work Area</b>			
O	8,11	<input type="checkbox"/>	1.3.1 Adequate for workload and storage.
K	11	<input type="checkbox"/>	1.3.2 Clean, well-lighted.
K	11	<input type="checkbox"/>	1.3.3 Adequate temperature control.
O	11	<input type="checkbox"/>	1.3.4 All work surfaces are nonporous, easily cleaned and disinfected.
K	11	<input type="checkbox"/>	1.3.5 Microbiological quality of the air is fewer than 15 colonies for a 15 minute exposure and determined monthly. The results are recorded and records maintained.
<b>1.4 Laboratory Equipment</b>			
O	9	<input type="checkbox"/>	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"/>	1.4.2 pH electrodes consisting of pH half-cell and reference half-cell or equivalent combination electrode free from (Ag/AgCl) or contains an ion exchange barrier preventing passage of Ag ions into the medium which may affect the accuracy of the pH reading.
K	11	<input type="checkbox"/>	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"/>	1.4.4 pH meter is calibrated daily or with each use Results are recorded and records maintained.
K	11	<input type="checkbox"/>	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 and discarded.
O	8,15	<input type="checkbox"/>	1.4.6 Electrode acceptability is determined daily or with each use by the millivolt

			procedure or through determination of the slope. ( <i>Circle the method used.</i> )
K	9	<input type="checkbox"/>	1.4.7 Balance provides a sensitivity of at least 0.1 g at weights of use.
K	11,13	<input type="checkbox"/>	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.
K	11	<input type="checkbox"/>	1.4.9 Refrigerator temperature(s) are monitored at least once daily on workdays. Results are recorded and records maintained.
K	1	<input type="checkbox"/>	1.4.10 Refrigerator temperature is maintained at 0 to 4°C.
C	9	<input type="checkbox"/>	1.4.11 <b>The temperature of the incubator is maintained at 35 ± 0.5°C.</b>
C	11	<input type="checkbox"/>	1.4.12 <b>Thermometers used in the air incubator(s) are graduated in at least 0.1°C increments.</b>
K	9	<input type="checkbox"/>	1.4.13 Working thermometers are located on top and bottom shelves or appropriately placed based on the results of spatial temperature checks.
C	11	<input type="checkbox"/>	1.4.14 <b>Temperature of the waterbath is maintained at 44.5 ± 0.2°C under all loading conditions.</b>
C	9	<input type="checkbox"/>	1.4.15 <b>The thermometers used in the waterbath are graduated in at least 0.1°C increments.</b>
C	13	<input type="checkbox"/>	1.4.16 <b>The waterbath has adequate capacity for workload.</b>
K	9	<input type="checkbox"/>	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"/>	1.4.18 Air incubator/waterbath temperatures are taken twice daily on workdays. The results are recorded and records maintained.
C	4	<input type="checkbox"/>	1.4.19 <b>All working thermometers are appropriately immersed.</b>
C	29	<input type="checkbox"/>	1.4.20 <b>Working thermometers are either: calibrated mercury-in-glass thermometers, calibrated non-mercury-in-glass thermometers, or appropriately calibrated electronic devices, including Resistance Temperature Devices (RTDs) and Platinum Resistance Devices (PTDs).</b>
C	11	<input type="checkbox"/>	1.4.21 <b>A mercury-in-glass standards thermometer has been calibrated by NIST or 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.</b>
K	9	<input type="checkbox"/>	1.4.22 Standards thermometers are checked annually for accuracy by ice point determination. Results recorded and maintained.  Date of most recent determination_____.
C	29	<input type="checkbox"/>	1.4.23 <b>Either mercury-in-glass thermometers, non-mercury-in-glass thermometers having the accuracy (uncertainty), tolerance and response time of mercury or low drift electronic resistance thermometers with an accuracy of ≤ ±0.05°C are used as the laboratory standards thermometer. (<i>Circle the thermometer type used.</i>)</b>
K	13, <u>33</u>	<input type="checkbox"/>	1.4.24 Incubator and water bath working thermometers are <del>checked</del> - <u>verified</u> annually against the standards thermometer at the temperatures at which they are used. <u>Discard working temperature-sensing devices that differ by &gt;1°C from the reference/standards device.</u> Results are recorded and records maintained.
O	11	<input type="checkbox"/>	1.4.25 Appropriate pipet aids are available and used to inoculate samples. Mouth pipetting is not permitted.
<b>1.5 Labware and Glassware Washing</b>			
O	9	<input type="checkbox"/>	1.5.1 Utensils and containers are clean borosilicate glass, stainless steel or other noncorroding materials.
K	9	<input type="checkbox"/>	1.5.2 Culture tubes are of a suitable size to accommodate the volume for nutritive ingredients and samples.
K	9	<input type="checkbox"/>	1.5.3 Sample containers are made of glass or some other inert material.
O	9	<input type="checkbox"/>	1.5.4 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"/>	1.5.5	Graduations are indelibly marked on dilution bottles and tubes or an acceptable alternative method is used to ensure appropriate volumes.
C	9	<input type="checkbox"/>	1.5.6	<b>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 aliquots; nor, are pipets larger than 1.1mL used to deliver 0.1 mL aliquots.</b>
K	9	<input type="checkbox"/>	1.5.7	Reusable sample containers are capable of being properly washed and sterilized.
K	9	<input type="checkbox"/>	1.5.8	In washing reusable pipettes, 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.
C	2	<input type="checkbox"/>	1.5.9	<b>An alkaline or acidic detergent is used for washing glassware/labware.</b>
C	11	<input type="checkbox"/>	1.5.10	<b>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% bromothymol blue. Results are recorded and records maintained.</b>
