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 Update Microbiology Laboratory Evaluation Checklist. Please find the updated Microbiology Laboratory Checklist attached - word document titled "Revised Microbiology Checklist 11-08-2010.doc".
A summary of the changes is:

- 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: 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.

Cost Information (if available): None

Action by 2011 Laboratory Methods Review Committee Recommended referral of Proposal 11-108 to the appropriate committee as determined by the Conference Chairman.

Action by 2011 Task Force I Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 11-108.

Action by 2011 General Assembly Adopted recommendation of 2011 Task Force I on Proposal 11-108.

Action by FDA February 26, 2012 Concurred with Conference action on Proposal 11-108.

PUBLIC HEALTH SERVICE
U.S. FOOD AND DRUG ADMINISTRATION
~~**SHELLFISH PROGRAM IMPLEMENTATION BRANCH**~~ **OFFICE OF FOOD SAFETY**
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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	1.1 **Quality Assurance (QA) Plan**

			~~1.~~ 1.1.1 Written Plan (Check those items which apply.)
		☐	a. Organization of the laboratory.
		☐	b. Staff training requirements.
		☐	c. Standard operating procedures.
		☐	d. Internal quality control measures for equipment, their calibration, maintenance, repair, ~~and for~~ performance ~~checks~~ and rejection criteria established
		☐	e. Laboratory safety.
		☐	f. Internal performance assessment.
		☐	g. External performance assessment.
C	**8**	☐	~~6.~~ **1.1.2 QA Plan Implemented**
K	11	☐	~~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	☐	~~2.~~ **1.2.1 In state/county laboratories, the supervisor meets the state/county educational and experience requirements for managing a public health laboratory**
K	State's Human Resources Department	☐	~~3.~~ 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	☐	~~4.~~ **1.2.3 In ~~private~~ commercial 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	☐	~~5.~~ 1.2.4 In ~~private~~ 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.
		1.3 Work Area	
O	8,11	☐	~~1.~~ 1.3.1 Adequate for workload and storage.
K	11	☐	~~2.~~ 1.3.2 Clean, well lighted.
K	11	☐	~~3.~~ 1.3.3 Adequate temperature control.
O	11	☐	~~4.~~ 1.3.4 All work surfaces are nonporous, easily cleaned and disinfected.
K	11	☐	~~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	☐	~~6. Pipette aid used, mouth pipetting not permitted.~~ Moved to equipment 1.4.25
		1.4 Laboratory Equipment	
O	9	☐	~~1.~~ 1.4.1 To determine the pH of prepared media, the pH meter has a standard accuracy of 0.1 units.
O	14	☐	~~2.~~ 1.4.2 pH electrodes consisting of pH half cell and reference half cell or equivalent combination electrode/triode (free from silver/silver chloride (Ag/AgCl) or contains an ion exchange barrier ~~preventing passage of Ag ions into the medium which may effect the accuracy of the pH reading~~) to prevent the passage of silver (Ag) ions into the substance being measured.
K	11	☐	~~3.~~ 1.4.3 The effect of temperature on the pH is compensated for by an ATC probe or by manual adjustment.
K	8	☐	~~4.~~ 1.4.4 pH meter is calibrated daily or with each use ~~and records are maintained.~~ Results are recorded and records maintained.
K	11	☐	~~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	☐	~~6. Electrode effectiveness is determined daily or with each use.~~ ~~Method of determination _____.~~ 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.*)
K	9	☐	~~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	☐	~~8. Balance checked monthly using NIST Class S or ASTM Class 1 or 2 weights or equivalent and records are maintained.~~ 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	☐	~~9.~~ 1.4.9. Refrigerator temperature (s) monitored at least once daily on workdays ~~and recorded.~~ Results are recorded and records maintained
K	1	☐	~~10.~~ 1.4.10 Refrigerator temperature maintained at 0° to 4°C.
C	**9**	☐	**~~11.~~ 1.4.11 The temperature of the incubator is maintained at 35 ± 0.5°C.**
C	**11**	☐	**~~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	☐	~~13.~~ 1.4.13 Working thermometers are located on top and bottom shelves of use in the air incubator(s).
C	**11**	☐	**~~14.~~ 1.4.14 Temperature of the waterbath is maintained at 44.5 ± 0.2°C under ~~any~~ all loading ~~capacity~~ conditions.**
C	**9**	☐	**~~15.~~ 1.4.15 The thermometers used in the waterbath are graduated in 0.1°C increments.**
~~O~~ C	**13**	☐	**~~16.~~ 1.4.16 The waterbath has adequate capacity for workload.**
K	9	☐	~~17.~~ 1.4.17 The level of water in the waterbath covers the level of liquid in the incubating tubes.
K	8, 11	☐	~~18.~~ 1.4.18 Air incubator/waterbath temperatures are taken twice daily ~~and recorded~~ on workdays. The results are recorded and records maintained.
~~K~~	~~13~~	~~☐~~	~~19. Working thermometers are tagged with identification, date of calibration, calibrated temperature and correction factor.~~
~~K~~ C	**4**	☐	**~~20.~~ 1.4.19 All working thermometers are appropriately immersed.**
C	**29**	☐	**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.**
~~K~~ C	**11**	☐	~~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.~~ **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.**
K	9	☐	~~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 _____.
C	**29**	☐	**1.4.23 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. (*Circle the thermometer type used.*)
K	13	☐	~~23.~~ 1.4.24 Incubator and waterbath working thermometers are checked annually against the standards thermometer at the temperatures at which they are used. Results are recorded and records maintained.
O	11	☐	1.4.25 Appropriate pipet aids are available and used to inoculate samples. Mouth pipetting is not permitted.
		1.5 Labware and Glassware Washing	
O	9	☐	~~1.~~ 1.5.1 Utensils and containers are clean borosilicate glass, stainless steel or other noncorroding materials
K	9	☐	~~2.~~ 1.5.2 Culture tubes are of a suitable size to accommodate the volume for nutritive ingredients and samples
K	9	☐	~~3.~~ 1.5.3 Sample containers are made of glass or some other inert material ~~(ie polypropylene)~~.
O	9	☐	~~4.~~ 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	☐	~~5.~~ 1.5.5 Graduations are indelibly marked on dilution bottles and tubes or an acceptable alternative method is used to ensure appropriate volumes.
~~K~~ C	**9**	☐	~~6.~~ **1.5.6 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 1mL used to deliver 0.1 mL aliquots.**
K	9	☐	~~7.~~ 1.5.7 Reusable sample containers are capable of being properly washed and sterilized.
K	9	☐	~~8.~~ 1.5.8 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.
~~C~~	~~9~~	☐	~~9. 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.~~ ~~Date of most recent testing_____~~ ~~Average difference between Groups A and B_____~~ ~~Average difference between Groups B and D_____~~ ~~Detergent Brand_____ Lot #_____~~
C	2	☐	**1.5.9 An alkaline or acidic detergent is used for washing glassware/labware.**
~~K~~ C	**11**	☐	~~10. 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.~~ **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% bromothymol blue. Results are recorded and records maintained.**
		1.6 Sterilization and Decontamination	
~~O~~ K	9	☐	~~1.~~ 1.6.1 Autoclave(s) are of sufficient size to accommodate the workload.
O	8	☐	~~2.~~ 1.6.2 Routine autoclave maintenance performed ~~(e.g. pressure relief valves, exhaust trap, chamber drain)~~ and the records maintained.
~~O~~	~~8~~	☐	~~3. Autoclave(s) and/or steam generators serviced annually or as needed by qualified technician and records maintained.~~

