

Laboratory Evaluation Checklist - Microbiology

**PUBLIC HEALTH SERVICE
U.S. FOOD AND DRUG ADMINISTRATION
SHELLFISH PROGRAM IMPLEMENTATION BRANCH
SHELLFISH SAFETY TEAM
5100 PAINT BRANCH PARKWAY
COLLEGE PARK, MD 20740-3835
TEL. 301-436-2151/2147 FAX 301-436-2672**

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]
PART 1 - QUALITY ASSURANCE			
CODE	REF		ITEM
			Quality Assurance Plan
K	8,11	√	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 calibration, maintenance, repair and for performance checks. e. Laboratory safety f. Internal performance assessment g. External performance assessment
C	State's Human Resources Department		2. In state laboratories, the supervisor meets the state educational and experience requirements for managing a public health laboratory.
K	State's Human Resources Department		3. In state laboratories, the analyst(s) meets the state educational and experience requirements for processing samples in a public health laboratory.
C	USDA Microbiology & EELAP		4. In private 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. In private laboratories, the analyst(s) must have at least a high school diploma and shall have at least three months of experience in laboratory sciences.
C	8		6. QA Plan Implemented
K	11		7. Participates in a proficiency testing program annually. Specify Program(s) _____
			Work Area
O	8, 11		1. Adequate for workload and storage.
K	11		2. Clean, well lighted.
K	11		3. Adequate temperature control.
O	11		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.
O	11		6. Pipette aid used, mouth pipetting not permitted.
			Equipment
O	9		1. To determine the pH of prepared media, the pH meter has a standard accuracy of 0.1 units.
O	14		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		3. The effect of temperature on the pH is compensated for by an ATC probe or by manual adjustment.

K	8		4.	pH meter is calibrated daily or with each use and records are maintained.
K	11		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 _____.
K	9		7.	Balance provides a sensitivity of at least 0.1 g at a load of 150 g.
K	11,13		8.	Balance checked monthly using NIST Class S or ASTM Class 1 or 2 weights or equivalent and records are maintained.
K	11		9.	Refrigerator temperature(s) monitored at least once daily and recorded.
K	1		10.	Refrigerator temperature maintained at 0° to 4° C.
C	9		11.	The temperature of the incubator is maintained at 35 ± 0.5°C.
C	11		12.	Thermometers used in the air incubator(s) are graduated at no greater than 0.5°C increments.
K	9		13.	Working thermometer located on top and bottom shelves of use in the air incubator(s).
C	11		14.	Temperature of the waterbath is maintained at 44.5 ± 0.2°C under any loading capacity.
C	9		15.	The thermometers used in the waterbath are graduated in 0.1°C increments.
O	13		16.	The waterbath has adequate capacity for workload.
K	9		17.	The level of water in the waterbath covers the level of liquid in the incubating tubes.
K	8, 11		18.	Air incubator/waterbath temperatures are taken twice daily and recorded.
K	13		19.	Working thermometers are tagged with identification, date of calibration, calibrated temperature and correction factor.
K	4		20.	All working thermometers are appropriately immersed.
K	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.
K	9		22.	Standards thermometer is checked annually for accuracy by ice point determination. Results recorded and maintained. Date of most recent determination _____.
K	13		23.	Incubator and waterbath working thermometers are checked annually against the standards thermometer at the temperatures at which they are used. Records maintained.
Labware and Glassware Washing				
O	9		1.	Utensils and containers are clean borosilicate glass, stainless steel or other noncorroding materials
K	9		2.	Culture tubes are of a suitable size to accommodate the volume for nutritive ingredients and samples
K	9		3.	Sample containers are made of glass or some other inert material (i.e. polypropylene).
O	9		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.	Graduations are indelibly marked on dilution bottles and tubes or an acceptable alternative method is used to ensure appropriate volumes.
K	9		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; nor, are pipets larger than 1ml used to deliver 0.1ml.
K	9		7.	Reusable sample containers are capable of being properly washed and sterilized.
K	9		8.	In washing reusable pipets, 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 or when detergent (brand or lot) is changed by the Inhibitory Residue Test as described in the current edition of <u>Standard Methods for the Examination of Water and Wastewater</u>. 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 # _____
K	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.
			Sterilization and Decontamination
O	9	1.	Autoclave(s) are of sufficient size to accommodate the workload.
O	8	2.	Routine autoclave maintenance performed (e.g. pressure relief valves, exhaust trap, chamber drain) and records maintained.
O	8	3.	Autoclave(s) and/or steam generators serviced annually or as needed by qualified technician and records maintained.
C	11	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).
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.
K	16	6.	The autoclave standards thermometer is checked every five years for accuracy at either 121°C or at the steam point. Date of most recent determination _____
K	1	7.	Working autoclave thermometers are checked against the autoclave standards thermometer at 121° C yearly. Date of last check _____ Method _____
K	11	8.	Spore suspensions are used monthly to evaluate the effectiveness of the autoclave sterilization process. Results recorded.
O	11	9.	Heat sensitive tape is used with each autoclave batch.
K	11, 13	10.	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.	For dry heat sterilized material, the hot-air sterilizing oven provides heating and sterilizing temperature in the range of 160° to 180° C.
K	9	12.	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.	Records of temperatures and exposure times are maintained for the operation of the hot-air sterilizing oven during use.
K	11	14.	Spore strips are used quarterly to evaluate the effectiveness of the sterilization process in the hot-air oven. Records are maintained.
K	11	15.	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	1	16.	The sterility of reusable/disposable sample containers is determined for each batch/lot.
K	9	17.	Reusable pipettes are stored and sterilized in aluminum or stainless steel canisters or equivalent alternative.
K	9	18.	Reusable pipettes (in canisters) are sterilized in a hot-air oven at 170° C for 2 hours.
O	2	19.	The sterility of reusable/disposable pipettes is determined with each batch/lot. Results are recorded and maintained.