<b>1.6 Sterilization and Decontamination</b>				
K	9	<input type="checkbox"/>	1.6.1	Autoclave(s) are of sufficient size to accommodate the workload.
O	8	<input type="checkbox"/>	1.6.2	Routine autoclave maintenance is performed and the records are maintained.
C	11, 30	<input type="checkbox"/>	1.6.3	<b>The autoclave provides a sterilizing temperature of 121± 2°C as determined for each load using a calibrated maximum registering thermometer. 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.</b>
K	11	<input type="checkbox"/>	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.
K	16	<input type="checkbox"/>	1.6.5	The autoclave standards thermometer is checked every five (5) years for accuracy at 121°C by a qualified calibration laboratory; or, is checked in-house at the steam point (100°C) if it has been previously calibrated at both 100°C and 121°C. Any change in temperature at the steam point changes the calibrated temperature at 121°C by the same magnitude.  Date of most recent determination _____
K	1	<input type="checkbox"/>	1.6.6	Working autoclave thermometers are checked against the autoclave standards thermometer at 121°C yearly.  Date of last check _____ Method _____
K	11	<input type="checkbox"/>	1.6.7	Spore strips/suspensions appropriate for use in an autoclave media cycle are used monthly according to manufacturer's instructions to evaluate the effectiveness of the sterilization process. Results are recorded and the records maintained.
O	11	<input type="checkbox"/>	1.6.8	Heat sensitive tape is used with each autoclave batch.
K	11, 13	<input type="checkbox"/>	1.6.9	Autoclave sterilization records including length of sterilization, total heat exposure time and chamber temperature are maintained. Type of record: Autoclave log, computer printout or chart recorder tracings. (Circle appropriate type or types.)
K	11	<input type="checkbox"/>	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.
K	9	<input type="checkbox"/>	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
K	13	<input type="checkbox"/>	1.6.12	Records of temperatures and exposure times are maintained for the operation of the hot-air sterilizing oven during use.
K	11	<input type="checkbox"/>	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.
K	11	<input type="checkbox"/>	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.
C	1	<input type="checkbox"/>	1.6.15 <b>The sterility of reusable sample containers is determined for each load sterilized. The results are recorded and the records maintained.</b>
C	1	<input type="checkbox"/>	1.6.16 <b>The sterility of pre-sterilized disposable sample containers is determined for each lot received. Results are recorded and the records maintained.</b>
K	9	<input type="checkbox"/>	1.6.17 Reusable pipettes are stored and sterilized in aluminum or stainless steel canisters.
K	9	<input type="checkbox"/>	1.6.18 Reusable pipettes (in canisters) are sterilized in a hot-air oven at 170°C for 2 hours.
C	2	<input type="checkbox"/>	1.6.19 <b>The sterility of reusable pipettes is determined with each load sterilized. Results are recorded and records maintained.</b>
C	2	<input type="checkbox"/>	1.6.20 <b>The sterility of pre-sterilized disposable pipettes is determined with each lot received. Results are recorded and the records maintained.</b>
K	18	<input type="checkbox"/>	1.6.21 Hardwood applicator transfer sticks are properly sterilized.  Method of sterilization _____
C	2	<input type="checkbox"/>	1.6.22 <b>The sterility of the hardwood applicator transfer sticks is checked routinely. Results are recorded and the records maintained.</b>
O	13	<input type="checkbox"/>	1.6.23 Spent broth cultures and agar plates are decontaminated by autoclaving for at least 30 minutes before conventional disposal.
<b>1.7 Media Preparation</b>			
K	3, 5	<input type="checkbox"/>	1.7.1 Media is commercially dehydrated except in the case of medium A-1 which must be prepared from the individual components and modified MacConkey agar which may be prepared from its components.
K	11	<input type="checkbox"/>	1.7.2 Media is prepared according to manufacturer's instructions.
O	11	<input type="checkbox"/>	1.7.3 Dehydrated media and media components are properly stored in a cool, clean, dry place.
O	11	<input type="checkbox"/>	1.7.4 Dehydrated media are labeled with date of receipt and date opened.
C	12	<input type="checkbox"/>	1.7.5 <b>Caked or expired media or media components are discarded.</b>
C	11	<input type="checkbox"/>	1.7.6 <b>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.</b>
C	11	<input type="checkbox"/>	1.7.7 <b>Reagent water is analyzed for residual chlorine monthly and is at a non-detectable level (&lt; 0.1 mg/L). Results are recorded and the records maintained.</b>  Specify method of determination _____.
K	11	<input type="checkbox"/>	1.7.8 Reagent water contains <100 CFU/mL as determined monthly using the heterotrophic plate count method. Results are recorded and the records maintained.
K	11	<input type="checkbox"/>	1.7.9 Media prepared from commercial dehydrated components are sterilized according to the manufacturer's instructions.
K	9	<input type="checkbox"/>	1.7.10 The volume and concentration of media in the tube are suitable for the amount of sample inoculated.
C	11	<input type="checkbox"/>	1.7.11 <b>Total time of exposure of sugar broths to autoclave temperatures does not exceed 45 minutes.</b>
C	1	<input type="checkbox"/>	1.7.12 <b>Media sterility is determined for each load sterilized. Results are recorded and the records maintained.</b>
C	1	<input type="checkbox"/>	1.7.13 <b>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 when the medium is</b>