C	**11, 30**	☐	~~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).~~ **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.**
K	11	☐	~~5. An autoclave standards thermometer has been calibrated by the National Institute of Standards and Technology (NIST) or its equivalent at 121° C.~~ 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	☐	~~6. The autoclave standards thermometer is checked every five years for accuracy at either 121° C or at the steam point.~~ 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. Date of most recent determination_____
K	1	☐	~~7.~~ 1.6.6 Working autoclave thermometers are checked against the autoclave standards thermometer at 121°C yearly. Date of last check _____ Method _____
K	11	☐	~~8.~~ 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.
O	11	☐	~~9.~~ 1.6.8 Heat sensitive tape is used with each autoclave batch.
K	11, 13	☐	~~10.~~ 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	☐	~~11.~~ 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	☐	~~12.~~ 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.~~
K	13	☐	~~13.~~ 1.6.12 Records of temperatures and exposure times are maintained for the operation of the hot-air sterilizing oven during use.
K	11	☐	~~14.~~ 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	☐	~~15.~~ 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.
~~O~~ C	**1**	☐	~~16. The sterility of reusable/disposable sample containers is determined for each batch/lot.~~ **1.6.15 The sterility of reusable sample containers is determined for each load sterilized. The results are recorded and the records maintained.**
C	1	☐	**1.6.16 The sterility of pre-sterilized disposable sample containers is determined for each lot received. Results are recorded and the records maintained.**
K	9	☐	~~17.~~ 1.6.17 Reusable pipettes are stored and sterilized in aluminum or stainless steel canisters ~~or equivalent alternative~~ .