K	18		20.	Hardwood applicators transfer sticks are properly sterilized.
O	13		21.	Spent broth cultures and agar plates are decontaminated by autoclaving for at least 30 minutes before conventional disposal.
Media Preparation				
K	3, 5		1.	Media is commercially dehydrated except in the case of medium A-1 which is prepared from the individual components and modified MacConkey agar which may be prepared from its components.
O	11		2.	Dehydrated media and media components properly stored in cool, clean, dry place.
O	11		3.	Dehydrated media are labeled with date of receipt and date opened.
C	12		4.	Caked or expired media are discarded.
C	11		5.	Make-up water is distilled or deionized (<i>circle one</i>) and exceeds 0.5 megohm resistance or is less than 2μ Siemens/cm conductivity at 25° C to be tested and recorded monthly for resistance or conductivity (<i>circle the appropriate</i>).
C	11		6.	Make-up water is analyzed for residual chlorine monthly and is at a non-detectable level (\leq 0.1 ppm). Records are maintained. Specify method of determination
K	11		7.	Make-up water is free from trace (<0.05 mg/L) dissolved metals, specifically Cd, Cr, Cu, Ni, Pb, and Zn as determined annually with total heavy metal content < or equal to 1.0 mg/L and records are maintained.
K	11		8.	Make-up water contains <1000 CFU/ml as determined monthly using the heterotrophic plate count method and records are maintained.
K	11		9.	Media are sterilized according to the manufacturer's instructions.
K	9		10.	Volume and concentration of media in the tube are suitable for the amount of sample inoculated.
C	11		11.	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.
O	9		13.	buffered dilution water is used as the sample diluent.
K	11		14.	pH is determined after sterilization to ensure that it is consistent with manufacturer's requirements and records are maintained.
Storage of Prepared Culture Media				
O	9		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.	Brilliant green bile 2% broth and A-1 media are stored in the dark.
K	13		3.	Stored media are labeled with expiration date or sterilization date.
O	9		4.	Storage of prepared culture media at room temperature does not exceed 7 days.
O	2		5.	Storage under refrigeration of prepared media with loose fitting closures shall not exceed 1 month.
O	11		6.	Storage under refrigeration of prepared media with screw-cap closures does not exceed 3 months.
K	17		7.	All prepared media 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				
CODE	REF	ITEM		
Collection and Transportation of Samples				
C	11		1.	Containers are of suitable size to contain at least 100 ml and to allow headspace for shaking. Seawater samples are collected in clean, sterile, water tight, properly labeled sample containers.
K	1		2.	Sample identified with collectors name, harvest area, 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.
K	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.
Bacteriological Examination of Seawater by the APHA MPN			
C	9		1. Lactose broth or lauryl tryptose broth is used as the presumptive medium. (<i>circle appropriate one</i>)
C	9		2. Sample and dilutions of sample are mixed vigorously (25 times in a 12" arc in 7 seconds) before inoculation.
C	9		3. In a multiple dilution series not less than 3 tubes per dilution are used (5 tubes are recommended).
C	6		4. In a single dilution series not less than 12 tubes are used (for depuration at least 5 tubes are used).
K	6		5. 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 _____
K	9		6. Inoculated media are placed in an air incubator at 35 ± 0.5° C for up to 48 ± 3 hours.
K	2		7. Positive and negative control cultures accompany samples throughout the procedure. Records are maintained. Positive Control _____ Negative 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.
Confirmed Test for Seawater by APHA MPN			
C	9		1. Brilliant green bile 2% broth (BGB) is used as the confirmatory medium for total coliforms.
C	9		2. EC medium is used as the confirmatory medium for fecal coliforms.
K	9, 11		3. Transfers made to BGB/EC by either sterile loop or sterile hardwood applicator stick from positive presumptives incubated for 24 and 48 hours (<i>Circle the method of transfer</i>).
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. BGB tubes are incubated at 35 ± 0.5°C.
K	9		6. BGB tubes are read after 48 ± 3 hours of incubation.
C	9		7. EC tubes are incubated in a circulating waterbath at 44.5 ± 0.2° C for 24 ± 2 hours.
C	9		8. The presence of any amount of gas or effervescence in the culture tube constitutes a positive test.
Computation of Results			
K	9		1. Results of multiple dilution tests are read from tables in <i>Recommended Procedures</i> , 4 th Edition.
K	7		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	7, 9		3. Results are reported as MPN/100 ml of sample.
Bacteriological Examination of Seawater by the MA-1 Method			
C	5		1. Medium A-1 sterilized for 10 minutes at 121°C.
C	9		2. Sample and dilutions of sample are mixed vigorously (25 times in a 12" arc in 7 seconds) before inoculation.
C	9		3. In a multiple dilution series not less than 3 tubes per dilution are used (5 tubes are recommended).