			<b>made from its individual components.</b>	
O	9	<input type="checkbox"/>	1.7.14	Sterile phosphate buffered dilution water is used as the sample diluent.
K	11	<input type="checkbox"/>	1.7.15	The pH of the prepared media is determined after sterilization to ensure that it is consistent with manufacturer's requirements. Results are recorded and records are maintained.
<b>1.8 Storage of Prepared Culture Media</b>				
K	9	<input type="checkbox"/>	1.8.1	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"/>	1.8.2	Brilliant green bile 2% broth and A-1 media are stored in the dark.
K	13	<input type="checkbox"/>	1.8.3	Stored media are labeled with the storage expiration date or the sterilization date.
K	9	<input type="checkbox"/>	1.8.4	Storage of prepared culture media at room temperature does not exceed 7 days.
K	2	<input type="checkbox"/>	1.8.5	Storage under refrigeration of prepared culture media with loose fitting closures shall not exceed 1 month.
K	11	<input type="checkbox"/>	1.8.6	Storage under refrigeration of prepared culture media with screw-cap closures does not exceed 3 months.
K	17	<input type="checkbox"/>	1.8.7	All prepared MPN broth media stored under refrigeration must reach room temperature prior to use. Culture tubes containing any type of precipitate or Durham tubes containing air bubbles are discarded.
<b>PART II - SEAWATER SAMPLES</b>				
<b>2.1 Collection and Transportation of Samples</b>				
C	11	<input type="checkbox"/>	2.1.1	<b>Sample containers are of a suitable size to contain at least 110 mL of sample and to allow adequate headspace for proper shaking. Seawater samples are collected in clean, sterile, watertight, properly labeled sample containers.</b>
K	1	<input type="checkbox"/>	2.1.2	Samples are identified with collectors name, harvest area, sampling station, time and date of collection.
C	9	<input type="checkbox"/>	2.1.3	<b>Immediately after collection, seawater samples are placed in dry storage (ice chest or equivalent) capable of maintaining a temperature of 0 to 10°C with ice or cold packs for transport to the laboratory. Once received, the samples are placed in the refrigerator unless processed immediately.</b>
O	1	<input type="checkbox"/>	2.1.4	A temperature blank is used to represent the temperature of samples upon receipt at the laboratory. Temperature should be equivalent or less than that of the growing area waters. Results are recorded and maintained.
C	9	<input type="checkbox"/>	2.1.5	<b>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.</b>
<b>2.2 Bacteriological Examination of Seawater by the APHA MPN</b>				
C	9	<input type="checkbox"/>	2.2.1	Lactose broth or lauryl tryptose broth is used as the presumptive medium. <i>(Circle appropriate one.)</i>
C	2	<input type="checkbox"/>	2.2.2	The appropriate positive and negative productivity controls for the presumptive media are used. The results are recorded and the records maintained. Positive productivity control _____ Negative productivity control _____
C	9	<input type="checkbox"/>	2.2.3	Sample and dilutions of sample are shaken vigorously (25 times in a 12" arc in 7 seconds) before inoculation.
C	9	<input type="checkbox"/>	2.2.4	In a multiple dilution series not less than 3 tubes per dilution are used (5 tubes are recommended).
C	6	<input type="checkbox"/>	2.2.5	In a single dilution series not less than 12 tubes are used (for depuration at least 5 tubes are used).
C	6	<input type="checkbox"/>	2.2.6	In a single dilution series, the volumes analyzed are adequate to meet the needs of routine monitoring.  Sample volume inoculated _____

			<b>Range of MPN _____</b>
			<b>Strength of media used _____</b>
K	9	<input type="checkbox"/>	2.2.7 Inoculated tubes are incubated in air at $35 \pm 0.5^{\circ}\text{C}$ .
C	2	<input type="checkbox"/>	2.2.8 <b>Appropriately diluted process control cultures accompany the samples throughout both the presumptive and confirmed phases of incubation. Results are recorded and the records maintained.</b>
			<b>Positive process control _____ Negative process control _____</b>
K	9	<input type="checkbox"/>	2.2.9 Inoculated tubes are read after $24 \pm 2$ hours and $48 \pm 3$ hours of incubation and transferred at both time interval 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.
			<b>2.3 Confirmed Test for Seawater by APHA MPN</b>
C	9	<input type="checkbox"/>	2.3.1 <b>Brilliant green bile 2% broth (BGB) is used as the confirmatory medium for total coliforms.</b>
C	9	<input type="checkbox"/>	2.3.2 <b>EC medium is used as the confirmatory medium for fecal coliforms.</b>
C	2	<input type="checkbox"/>	2.3.3 <b>The appropriate positive and negative productivity controls for the presumptive media are used. The results are recorded and the records maintained.</b>
			<b>Positive productivity control _____ Negative productivity control _____</b>
K	9, 11	<input type="checkbox"/>	2.3.4 Transfers are made to BGB/EC by either sterile loop or sterile hardwood transfer stick from positive presumptive tubes incubated for 24 and 48 hours as appropriate. ( <i>Circle the method of transfer.</i> )
C	9	<input type="checkbox"/>	2.3.5 <b>BGB tubes are incubated at <math>35 \pm 0.5^{\circ}\text{C}</math>.</b>
K	9	<input type="checkbox"/>	2.3.6 BGB tubes are read after $48 \pm 3$ hours of incubation.
C	9	<input type="checkbox"/>	2.3.7 <b>EC tubes are incubated in a circulating waterbath maintained at <math>44.5 \pm 0.2^{\circ}\text{C}</math>.</b>
C	9	<input type="checkbox"/>	2.3.8 <b>EC tubes are read after <math>24 \pm 2</math> hours of incubation.</b>
C	9	<input type="checkbox"/>	2.3.9 <b>The presence of turbidity and any amount of gas or effervescence in the culture tube constitutes a positive test.</b>
			<b>2.4 Computation of Results – APHA MPN</b>
K	9	<input type="checkbox"/>	2.4.1 Results of multiple dilution tests are read from tables in <i>Recommended Procedures for the Examination of Sea Water and Shellfish</i> , Fourth Edition.
K	7	<input type="checkbox"/>	2.4.2 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".
C	7, 9	<input type="checkbox"/>	2.4.3 <b>Results are reported as MPN/100 mL of sample.</b>
			<b>2.5 Bacteriological Examination of Seawater by the MA-1 Method</b>
C	5	<input type="checkbox"/>	2.5.1 <b>A-1 medium complete is used in the analysis.</b>
C	2, 31	<input type="checkbox"/>	2.5.2 <b>A-1 medium without salicin is used in the analysis. Comparability testing supports use of A-1 medium without salicin. Study records are available.</b>
C	5	<input type="checkbox"/>	2.5.3 <b>A-1 medium sterilized for 10 minutes at <math>121^{\circ}\text{C}</math>.</b>
C	2	<input type="checkbox"/>	2.5.4 <b>The appropriate positive and negative productivity controls for the presumptive media are used. The results are recorded and the records maintained.</b>
			<b>Positive productivity control _____ Negative productivity control _____</b>
C	9	<input type="checkbox"/>	2.5.5 <b>Sample and dilutions of sample are shaken vigorously (25 times in a 12" arc in 7 seconds) before inoculation.</b>
C	9	<input type="checkbox"/>	2.5.6 <b>In a multiple dilution series not less than 3 tubes per dilution are used (5 tubes are recommended).</b>
C	6	<input type="checkbox"/>	2.5.7 <b>In a single dilution series at least 12 tubes are used.</b>