K	9	☐	~~18.~~ 1.6.18 Reusable pipettes (in canisters) are sterilized in a hot-air oven at 170°C for 2 hours.
~~O~~ C	2	☐	~~19. The sterility of reusable/disposable pipettes is determined with each batch/lot. Results are recorded and maintained.~~ **1.6.19 The sterility of reusable pipettes is determined with each load sterilized. Results are recorded and records maintained.**
C	2	☐	**1.6.20 The sterility of pre-sterilized disposable pipets is determined with each lot received. Results are recorded and the records maintained.**
K	18	☐	~~20.~~ 1.6.21 Hardwood applicator transfer sticks are properly sterilized. Method of sterilization _____
C	2	☐	**1.6.22 The sterility of the hardwood transfer sticks is checked routinely. Results are recorded and the records maintained.**
O	13	☐	~~21. Spent broth cultures and agar plates are decontaminated by autoclaving for at least 30 minutes before conventional disposal.~~ 1.6.23 Spent broth cultures and agar plates are decontaminated before disposal. Method _____.
		1.7 Media Preparation	
K	3, 5	☐	~~1.~~ 1.7.1 Media is commercially dehydrated except in the case of medium A-1 which ~~is~~ must be prepared from the individual components and modified MacConkey agar which may be prepared from its components.
O	11	☐	~~2.~~ 1.7.2 Dehydrated media and media components properly stored in cool, clean, dry place.
O	11	☐	~~3.~~ 1.7.3 Dehydrated media are labeled with the analyst's initials date of receipt and date opened.
C	12	☐	~~4.~~ **1.7.4 Caked or expired media or media components are discarded.**
C	11	☐	~~5. Make-up water is distilled or deionized *(circle one)* 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 *(circle the appropriate)*~~ **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.**
C	11	☐	~~6.~~ **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.** **Specify method of determination _____.**
~~K~~	~~11~~	☐	~~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	☐	~~8.~~ 1.7.7 ~~Make up~~ Reagent water contains ~~<1000~~ <100 CFU/mL as determined monthly using the heterotrophic plate count method. Results are recorded and the records maintained.
K	11	☐	~~9.~~ 1.7.8 Commercially prepared dehydrated media are sterilized according to the manufacturer's instructions.
K	9	☐	~~10.~~ 1.7.9 The volume and concentration of media in the tube are suitable for the amount of sample inoculated.
C	11	☐	~~11.~~ **1.7.10 Total time of exposure of sugar broths to autoclave temperatures does not exceed 45 minutes.**
C	1	☐	~~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.~~ **1.7.11 Media sterility is determined for each load sterilized. Results are recorded and the records maintained.**
C	1	☐	**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.**
O	9	☐	~~13.~~ 1.7.13 Sterile phosphate buffered dilution water is used as the sample diluent.
K	11	☐	~~14.~~ 1.7.14 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.
		1.8 Storage of Prepared Culture Media	
~~O~~ K	9	☐	~~1.~~ 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	☐	~~2.~~ 1.8.2 Brilliant green bile 2% broth and A-1 media are stored in the dark.
K	13	☐	~~3.~~ 1.8.3 Stored media are labeled with the storage expiration date or the sterilization date.
O	9	☐	~~4.~~ 1.8.4 Storage of prepared culture media at room temperature does not exceed 7 days.
O	2	☐	~~5.~~ 1.8.5 Storage under refrigeration of prepared broth media with loose fitting closures shall not exceed 1 month.
O	11	☐	~~6.~~ 1.8.6 Storage under refrigeration of prepared culture media with screw-cap closures does not exceed 3 months.
K	17	☐	~~7.~~ 1.8.7 All prepared ~~media~~ MPN broth 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			
		2.1 Collection and Transportation of Samples	
C	**11**	☐	**~~1.~~ 2.1.1 Sample containers are of a suitable size to contain at least ~~100~~ 110 mL of sample and to allow adequate headspace for proper shaking. Seawater samples are collected in clean, sterile, watertight, properly labeled sample containers.**
K	1	☐	~~2.~~ 2.1.2 Samples are identified with collectors name, harvest area, sampling station, time and date of collection.
C	**9**	☐	~~3. After collection, seawater samples shall be kept at a temperature between 0 and 10° C until examined.~~ **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.**
~~K~~ O	1	☐	~~4.~~ 2.1.4 A temperature blank is used to determine the temperature of samples upon receipt at the laboratory. Results are recorded and maintained.
C	**9**	☐	~~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.~~ **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.**
		2.2 Bacteriological Examination of Seawater by the APHA MPN	
C	**9**	☐	**~~1.~~ 2.2.1 Lactose broth or lauryl tryptose broth is used as the presumptive medium.** *(Circle appropriate one.)*