C	6		4.	In a single dilution series at least 12 tubes are used.
K	6		5.	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 _____
K	2		6.	Positive and negative control cultures accompany samples throughout the procedure. Records are maintained. Positive Control _____ Negative Control _____
C	2,5		7.	Inoculated media are placed in an air incubator at $35 \pm 0.5^\circ\text{C}$ for 3 ± 0.5 hours of resuscitation.
C	5		8.	After 3 ± 0.5 hours resuscitation at 35°C, inoculated media are incubated at $44.5 \pm 0.2^\circ\text{C}$ in a circulating waterbath for the remainder of the 24 ± 2 hours.
C	5		9.	The presence of any amount of gas or effervescence in the culture tube constitutes a positive test.
Computation of Results				
K	9		1.	Results of multiple dilution tests are read from tables in <i>Recommended Procedures</i> , 4 th Edition.
K	7		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	7, 9		3.	Results are reported as MPN/100 ml of sample.
PART III - SHELLFISH SAMPLES				
CODE	REF	ITEM		
Collection and Transportation of Samples				
C	9		1.	A representative sample of shellstock is collected.
K	9		2.	Shellstock is collected in clean, waterproof, puncture resistant containers.
K	9		3.	Shellstock labeled with collector's name, type of shellstock, the source, the harvest area, time, date and place (if market sample) of collection.
C	9		4.	Shellstock samples are maintained in dry storage between 0 and 10°C until examined.
C	1		5.	Examination of the sample is initiated as soon as possible after collection. However, shellfish samples are not examined if the time interval between collection and examination exceeds 24 hours.
Preparation of Shellstock for Examination				
K	2,11		1.	Shucking knives, scrub brushes and blender jars are (autoclave) sterilized for 15 minutes prior to use.
O	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.
O	2		4.	The faucet used to provide the potable water for rinsing the shellstock does not contain an aerator.
K	9		5.	Shellstock are scrubbed with a stiff, sterile brush and rinsed under water of drinking water quality.
O	9		6.	Shellstock are allowed to drain in a clean container or on clean towels prior to opening.
K	9		7.	Prior to opening, the hands (or gloved hands) of the analyst are thoroughly washed with soap and water and rinsed in 70% alcohol.
K	9		8.	Shellstock are not shucked directly through the hinge.
C	9		9.	Contents of shellstock (liquor and meat) are shucked into a sterile, tared blender jar or other sterile container.
K	9		10.	At least 200 grams of shellfish meat is used for analysis.
K	2, 19		11.	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		12.	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.
C	9		14.	Samples are blended at high speed for 60 to 120 seconds.
K	9		15.	For other shellstock, APHA <i>Recommended Procedures</i> are followed for the examination of freshly shucked and frozen shellfish meats.
Bacteriological Examination of Seawater by Membrane Filtration using mTEC Agar Equipment				
C	23, 24		1.	When used for elevated temperature incubation, the temperature of the hot air incubator is maintained at 44.5+0.5°C under any loading capacity.
C	23		2.	When using a waterbath for elevated temperature incubation, the level of the water completely covers the plates.
C	23		3.	Pre-sterilized plastic or sterile glass culture plates that are clear, flat bottomed, free of bubbles and scratches are used.
K	11		4.	Colonies are counted with the aid of magnification.
C	11, 23		5.	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	2		6.	Lot number, date of receipt and if provided the expiration date of the membrane filters are recorded.
K	2, 11		7.	New lots of membrane filters are checked by comparing recovery of fecal coliform organisms against membrane filters from previously acceptable lots.
	2		8.	The sterility of each lot or autoclave batch of membrane filters are checked before use.
K	2		9.	Membrane filters which are beyond their expiration date are not used.
O	11		10.	Forceps tips are clean.
O	11		11.	Forceps tips are smooth without pitting or corrugations to damage the filters being manipulated.
K	11		12.	Forceps are dipped in alcohol and flame sterilized between sample filters.
K	11		13.	If indelible graduation marks are used on clear glass or plastic funnels to measure sample volumes, their accuracy is checked 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.	Membrane filtration units are made of stainless steel, glass or autoclavable plastic free of scratches, corrosion and leaks.
C	11		15.	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.	A UV sterilization unit is used to disinfect filter assemblies between sample and filtration runs.
K	11		17.	If used, the effectiveness of the UV sterilization unit is determined by biological testing monthly. Results are recorded and records maintained.
Media Preparation and Storage				
K	11		1.	Phosphate buffered saline is used as the sample diluent.
C	11		2.	Phosphate buffered saline is properly sterilized.
K	23		3.	A sufficient amount of medium (4-5 ml) is used in each plate.
O	11		4.	Refrigerated prepared plates are stored for no more than 2 weeks in sealed plastic bags or containers to minimize evaporation.
Sample Analysis				
C	24		1.	mTEC agar is used.
C	23		2.	The sample is mixed vigorously (25 times in a 12" arc in 7 seconds) before filtration.
C	23		3.	The membrane is placed grid side up within the sterile filter apparatus.
C	23, 25		4.	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.	Sample volumes are filtered under vacuum.