C	6	<input type="checkbox"/>	2.5.8	In a single dilution series, the volumes analyzed are adequate to meet the needs of routine monitoring. Sample volume inoculated _____ Range of MPN _____ Strength of media used _____
C	2	<input type="checkbox"/>	2.5.9	Appropriately diluted process control cultures accompany the samples <i>throughout both resuscitation and waterbath incubation</i> Results are recorded and the records maintained. Positive process control _____ Negative process control _____
C	2,5	<input type="checkbox"/>	2.5.10	Inoculated tubes are placed in an air incubator at $35 \pm 0.5^{\circ}\text{C}$ for $3 \pm 0.5$ hours of resuscitation.
C	5	<input type="checkbox"/>	2.5.11	After $3 \pm 0.5$ hours resuscitation at $35^{\circ}\text{C}$ , inoculated tubes are incubated at $44.5 \pm 0.2^{\circ}\text{C}$ in a circulating waterbath for the remainder of the $24 \pm 2$ hours.
C	5	<input type="checkbox"/>	2.5.12	The presence of turbidity and any amount of gas or effervescence in the culture tube constitutes a positive test.
<b>2.6 Computation of Results – APHA MPN</b>				
K	9	<input type="checkbox"/>	2.6.1	Results of multiple dilution tests are read from tables in <i>Recommended Procedures for the Examination of Sea Water and Shellfish</i> , 4 <sup>th</sup> Edition.
K	7	<input type="checkbox"/>	2.6.2	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".
C	7, 9	<input type="checkbox"/>	2.6.3	Results are reported as MPN/100 mL of sample.
<b>2.7 Bacteriological Analysis of Seawater by Membrane Filtration (MF) using mTEC Agar - Materials and Equipment</b>				
C	23, 24	<input type="checkbox"/>	2.7.1	When used for elevated temperature incubation in conjunction with ethafoam resuscitation, the temperature of the hot air incubator is maintained at $44.5 \pm 0.5^{\circ}\text{C}$ under any loading capacity.
C	23	<input type="checkbox"/>	2.7.2	When using a waterbath for elevated temperature incubation, the level of the water completely covers the plates.
C	23	<input type="checkbox"/>	2.7.3	Pre-sterilized plastic or sterile glass culture plates that are clear, flat bottomed, free of bubbles and scratches with tight fitting lids are used.
C	2	<input type="checkbox"/>	2.7.4	The sterility of pre-sterilized culture plates is determined for each lot received. Results are recorded and the records maintained.
K	11	<input type="checkbox"/>	2.7.5	Colonies are counted with the aid of magnification.
C	11, 23	<input type="checkbox"/>	2.7.6	Membrane filters are made from cellulose ester material, white, grid marked, 47 mm in diameter with a pore size of $0.45 \mu\text{m}$ and certified by the manufacturer for fecal coliform analyses.
C	2	<input type="checkbox"/>	2.7.7	Lot number, date of receipt and if provided the expiration date of the membrane filters are recorded and records maintained.
C	2	<input type="checkbox"/>	2.7.8	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.
K	2, 11	<input type="checkbox"/>	2.7.9	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"/>	2.7.10	The sterility of each lot or autoclave batch of membrane filters are checked before use.
K	2	<input type="checkbox"/>	2.7.11	Membrane filters which are beyond their expiration date are not used.
O	11	<input type="checkbox"/>	2.7.12	Forceps tips are clean.
O	11	<input type="checkbox"/>	2.7.13	Forceps tips are smooth without pitting or corrugations to damage the filters being manipulated.