C	2	☐	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. Positive productivity control _____ Negative productivity control _____
C	9	☐	2. 2.2.3 **Sample and dilutions of sample are shaken ~~mixed~~ vigorously (25 times in a 12" arc in 7 seconds) before inoculation.**
C	9	☐	3. 2.2.4 **In a multiple dilution series not less than 3 tubes per dilution are used (5 tubes are recommended).**
C	6	☐	4. 2.2.5 **In a single dilution series not less than 12 tubes are used (for depuration at least 5 tubes are used).**
~~K~~ C	6	☐	5. 2.2.6 **In a single dilution series, the volumes analyzed ~~examined~~ are adequate to meet the needs of routine monitoring.** **Sample volume inoculated _____** **Range of MPN_____** **Strength of media used_____**
K	9	☐	6. 2.2.72.2.7 Inoculated ~~media~~ tubes are ~~placed in an air incubator~~incubated in air at 35~~+~~± 0.5°C ~~for up to 48 ± 3 hours~~.
~~K~~ C	2	☐	7. Positive and negative control cultures accompany samples throughout the procedure. Records are maintained. 2.2.8 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	☐	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 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
		2.3 **Confirmed Test for Seawater by APHA MPN**	
C	9	☐	1. 2.3.1 **Brilliant green bile 2% broth (BGB) is used as the confirmatory medium for total coliforms.**
C	9	☐	2. 2.3.2 **EC medium is used as the confirmatory medium for fecal coliforms.**
C	2	☐	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. Positive productivity control _____ Negative productivity control _____
K	9, 11	☐	3. 2.3.4 Transfers are made to BGB/EC by either sterile loop or sterile hardwood transfer stick from positive presumptive~~s~~ tubes incubated for 24 and 48 hours as appropriate . *(Circle the method of transfer.)*
~~K~~	~~2~~	☐	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	☐	5. 2.3.5 **BGB tubes are incubated at 35 ± 0.5°C.**
K	9	☐	6. 2.3.6 BGB tubes are read after 48 ± 3 hours of incubation.
C	9	☐	7. 2.3.7 **EC tubes are incubated in a circulating waterbath maintained at 44.5 ± 0.2°C ~~for 24 ± 2 hours~~.**
C	9	☐	2.3.8 EC tubes are read after 24 ± 2 hours of incubation.
C	9	☐	8. 2.3.9 **The presence of turbidity and any amount of gas or effervescence in the culture tube constitutes a positive test.**

		2.4 Computation of Results – APHA MPN	
K	9	☐	~~1.~~ 2.4.1 Results of multiple dilution tests are read from tables in *Recommended Procedures for the Examination of Sea Water and Shellfish*,Fourth ~~4~~th Edition.
K	7	☐	~~2.~~ 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".
~~K~~C	7, 9	☐	**~~3.~~ 2.4.3 Results are reported as MPN/100 mL of sample.**
		2.5 Bacteriological Examination of Seawater by the MA-1 Method	
C	5	☐	**2.5.1 A-1 medium complete is used in the analysis.**
C	2, 31	☐	**2.5.2 A-1 medium without salicin is used in the analysis. 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.**
C	5	☐	**~~1.~~ 2.5.3 A-1 medium sterilized for 10 minutes at 121°C.**
C	2	☐	**2.5.4 The media productivity controls used are properly diluted and appropriate for use with A-1 medium. The results are recorded and the results maintained.** **Positive productivity control _____ Negative productivity control _____**
C	9	☐	**~~2.~~ 2.5.5 Sample and dilutions of sample are shaken ~~mixed~~ vigorously (25 times in a 12" arc in 7 seconds) before inoculation.**
C	9	☐	**~~3.~~ 2.5.6 In a multiple dilution series not less than 3 tubes per dilution are used (5 tubes are recommended).**
C	6	☐	**~~4.~~ 2.5.7 In a single dilution series at least 12 tubes are used.**
~~K~~C	6	☐	**~~5.~~ 2.5.8 In a single dilution series, the volumes analyzed ~~examined~~ are adequate to meet the needs of routine monitoring.** **Sample volume inoculated _____** **Range of MPN _____** **Strength of media used _____**
~~K~~C	2	☐	~~6. Positive and negative control cultures accompany samples throughout the procedure. Records are maintained.~~ **2.5.9 Appropriately diluted process control cultures accompany the samples *throughout both resuscitation and waterbath incubation* Results are recorded and the records maintained.** **Positive process control _____ Negative process control _____**
C	2,5	☐	**~~7.~~ 2.5.10 Inoculated ~~media~~ tubes are placed in an air incubator at 35 ± 0.5°C for 3 ± 0.5 hours of resuscitation.**
C	5	☐	**~~8.~~ 2.5.11 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	☐	**~~9.~~ 2.5.12 The presence of turbidity and any amount of gas or effervescence in the culture tube constitutes a positive test.**
		2.6 Computation of Results - MPN	
K	9	☐	~~1.~~ 2.6.1 Results of multiple dilution tests are read from tables in *Recommended Procedures for the Examination of Sea Water and Shellfish*, 4th Edition.
K	7	☐	~~2.~~ 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".
~~K~~ C	7, 9	☐	**~~3.~~ 2.6.3 Results are reported as MPN/100 mL of sample.**
		2.7 Bacteriological ~~Examination~~ Analysis of Seawater by Membrane Filtration	