K	26		6.	The pressure of the vacuum pump does not exceed 15 psi.
C	23, 26		7.	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.	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.	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	2, 11		10.	Positive and negative control cultures treated like samples accompany test samples throughout the procedure. Positive control _____ Negative control _____ Results are recorded and records maintained.
C	11, 23, 24		11.	Inoculated plates are placed inverted either 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.
C	11, 23, 24		12.	After 2 hours of resuscitation at 35°C watertight 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.
Computation of Results				
C	23		1.	All yellow, yellow-green or yellow-brown colonies are counted.
C	23		2.	Only plates having 80 or fewer colonies are counted. If it is necessary to use plates having more than 80 colonies, counts are given as >80 x 100/the volume filtered.
K	23, 11		3.	The number of fecal coliforms is calculated by the following equation: Number of fecal coliforms per 100 ml =[number of colonies counted/volume of sample filtered in ml] x 100.
K	23, 11		4.	Results are reported as CFU/100 ml of sample.
MPN Analysis for Fecal Coliform Organisms, Presumptive Test, APHA				
C	9		1.	Appropriate strength lactose or lauryl tryptose broth is used as presumptive media in the analysis. (circle appropriate choice)
K	9		2.	Immediately (within 2 minutes) after blending, the ground sample is diluted and inoculated into tubes of presumptive media.
C	9		3.	No fewer than 5 tubes per dilution are used in a multiple dilution MPN series.
C	9		4.	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).
K	6		5.	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. Positive Control _____ Negative Control _____
K	9		7.	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.