K	11	<input type="checkbox"/>	2.7.14	Forceps are dipped in alcohol and flame sterilized between sample filters.
K	11	<input type="checkbox"/>	2.7.15	If indelible graduation marks are used on clear glass or plastic funnels to measure sample volumes, their accuracy is checked gravimetrically or 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"/>	2.7.16	Membrane filtration units are made of stainless steel, glass or autoclavable plastic free of scratches, corrosion and leaks.
C	11	<input type="checkbox"/>	2.7.17	<b>Membrane filter assemblies are autoclave sterilized for 15 minutes at 121°C prior to the start of a filtration series.</b>
O	11, 23, 26	<input type="checkbox"/>	2.7.18	A UV sterilization unit is used to disinfect filter assemblies between sample and filtration runs.
K	11	<input type="checkbox"/>	2.7.19	The effectiveness of the UV sterilization unit is determined by biological testing monthly. Results are recorded and records maintained.
K	2	<input type="checkbox"/>	2.7.20	Maintenance of the UV sterilization unit is performed as needed. This maintenance is documented and the records maintained.
<b>2.8 Media Preparation and Storage – MF using mTEC Agar</b>				
K	11	<input type="checkbox"/>	2.8.1	Phosphate buffered saline is used as the sample diluent and filter funnel rinse.
C	11	<input type="checkbox"/>	2.8.2	<b>The phosphate buffered saline is properly sterilized.</b>
K	23	<input type="checkbox"/>	2.8.3	A sufficient amount of medium (4-5 mL) is used in each plate.
O	11	<input type="checkbox"/>	2.8.4	Refrigerated prepared plates are stored for no more than 2 weeks in sealed plastic bags or containers to minimize evaporation.
<b>2.9 Sample Analyses - MF using mTEC Agar</b>				
C	24	<input type="checkbox"/>	2.9.1	<b>mTEC agar is used.</b>
C	2	<input type="checkbox"/>	2.9.2	<b>The appropriate positive and negative productivity controls for the presumptive media are used. The results are recorded and the records maintained.</b> Positive productivity control _____ Negative productivity control _____
C	23	<input type="checkbox"/>	2.9.3	<b>The sample is shaken vigorously (25 times in a 12" arc in 7 seconds) before filtration.</b>
C	23	<input type="checkbox"/>	2.9.4	<b>The membrane is placed grid side up within the sterile filter apparatus.</b>
C	23, 25	<input type="checkbox"/>	2.9.5	<b>Sample volumes tested are consistent with the sampling regime employed (i.e., half log or other appropriate dilutions are used with systematic random sampling).</b>
C	23	<input type="checkbox"/>	2.9.6	<b>Sample volumes are filtered under vacuum.</b>
K	26	<input type="checkbox"/>	2.9.7	The pressure of the vacuum pump does not exceed 15 psi.
C	23, 26	<input type="checkbox"/>	2.9.8	<b>The sides of the filter funnel are rinsed at least twice with 20-30 mL of sterile phosphate buffered saline after sample filtration.</b>
C	23	<input type="checkbox"/>	2.9.9	<b>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.</b>
C	11	<input type="checkbox"/>	2.9.10	<b>Blanks are run at the beginning of filtration, after every 10<sup>th</sup> 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).</b>
C	2, 11	<input type="checkbox"/>	2.9.11	<b>Appropriately diluted process control cultures accompany the samples throughout both resuscitation and elevated temperature incubation. Results are recorded and the records maintained.</b>  Positive process control _____ Negative process control _____
C	11, 23, 24	<input type="checkbox"/>	2.9.12	<b>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.</b>

C	11, 23, 24	<input type="checkbox"/>	2.9.13	After 2 hours of resuscitation at 35°C, 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.
			<b>2.10 Computation of Results - MF using mTEC Agar</b>	
C	23	<input type="checkbox"/>	2.10.1	All yellow, yellow-green or yellow-brown colonies are counted.
C	23	<input type="checkbox"/>	2.10.2	Only plates having 80 or fewer colonies are counted. If it is unavoidable to use plates having more than 80 colonies, counts are given as >80 x 100/the volume of sample filtered.
C	2, 11, 23	<input type="checkbox"/>	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.
C	23, 11	<input type="checkbox"/>	2.10.4	The number of fecal coliforms is calculated by the following equation:  Number of fecal coliforms per 100 mL = [number of colonies counted per plate used in the count / volume (s) of sample filtered in ml] x 100.
C	23, 11	<input type="checkbox"/>	2.10.5	Results are reported as CFU/100 mL of sample.
<b>PART III - SHELLFISH SAMPLES</b>				
			<b>3.1 Collection and Transportation of Samples</b>	
C	9	<input type="checkbox"/>	3.1.1	A representative sample of shellstock is collected.
K	9	<input type="checkbox"/>	3.1.2	Shellstock samples are collected in clean, waterproof, puncture resistant containers loosely sealed.
K	9	<input type="checkbox"/>	3.1.3	Shellstock samples are labeled with collector's name, type of shellstock, the source or harvest area, sampling station, time, date and place (if applicable ) of collection.
C	9	<input type="checkbox"/>	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.
C	1	<input type="checkbox"/>	3.1.5	Analysis of the samples is initiated as soon as possible after collection. Shellfish samples are not tested if the time interval between collection and analysis exceeds 24 hours.
			<b>3.2 Preparation of Shellfish for Examination</b>	
K	2,11	<input type="checkbox"/>	3.2.1	Shucking knives, scrub brushes and blender jars are (autoclave) sterilized for 15 minutes prior to use.
O	2	<input type="checkbox"/>	3.2.2	Blades of shucking knives are not corroded.
O	9	<input type="checkbox"/>	3.2.3	The hands of the analyst are thoroughly washed with soap and water immediately prior to cleaning the shells of debris.
O	2	<input type="checkbox"/>	3.2.4	The faucet used for rinsing the shellstock does not contain an aerator.
K	9	<input type="checkbox"/>	3.2.5	Shellstock are scrubbed with a stiff, sterile brush and rinsed under tap water of drinking water quality.
O	9	<input type="checkbox"/>	3.2.6	Shellstock are allowed to drain in a clean container or on clean towels prior to opening.
K	9	<input type="checkbox"/>	3.2.7	Immediately 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"/>	3.2.8	Shellstock are not shucked directly through the hinge.
C	9	<input type="checkbox"/>	3.2.9	Contents of shellstock (liquor and meat) are shucked into a sterile, tared blender jar or other sterile container.
K	9	<input type="checkbox"/>	3.2.10	At least 200 grams of shellfish meat or a quantity of meat sufficient to cover the blender blades is used for the analysis.
K	9	<input type="checkbox"/>	3.2.11	A representative sample of at least 12 shellfish is used for the analysis.
K	2	<input type="checkbox"/>	3.2.12	The sample is weighed to the nearest 0.1 gram and an equal amount by weight of diluent is added.
O	9	<input type="checkbox"/>	3.2.13	Sterile phosphate buffered dilution water is used as the sample diluent.