			(MF) using mTEC Agar -Materials and Equipment
C	**23, 24**	☐	**1. 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 ± 0.5°C under any loading capacity.**
C	**23**	☐	**2. 2.7.2 When using a waterbath for elevated temperature incubation, the level of the water completely covers the plates.**
C	**23**	☐	**3. 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**	☐	**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	☐	4. 2.7.5 Colonies are counted with the aid of magnification.
C	**11, 23**	☐	**5. 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 µm and certified by the manufacturer for fecal coliform analyses.**
O C	**2**	☐	**6. 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**	☐	**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	☐	7. 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**	☐	**8. 2.7.10 The sterility of each lot or autoclave batch of membrane filters are checked before use.**
K	2	☐	9. 2.7.11 Membrane filters which are beyond their expiration date are not used.
O	11	☐	10. 2.7.12 Forceps tips are clean.
O	11	☐	11. 2.7.13 Forceps tips are smooth without pitting or corrugations to damage the filters being manipulated.
K	11	☐	12. 2.7.14 Forceps are dipped in alcohol and flame sterilized between sample filters.
K	11	☐	13. 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	☐	14. 2.7.16 Membrane filtration units are made of stainless steel, glass or autoclavable plastic free of scratches, corrosion and leaks.
C	**11**	☐	**15. 2.7.17 Membrane filter assemblies are autoclave sterilized for 15 minutes at 121°C prior to the start of a filtration series.**
O	11, 23, 26	☐	16. 2.7.18 A UV sterilization unit is used to disinfect filter assemblies between sample and filtration runs.
K	11	☐	17. 2.7.19 ~~If used,~~ The effectiveness of the UV sterilization unit is determined by biological testing monthly. Results are recorded and records maintained.
K	2	☐	2.7.20 Maintenance of the UV sterilization unit is performed as needed. This maintenance is documented and the records maintained.
			2.8 Media Preparation and Storage – MF using mTEC Agar
K	11	☐	1. 2.8.1 Phosphate buffered saline is used as the sample diluent and filter funnel rinse.
C	**11**	☐	**2. 2.8.2 The phosphate buffered saline is properly sterilized.**
K	23	☐	3. 2.8.3 A sufficient amount of medium (4-5 mL) is used in each plate.
O	11	☐	4. 2.8.4 Refrigerated prepared plates are stored for no more than 2 weeks in sealed plastic bags or containers to minimize evaporation.
			2.9 Sample Analyses -MF using mTEC Agar
C	**24**	☐	**1. 2.9.1 mTEC agar is used.**

C	2	☐	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. Positive productivity control _____ Negative productivity control _____
C	23	☐	2. 2.9.3 **The sample is ~~mixed~~ shaken vigorously (25 times in a 12″ arc in 7 seconds) before filtration.**
C	23	☐	3. 2.9.4 **The membrane is placed grid side up within the sterile filter apparatus.**
C	23, 25	☐	4. 2.9.5 **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	☐	5. 2.9.6 **Sample volumes are filtered under vacuum.**
K	26	☐	6. 2.9.7 The pressure of the vacuum pump does not exceed 15 psi.
C	23, 26	☐	7. 2.9.8 **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	☐	8. 2.9.9 **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	☐	9. 2.9.10 **Blanks are run at the beginning of filtration, after every 10th 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).**
~~K~~C	2, 11	☐	10. Positive and negative control cultures accompany samples throughout the procedure. Records are maintained. 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. **Positive process control _____ Negative process control _____**
C	11, 23, 24	☐	11. 2.9.12 ~~Innoculated 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.~~ 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 .
C	11, 23, 24	☐	12. 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.** ~~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.~~
		2.10 **Computation of Results- MF using mTEC Agar**	
C	23	☐	1. 2.10.1 **All yellow, yellow-green or yellow-brown colonies are counted.**
C	23	☐	2. 2.10.2 **Only plates having 80 or fewer colonies are counted. If it is unavoidable ~~necessary~~ to use plates having more than 80 colonies, counts are given as >80 x 100/the volume of sample filtered.**
C	2, 11, 23	☐	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.
K	23, 11	☐	3. 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.
~~K~~C	23, 11	☐	~~4.~~ **2.10.5 Results are reported as CFU/100 mL of sample.**
PART III - SHELLFISH SAMPLES			
		3.1 Collection and Transportation of Samples	
C	9	☐	~~1.~~ **3.1.1 A representative sample of shellstock is collected.**
K	9	☐	~~2.~~ 3.1.2 Shellstock samples are ~~is~~ collected in clean, waterproof, puncture resistant containers loosely sealed .
K	9	☐	~~3.~~ 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 ~~market sample~~) of collection.
C	9	☐	~~4. Shellstock samples are maintained in dry storage between 0 and 10° C until examined.~~ **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	☐	~~5.~~ **3.1.5 ~~Examination~~ Analysis of the sample~~s~~ 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.**
		3.2 Preparation of Shellfish for Examination	
K	2,11	☐	~~1.~~ 3.2.1 Shucking knives, scrub brushes and blender jars are (autoclave) sterilized for 15 minutes prior to use.
O	2	☐	~~2.~~ 3.2.2 Blades of shucking knives are not corroded.
O	9	☐	~~3. Prior to scrubbing and rinsing debris off shellstock , the hands of the analyst are thoroughly washed with soap and water.~~ 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	☐	~~4.~~ 3.2.4 The faucet used ~~to provide the potable water~~ for rinsing the shellstock does not contain an aerator.
K	9	☐	~~5.~~ 3.2.5 Shellstock are scrubbed with a stiff, sterile brush and rinsed under tap water of drinking water quality.
C	2	☐	**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.**
O	9	☐	~~6.~~ 3.2.7 Shellstock are allowed to drain in a clean container or on clean towels prior to opening.
K	9	☐	~~7.~~ 3.2.8 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	☐	~~8.~~ **3.2.9 Shellstock are not shucked directly through the hinge.**
C	9	☐	~~9.~~ **3.2.10 Contents of shellstock (liquor and meat) are shucked into a sterile, tared blender jar or other sterile container.**
K	9	☐	~~10.~~ 3.2.11 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	☐	3.2.12 A representative sample of at least 12 shellfish is used for the analysis.
K	2, 19	☐	~~11.~~ 3.2.13 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	☐	1~~2.~~ 3.2.14 Sterile phosphate buffered dilution water is used as the sample diluent.
~~K~~	~~3~~	☐	~~13. Sterile phosphate buffered saline is used as a sample diluent for the ETCP procedure.~~ Moved to ETCP section
C	9	☐	~~14.~~ **3.2.15 Samples are blended at high speed for 60 to 120 seconds until homogenous.**
K	9	☐	~~15. For other shellstock, APHA *Recommended Procedures* are followed for the examination of freshly shucked and frozen shellfish meats.~~

			3.2.16 APHA *Recommended Procedures for the Examination of Sea Water And Shellfish*, 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**	☐	~~1.~~ **3.3.1 Appropriate strength lactose or lauryl tryptose broth is used as presumptive media in the analysis.** ~~*(circle appropriate choice)*~~ ***(Circle the medium used.)***
C	**2**	☐	**3.3.2 The media productivity controls utilized are properly diluted and appropriate for the presumptive medium being used. The results are recorded and the records maintained.** **Positive productivity control _____ Negative productivity control _____**
K	9	☐	~~2.~~ 3.3.3 Immediately (within 2 minutes) after blending, the ground sample is diluted and inoculated into tubes of presumptive media.
C	**9**	☐	~~3.~~ **3.3.4 No fewer than 5 tubes per dilution are used in a multiple dilution MPN series.**
C	**9**	☐	~~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). All successive dilutions are prepared conventionally.**
K	6	☐	~~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**	☐	~~6. Positive and negative control cultures accompany samples throughout the procedure. Records are maintained.~~ **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	☐	~~7.~~ 3.3.8 Inoculated media are incubated at 35 ± 0.5°C.
K	10	☐	~~8. Presumptive tubes are read at 24 ± 2 hours of incubation and transferred if positive.~~ 3.3.9 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.
		3.4 Confirmed Test for Fecal Coliforms - APHA	
C	**9**	☐	~~1.~~ **3.4.1 EC medium is used as the confirmatory medium.**
C	**2**	☐	**3.4.2 The media productivity controls utilized are properly diluted and appropriate for use with EC medium. The results are recorded and the records maintained.** **Positive productivity control _____ Negative productivity control _____**
K	9, 11	☐	~~2.~~ 3.4.3 Transfers are made to EC medium by either sterile loop or hardwood sterile ~~applicator~~ transfer sticks from positive presumptives ~~incubated for 24 hours~~. *(Circle the method of transfer.)*
C	**9**	☐	~~3.~~ **3.4.4 EC tubes are incubated in a circulating waterbath at 44.5 ± 0.2°C** ~~for 24 ± 2 hours.~~
K	9	☐	~~4.~~ 3.4.5 EC tubes are read for gas production after 24 ± 2 hours of incubation.
C	**9**	☐	~~5.~~ **3.4.6 The presence of turbidity and any amount of gas and/or effervescence in**