C	9	<input type="checkbox"/>	3.2.14	Samples are blended at high speed for 60 to 120 seconds until homogenous.
K	9	<input type="checkbox"/>	3.2.15	APHA <i>Recommended Procedures for the Examination of Sea Water And Shellfish</i> , Fourth Edition is followed for the analysis of previously shucked and frozen shellfish meats.
<b>3.3 MPN Analysis for Fecal Coliform Organisms, Presumptive Test, APHA</b>				
C	9	<input type="checkbox"/>	3.3.1	Appropriate strength lactose or lauryl tryptose broth is used as presumptive media in the analysis. <i>(Circle the medium used.)</i>
C	2	<input type="checkbox"/>	3.3.2	The appropriate positive and negative productivity controls for the presumptive media are used. The results are recorded and the records maintained. Positive productivity control _____ Negative productivity control _____
K	9	<input type="checkbox"/>	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"/>	3.3.4	No fewer than 5 tubes per dilution are used in a multiple dilution MPN series.
C	9	<input type="checkbox"/>	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., 20 ml of 1:1 diluted sample to 80 ml of diluent or the equivalent for 0.1 g portion). All successive dilutions are prepared conventionally.
K	6	<input type="checkbox"/>	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"/>	3.3.7	Appropriately diluted process control cultures accompany the samples throughout both the presumptive and confirmed phases of incubation. Results are recorded and the records maintained. Positive Process control _____ Negative Process control _____
K	9	<input type="checkbox"/>	3.3.8	Inoculated media are incubated at $35 \pm 0.5^{\circ}\text{C}$ .
K	10	<input type="checkbox"/>	3.3.9	Tubes are read after $24 \pm 2$ 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.
<b>3.4 Confirmed Test for Fecal Coliforms - APHA</b>				
C	9	<input type="checkbox"/>	3.4.1	EC medium is used as the confirmatory medium.
C	2	<input type="checkbox"/>	3.4.2	The appropriate positive and negative productivity controls for the presumptive media are used. The results are recorded and the records maintained. Positive productivity control _____ Negative productivity control _____
K	9, 11	<input type="checkbox"/>	3.4.3	Transfers are made to EC medium by either sterile loop or hardwood sterile transfer sticks from positive presumptives. <i>(Circle the method of transfer.)</i>
C	9	<input type="checkbox"/>	3.4.4	EC tubes are incubated in a circulating waterbath at $44.5 \pm 0.2^{\circ}\text{C}$
K	9	<input type="checkbox"/>	3.4.5	EC tubes are read for gas production after $24 \pm 2$ hours of incubation.
C	9	<input type="checkbox"/>	3.4.6	The presence of turbidity and any amount of gas and/or effervescence in the Durham tube constitutes a positive test.
<b>3.5 Computation of Results for MPN Analyses</b>				
K	9	<input type="checkbox"/>	3.5.1	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"/>	3.5.2	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".
C	9	<input type="checkbox"/>	3.5.3	Results are reported as MPN/100 grams of sample.

		<b>3.6 Standard Plate Count Method</b>		
O	20	<input type="checkbox"/>	3.6.1	A standard plate count (SPC) analysis may be performed in conjunction with the analysis for fecal coliform organisms.
K	9	<input type="checkbox"/>	3.6.2	In the standard plate count procedure at least four plates are used, duplicates of two dilutions. One of the dilutions should produce colonies of 30 to 300 per plate.
K	2	<input type="checkbox"/>	3.6.3	Fifteen to 20 mL of tempered sterile plate count agar is used per plate.
C	9	<input type="checkbox"/>	3.6.4	<b>Agar tempering bath maintains the agar at 44-46°C.</b>
C	9	<input type="checkbox"/>	3.6.5	<b>An agar based temperature control having a similar volume and shape as the tempering plate count agar is used in the tempering bath.</b>
K	9	<input type="checkbox"/>	3.6.6	Samples or sample dilutions to be plated are shaken vigorously (25 times in a 12" arc in 7 seconds) before plating.
C	9	<input type="checkbox"/>	3.6.7	<b>Not more than 1 mL nor less than 0.1 mL of sample or sample dilution is plated.</b>
K	11	<input type="checkbox"/>	3.6.8	Control plates are used to check air quality and the sterility of the agar and the diluent.
K	9,21	<input type="checkbox"/>	3.6.9	Solidified plates are incubated at $35 \pm 0.5^{\circ}\text{C}$ for $48 \pm 3$ hours inverted and stacked no more than four high.
K	9	<input type="checkbox"/>	3.6.10	Quebec Colony Counter or its equivalent is used to provide the necessary magnification and visibility for counting plates.
K	1	<input type="checkbox"/>	3.6.11	A hand tally or its equivalent is used for accuracy in counting.
		<b>3.7 Computation of Results -SPC</b>		
K	9	<input type="checkbox"/>	3.7.1	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> , Fourth Edition.
C	19	<input type="checkbox"/>	3.7.2	<b>Colony counts are reported as CFU/g of sample.</b>
		<b>3.8 Bacteriological Analysis of Shellfish Using the ETCP</b>		
C	2,3	<input type="checkbox"/>	3.8.1	<b>Prepared modified MacConkey agar is used on the day that it is made.</b>
K	3	<input type="checkbox"/>	3.8.2	Double strength modified MacConkey agar is used.
C	3	<input type="checkbox"/>	3.8.3	<b>Prepared double strength modified MacConkey agar is heated to boiling, removed from the heat, and boiled again. This agar is never autoclaved.</b>
K	2, 3	<input type="checkbox"/>	3.8.4	Twice boiled, double strength modified MacConkey agar and is maintained in a tempering bath at 45 to 50°C until used.
K	2, 3	<input type="checkbox"/>	3.8.5	Phosphate buffered saline is used as the sample diluent in the ETCP.
C	2, 3	<input type="checkbox"/>	3.8.6	<b>The phosphate buffered saline is tempered at 45 - 50°C to prevent premature solidification of the agar.</b>
C	9	<input type="checkbox"/>	3.8.7	<b>The sample homogenate is cultured within 2 minutes of blending.</b>
C	2,3	<input type="checkbox"/>	3.8.8	<b>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.</b>
K	3	<input type="checkbox"/>	3.8.9	Sixty (60) mL of tempered, twice boiled double strength Modified MacConkey Agar is added.
K	2,3, 22	<input type="checkbox"/>	3.8.10	The container is gently swirled or slowly inverted once to mix the contents, which are subsequently distributed uniformly over six plates.
C	1	<input type="checkbox"/>	3.8.11	<b>Media and diluent sterility are determined with each use. Results are recorded and the records maintained.</b>
C	1	<input type="checkbox"/>	3.8.12	<b>Media productivity is determined using media appropriate properly diluted pour plated positive and negative control cultures for each batch of Modified MacConkey agar prepared.</b> Positive control culture _____ Negative control culture _____
C	3, 13	<input type="checkbox"/>	3.8.13	<b>When solidified, the plates are placed inverted into an air incubator at <math>45.5 \pm 0.5^{\circ}\text{C}</math> for 18 to 30 hours of incubation.</b>
C	2	<input type="checkbox"/>	3.8.14	<b>Plates are stacked no more than three high in the incubator.</b>