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

C	**2,3**	☐	~~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.~~ **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.**
K	3	☐	~~6.~~ 3.8.9 Sixty (60) mL of tempered, twice boiled double strength Modified MacConkey Agar is added.
K	2,3, 22	☐	~~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**	☐	~~8.~~ **3.8.11 Media and diluent sterility are determined with each use. Results are recorded and the records maintained.**
C	**1**	☐	~~9. To determine media productivity, positive and negative control cultures are pour plated in an appropriate concentration to accompany samples throughout the procedure.~~ **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.** **Positive control culture _____ Negative control culture _____**
C	**3, 13**	☐	~~10. Plates are incubated inverted within 3 hours of plating in air at 45.5 ± 0.5° C for 18 to 30 hours. Plates are stacked not more than four high.~~ **3.8.13 When solidified the plates are placed inverted into an air incubator at 45.5 ± 0.5°C for 18 to 30 hours of incubation.**
C	**2**	☐	**3.8.14 Plates are stacked no more than three high in the incubator.**
C	**2**	☐	**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.** **Positive process control_____Negative process control_____**
C	**3**		~~11.~~ **3.8.16 Incubator temperature is maintained at 45.5 ± 0.5°C.**
		3.9 Computation ~~Expression~~ of Results - ETCP	
K	11	☐	~~1.~~ 3.9.1 Quebec Colony counter or its equivalent is used to provide the necessary magnification and visibility for counting.
O	1	☐	~~2.~~ 3.9.2 A hand tally or its equivalent is used to aid in counting.
C	**3, 6**	☐	~~3.~~ **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~~ **.**
C	**3**	☐	**3.9.4 Results are reported as CFU/100 grams of sample.**
		Bacteriological Examination of Soft-shelled Clams and American Oysters for Male Specific Coliphage (MSC)	
		3.10 MSC Equipment and Supplies	
K	30	☐	~~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**	☐	~~2.~~ **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.**
C	**27, 28**	☐	~~3.~~ **3.10.3 The tempering bath(s) must be able to maintain the temperature within 2°C of the set temperature.**
K	9	☐	~~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**	☐	~~5.~~ **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.**

K	1	☐	~~6.~~ 3.10.6 The sterility of each lot of pre-sterilized syringes and syringe filters is determined. Results are recorded and records maintained.
K	1	☐	~~7.~~ 3.10.7 The sterility of each batch of reusable glass syringes is determined. Results are recorded and records maintained.
C	**27, 28**	☐	**~~8.~~ 3.10.8 The balance used provides a sensitivity of at least 10 mg.**
C	**27, 28**	☐	**~~9.~~ 3.10.9 The temperature of the incubator used is maintained between 35 – 37°C.**
C	**28**	☐	**~~10.~~ 3.10.10 Sterile disposable 50 mL centrifuge tubes are used and their sterility is determined with each lot. Results are recorded and records maintained.**
		3.11 MSC Media Preparation	
K	28	☐	~~1.~~ 3.11.1 Media preparation and sterilization is according to the validated method.
K	27, 28	☐	~~2.~~ 3.11.2 Bottom agar, double strength soft agar and growth broth are prepared from their individual components.
K	27, 28	☐	~~3.~~ 3.11.3 Soft agar is prepared double strength in volumes of 2.5 mL.
C	**27, 28**	☐	**~~4.~~ 3.11.4 The streptomycin and ampicillin solutions are added to tempered bottom agar.**
O	27, 28	☐	~~5.~~ 3.11.5 Storage of the bottom agar under refrigeration does not exceed 1 month.
K	27, 28	☐	~~6.~~ 3.11.6 Unsterilized soft agar is stored at -20°C for up to 3 months.
K	27, 28	☐	~~7.~~ 3.11.7 The soft agar is removed from the freezer and sterilized for 15 minutes at 121°C before use.
K	27, 28	☐	~~8.~~ 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	☐	~~9.~~ 3.11.9 Bottom agar plates are allowed to reach room temperature before use.
		3.12 Preparation of the Soft-Shelled Clams and American Oysters for MSC Analysis	
K	2,11	☐	~~1.~~ 3.12.1 Shucking knives, scrub brushes and blender jars are autoclave sterilized for 15 minutes prior to use.
O	2	☐	~~2.~~ 3.12.2 The blades of shucking knives are not corroded.
O	9	☐	~~3.~~ 3.12.3 The hands of the analyst are thoroughly washed with soap and water immediately prior to ~~scrubbing and rinsing~~ cleaning the shells of debris ~~off the shellfish~~.
O	2	☐	~~4.~~ 3.12.4 The faucet used for rinsing the shellfish does not contain an aerator.
K	9	☐	~~5.~~ 3.12.5 The shellfish are scrubbed with a stiff, sterile brush and rinsed under tap water of drinking water quality.
C	**2**	☐	**3.12.6 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.**
O	9	☐	~~6.~~ 3.12.7 The shellfish are allowed to drain in a clean container or on clean towels unlayered prior to shucking.
K	9	☐	~~7.~~ 3.12.8 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**	☐	**~~8.~~ 3.12.9 Shellfish are not shucked through the hinge.**
C	**9**	☐	**~~9.~~ 3.12.10 The contents of shellfish (liquor and meat) are shucked into a sterile, tared blender jar or other sterile container.**
K	9	☐	~~10.~~ 3.12.11 A representative sample of at least 12 shellfish is used for the analysis.
~~C~~ K	2, 19	☐	~~11.~~ 3.12.12 The sample is weighed to the nearest 0.1 gram.
		3.13 MSC Sample Analysis	
C	**28**	☐	**~~1.~~ 3.13.1 *E.coli Famp* ATCC 700891 is the bacterial host strain used in this procedure.**
K	27, 28	☐	~~2.~~ 3.13.2 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	☐	~~3.~~ 3.13.3 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	☐	~~4.~~ 3.13.4 **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	☐	~~5.~~ 3.13.5 **After inoculation, the host cell growth broth culture is not shaken.**
C	28	☐	~~6.~~ 3.13.6 **A 2:1 mixture of growth broth to shellfish tissue is used for eluting the MSC.**
C	28	☐	~~7.~~ 3.13.7 **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	☐	~~8.~~ 3.13.8 **The elution mixture is homogenized at high speed for 180 seconds.**
C	28	☐	~~9.~~ 3.13.9 **Immediately after blending, 33 grams of the homogenized elution mixture are weighed into centrifuge tubes.**
C	28	☐	~~10.~~ 3.13.10 **The homogenized elution mixture is centrifuged for 15 minutes at 9000 x g at 4°C.**
C	27, 28	☐	~~11.~~ 3.13.11 **The supernatant is pipetted off, weighed and the weight recorded.**
C	27, 28	☐	~~12.~~ 3.13.12 **The supernatant is allowed to warm to room temperature about 20 to 30 minutes.**
K	27, 28	☐	~~13.~~ 3.13.13 The autoclaved soft agar is tempered and held at 50 – 52°C throughout the period of sample analysis.
K	27, 28	☐	~~14.~~ 3.13.14 Two hundred microliters (0.2 mL) of log phase host strain *E coli* is added to the tempering soft agar immediately prior to adding the sample supernatant.
K	27, 28	☐	~~15.~~ 3.13.15 The sample supernatant is shaken or vortexed before being added to the tempering soft agar.
C	27, 28	☐	~~16.~~ 3.13.16 **2.5 mL of sample supernatant is added to each tube of tempering soft agar.**
C	27, 28	☐	~~17.~~ 3.13.17 **The soft agar/sample supernatant/host cell mixture is gently rolled between the palms of the hands to mix.**
C	27, 28	☐	~~18.~~ 3.13.18 **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	☐	~~19.~~ 3.13.19 **Ten (10) plates are used, 2.5 mL per plate for a total of 25 mL of supernatant analyzed per sample.**
K	27, 28	☐	~~20.~~ 3.13.20 Negative and positive control plates are prepared and accompany each set of samples analyzed. The results are recorded and records maintained.
K	27, 28	☐	~~21.~~ 3.13.21 Growth broth is used as the negative control or blank.
K	27, 28	☐	~~22.~~ 3.13.22 Type strain MS2 (ATCC 15597) male specific bacteriophage appropriately diluted to provide countable low levels of phage is used as the positive control.
K		☐	~~23.~~ 3.13.23 A negative control plate is plated at the beginning and end of each set of samples analyzed.
K	27, 28	☐	~~24.~~ 3.13.24 The positive control is plated after all the samples are ~~analyzed~~ inoculated and immediately prior to the final negative control.
C	27, 28	☐	~~25.~~ 3.13.25 **All plates are incubated at 35 – 37°C for 16 to 20 hours.**
		3.14 Computation of Results - MSC	
C	27	☐	~~1.~~ 3.14.1 **Circular zones of clearing or plaques of any diameter in the lawn of host bacteria are counted.**
C	28	☐	~~2.~~ 3.14.2 **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	☐	~~3.~~ 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	☐	~~4.~~ 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

Page	Item	Observation	Documentation Required

LABORATORY STATUS	
LABORATORY	**DATE**
LABORATORY REPRESENTATIVE:	
MICROBIOLOGICAL COMPONENT: (Part I-III)	
A. Results 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. Criteria for Determining Laboratory Status of the Microbiological Component:

1. **Does Not Conform Status**: The Microbiological component of this laboratory is not in conformity with NSSP requirements if:

   a. The total # of Critical nonconformities is **≥ 4 or**

   b. The total # of Key nonconformities is **≥ 13 or**

   c. The total # of Critical, Key and Other is **≥ 18**

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**

C. Laboratory Status (*circle appropriate*)

Does Not Conform Provisionally Conforms Conforms

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. 2010-11-08