C	2	<input type="checkbox"/>	3.8.15	Appropriately diluted pour plated process control cultures <i>accompany each set of samples throughout incubation.</i> The results are recorded and the records maintained. Positive process control _____ Negative process control _____
<b>3.9 Computation of Results - ETCP</b>				
K	11	<input type="checkbox"/>	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"/>	3.9.2	A hand tally or its equivalent is used to aid in counting.
C	3, 6	<input type="checkbox"/>	3.9.3	<b>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.</b>
C	3	<input type="checkbox"/>	3.9.4	<b>Results are reported as CFU/100 grams of sample.</b>
<b>Bacteriological Examination of Soft-shelled Clams and American Oysters for Male Specific Coliphage (MSC)</b>				
<b>3.10 MSC Equipment and Supplies</b>				
K	30	<input type="checkbox"/>	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"/>	3.10.2	<b>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.</b>
K	9	<input type="checkbox"/>	3.10.3	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"/>	3.10.4	<b>Sterile 0.22 µm pore size syringe filters and pre-sterilized plastic or sterile glass syringes are used to sterilize the antibiotic solutions.</b>
K	1	<input type="checkbox"/>	3.10.5	The sterility of each lot of pre-sterilized syringes and syringe filters is determined. Results are recorded and records maintained.
K	1	<input type="checkbox"/>	3.10.6	The sterility of each batch of reusable glass syringes is determined. Results are recorded and records maintained.
C	27, 28	<input type="checkbox"/>	3.10.7	<b>The balance used provides a sensitivity of at least mg (0.01g.).</b>
C	27, 28	<input type="checkbox"/>	3.10.8	<b>The temperature of the incubator used is maintained at 36 ± 1°C.</b>
C	28	<input type="checkbox"/>	3.10.9	<b>Sterile disposable 50 mL centrifuge tubes are used and their sterility is determined with each lot. Results are recorded and records maintained.</b>
<b>3.11 MSC Media Preparation</b>				
K	28	<input type="checkbox"/>	3.11.1	Media preparation and sterilization is according to the validated method.
K	27, 28	<input type="checkbox"/>	3.11.2	Bottom agar, double strength soft agar and growth broth are prepared from their individual components.
K	27, 28	<input type="checkbox"/>	3.11.3	Soft agar is prepared double strength in volumes of 2.5 mL.
C	27, 28	<input type="checkbox"/>	3.11.4	<b>The streptomycin and ampicillin solutions are added to tempered bottom agar and vortex for 2 minutes on stir plate.</b>
O	27, 28	<input type="checkbox"/>	3.11.5	Storage of the bottom agar under refrigeration does not exceed 1 month.
K	27, 28	<input type="checkbox"/>	3.11.6	Unsterilized soft agar is stored at -20 °C -15C for up to 3 months.
K	27, 28	<input type="checkbox"/>	3.11.7	The soft agar is removed from the freezer and sterilized for 15 minutes at 121°C before use.
K	27, 28	<input type="checkbox"/>	3.11.8	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"/>	3.11.9	Bottom agar plates are allowed to reach room temperature before use.
<b>3.12 Preparation of the Soft-Shelled Clams and American Oysters for MSC Analysis</b>				
K	2,11	<input type="checkbox"/>	3.12.1	Shucking knives, scrub brushes and blender jars are autoclave sterilized for 15 minutes prior to use.
O	2	<input type="checkbox"/>	3.12.2	The blades of shucking knives are not corroded.
O	9	<input type="checkbox"/>	3.12.3	The hands of the analyst are thoroughly washed with soap and water immediately prior to cleaning the shells of debris.
O	2	<input type="checkbox"/>	3.12.4	The faucet used for rinsing the shellfish does not contain an aerator.

K	9	<input type="checkbox"/>	3.12.5	The shellfish are scrubbed with a stiff, sterile brush and rinsed under tap water of drinking water quality.
O	9	<input type="checkbox"/>	3.12.6	The shellfish are allowed to drain in a clean container or on clean towels unlayered prior to shucking.
K	9	<input type="checkbox"/>	3.12.7	Immediately 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"/>	3.12.8	<b>Shellfish are not shucked through the hinge.</b>
C	9	<input type="checkbox"/>	3.12.9	<b>The contents of shellfish (liquor and meat) are shucked into a sterile, tared blender jar or other sterile container.</b>
K	9	<input type="checkbox"/>	3.12.10	A representative sample of at least 12 shellfish is used for the analysis.
K	2, 19	<input type="checkbox"/>	3.12.11	The sample is weighed to the nearest 0.1 gram.
<b>3.13 MSC Sample Analysis</b>				
C	28	<input type="checkbox"/>	3.13.1	<b>E.coli <i>Famp</i> ATCC 700891 is the bacterial host strain used in this procedure.</b>
K	27, 28	<input type="checkbox"/>	3.13.2	Host cell growth broth is tempered at $36 \pm 1^\circ\text{C}$ and vortexed (or shaken) to aerate prior to inoculation with host cells.
K	27, 28	<input type="checkbox"/>	3.13.3	Several host cell colonies are transferred to a tube of tempered, aerated growth broth and incubated at $36 \pm 1^\circ\text{C}$ for 4-6 hours to provide host cells in log phase growth for sample analysis.
C	27, 28	<input type="checkbox"/>	3.13.4	<b>After inoculation, the host cell growth broth culture is not shaken.</b>
C	28	<input type="checkbox"/>	3.13.5	<b>A 2:1 mixture of sterile growth broth to shellfish tissue is used for eluting the MSC.</b>
C	28	<input type="checkbox"/>	3.13.6	<b>The elution mixture is prepared w/v by weighing the sample and adding two equal portions of sterile growth broth by volume to the shellfish tissue.</b>
C	28	<input type="checkbox"/>	3.13.7	<b>The elution mixture is homogenized at high speed for 180 seconds.</b>
C	28	<input type="checkbox"/>	3.13.8	<b>Immediately after blending, 33 grams of the homogenized elution mixture are weighed into centrifuge tubes.</b>
C	28	<input type="checkbox"/>	3.13.9	<b>The homogenized elution mixture is centrifuged for 15 minutes at 9000 x g at <math>4^\circ\text{C}</math>.</b>
C	27, 28	<input type="checkbox"/>	3.13.10	<b>The supernatant is pipetted off, weighed and the weight recorded.</b>
C	27, 28	<input type="checkbox"/>	3.13.11	<b>The supernatant is allowed to warm to room temperature about 20 to 30 minutes.</b>
K	27, 28	<input type="checkbox"/>	3.13.12	The autoclaved soft agar is tempered and held at $51 \pm 1^\circ\text{C}$ throughout the period of sample analysis.
K	27, 28	<input type="checkbox"/>	3.13.13	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"/>	3.13.14	The sample supernatant is shaken or vortexed before being added to the tempering soft agar.
C	27, 28	<input type="checkbox"/>	3.13.15	<b>2.5 mL of sample supernatant is added to each tube of tempering soft agar.</b>
C	27, 28	<input type="checkbox"/>	3.13.16	<b>The soft agar/sample supernatant/host cell mixture is gently rolled between the palms of the hands to mix.</b>
C	27, 28	<input type="checkbox"/>	3.13.17	<b>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.</b>
C	28	<input type="checkbox"/>	3.13.18	<b>Ten (10) plates are used, 2.5 mL per plate for a total of 25 mL of supernatant analyzed per sample.</b>
K	27, 28	<input type="checkbox"/>	3.13.19	Negative and positive control plates are prepared and accompany each set of samples analyzed. The results are recorded and records maintained. Positive control _____
K	27, 28	<input type="checkbox"/>	3.13.20	Growth broth is used as the negative control or blank.
K	27, 28	<input type="checkbox"/>	3.13.21	Type strain MS2 (ATCC 15597) male specific bacteriophage appropriately diluted to provide countable low levels of phage is used as the positive control.
K	2	<input type="checkbox"/>	3.13.22	A negative control plate is plated at the beginning and end of each set of samples analyzed.



K	27, 28	<input type="checkbox"/>	3.13.23 The positive control is plated after all the samples are inoculated and immediately prior to the final negative control.
C	27, 28	<input type="checkbox"/>	3.13.24 All plates are incubated at $36 \pm 1^{\circ}\text{C}$ for $18 \pm 2$ hours.
<b>3.14 Computation of Results - MSC</b>			
C	27	<input type="checkbox"/>	3.14.1 Circular zones of clearing or plaques of any diameter in the lawn of host bacteria are counted.
C	28, 32	<input type="checkbox"/>	3.14.2 The working range of the method is 1 to 200 PFU per plate. When there are no plaques on all ten plates, the count is <6 PFU/100 grams for soft-shelled clams, <7 PFU/ 100 grams for American oysters, and <5 PFU/ 100 grams for quahog (hard) clams. If the density exceeds 200 PFU per plate on all plates, the count is given as > 20,000 PFU/100 grams.
K	28	<input type="checkbox"/>	3.14.3 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"/>	3.14.4 The MSC count is rounded off conventionally to give a whole number.

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## SHELLFISH LABORATORY EVALUATION CHECKLIST

## SUMMARY OF NONCONFORMITIES

[illegible]

<b>LABORATORY STATUS</b>	
<b>LABORATORY</b>	<b>DATE</b>
<b>LABORATORY REPRESENTATIVE:</b>	
<b>MICROBIOLOGICAL COMPONENT: (Part I-III)</b>	
<b>A. Results</b>	
Total # of Critical (C) Nonconformities in Parts I-III	_____
Total # of Key (K) Nonconformities in Parts I-III	_____
Total # of Critical, Key and Other (O)	_____
Nonconformities in Parts I-III	
<b>B. Criteria for Determining Laboratory Status of the Microbiological Component:</b>	
<p>1. <b>Does Not Conform Status:</b> The Microbiological component of this laboratory is not in conformity with NSSP requirements if:</p> <p style="margin-left: 40px;">a. The total # of Critical nonconformities is <math>\geq 4</math> <b>or</b></p> <p style="margin-left: 40px;">b. The total # of Key nonconformities is <math>\geq 13</math> <b>or</b></p> <p style="margin-left: 40px;">c. The total # of Critical, Key and Other is <math>\geq 18</math></p> <p>2. <b>Provisionally Conforms Status:</b> The microbiological component of this laboratory is determined to be provisionally conforming to NSSP requirements if the number of critical nonconformities is <math>\geq 1</math> <b>but</b> <math>\leq 3</math>.</p>	
<b>C. Laboratory Status (<i>circle appropriate</i>)</b>	
<b>Does Not Conform</b>	<b>Provisionally Conforms</b>
<b>Conforms</b>	
Acknowledgment by Laboratory Director/Supervisor:	
All corrective Action will be implemented and verifying substantiating documentation received by the Laboratory Evaluation Officer on or before _____.	
Laboratory Signature: _____	Date: _____
LEO Signature: _____	Date: _____

NSSP Form LAB-100 Microbiology Rev. October 2015