			Confirmed Test for Fecal Coliforms - APHA
C	9		1. EC medium is used as the confirmatory medium.
K	9, 11		2. Transfers are made to EC medium by either sterile loop or hardwood sterile applicator sticks from positive presumptives incubated for 24 hours (<i>circle the method of transfer</i>).
C	9		3. EC tubes are incubated in a circulating waterbath at 44.5 ± 0.2°C for 24 ± 2 hours.
K	9		4. EC tubes are read for gas production after 24 ± 2 hours of incubation.
C	9		5. The presence of any amount of gas or effervescence in the Durham tube constitutes a positive test.
			Computation of Results for MPN Analyses
K	9		1. Results of multiple dilution tests are read from tables in <i>Recommended Procedures</i> , 4 th Edition and multiplied by the appropriate dilution factor.
K	7		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	9		3. Results are reported as MPN/100 grams of sample.
			Standard Plate Count Method
O	20		1. A standard plate count analysis is performed in conjunction with the analysis for fecal coliform organisms.
K	9		2. In the standard plate count procedure at least four plates, duplicates of two dilutions are used to provide 30 to 300 colonies per plate.
K	2		3. Fifteen to 20 ml of tempered sterile plate count agar is used.
K	9		4. Agar tempering bath maintains the agar at 44 to 46°C.
O	9		5. Temperature control of the plate count agar is used in the tempering bath.
K	9		6. Not more than 1 ml nor less than 0.1 ml of sample or sample dilution is plated.
C	9		7. Samples or sample dilutions to be plated are mixed vigorously (25 times in a 12" arc in 7 seconds) before plating.
K	11		8. Control plates are used to check the sterility of the air, agar and the diluent.
K	9, 21		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. Quebec Colony Counter or its equivalent is used to provide the necessary magnification and visibility for counting plates.
K	1		11. A hand tally or its equivalent is used for accuracy in counting.
			Computation of Results
K	9		1. Colony counts determined in accordance with Part III, A, Sections 4.31 through 4.33 <i>Recommended Procedures</i> , 4 th Edition.
O	19		2. Colony counts reported as APC/g of sample.
			Bacteriological Examination of Shellfish Using the ETCP
K	9		1. Sample homogenate is cultured within 2 minutes of blending.
K	3		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.
K	2, 3		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.
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.
K	3		6. Sixty (60) ml of tempered, twice boiled double strength Modified MacConkey Agar is added.
K	2, 3, 22		7. The container is gently swirled or rotated to mix the contents, which are then, distributed uniformly over 6 to 8 Petri plates.
C	1		8. Media and diluent sterility are determined with each use. Results are recorded and 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. Positive control _____ Negative control _____
C	3, 13	10.	Plates are incubated inverted within 3 hours of plating in air at $45.5 \pm 0.5^{\circ}\text{C}$ for 18 to 30 hours. Plates are stacked not more than four high.
C	3	11.	Incubator temperature is maintained at $45.5 \pm 0.5^{\circ}\text{C}$.
Expression of Results			
K	11	1.	Quebec Colony counter or its equivalent is used to provide the necessary magnification and visibility.
O	1	2.	A hand tally or its equivalent is used to aid in counting.
C	3, 6	3.	All brick red colonies greater than 0.5mm 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.

REFERENCES

1.	American Public Health Association. 1984. <i>Compendium of Methods for the Microbiological Examination of Foods</i> , 2 nd Edition. APHA, Washington, D.C.
2.	Good Laboratory Practice.
3.	"Interim Guides for the Depuration of the Northern Quahog, <i>Mercenaria mercenaria</i> . 1968. Northeast Marine Health Sciences Laboratory, North Kingstown, RI.
4.	U.S. Department of Commerce. 1976. <i>NBS Monograph 150</i> . U.S. Department of Commerce, Washington, D.C.
5.	Association of Official Analytical Chemists (AOAC). 2000. <i>Official Methods of Analyses of the Association of Official Analytical Chemists</i> . 17 th Edition, Chapter 17.305, page 22. AOAC, Arlington, VA.
6.	Wilt, D.S. (ed.). 1974. <i>Proceedings of the 8th National Shellfish Sanitation Workshop</i> . U.S. Food and Drug Administration, Washington, D.C.
7.	U.S. Public Health Service (PHS). 1947. <i>Public Health Report</i> , Reprint #1621. PHS, Washington, D.C.
8.	Association of Official Analytical Chemists (AOAC). 1991. <i>Quality Assurance Principles for Analytical Laboratories</i> . AOAC, Arlington, VA.
9.	American Public Health Association (APHA). 1970. <i>Recommended Procedures for the Examination of Sea Water and Shellfish</i> , 4 th Edition. APHA, Washington, D.C.
10.	Interstate Shellfish Sanitation Conference (ISSC). 1986. <i>Shellfish Sanitation Interpretation #SS-39</i> . ISSC, Columbia, S.C.
11.	American Public Health Association (APHA). 1992. <i>Standard Methods for the Examination of Water and Wastewater</i> , 18 th Edition. APHA/AWWA/WEF, Washington, D.C.
12.	Title 21, Code of Federal Regulations, Part 58, <i>Good Laboratory Practice for Nonclinical Laboratory Study</i> . U.S. Government Printing, Washington, D.C.
13.	American Public Health Association (APHA). 1992. <i>Standard Methods for the Examination of Dairy Products</i> , 16 th Edition. APHA, Washington, D.C.
14.	Fisher, J. 1985. Measurement of pH. <i>American Laboratory</i> 16:54-60.
15.	Consult pH electrode product literature.
16.	Association of Official Analytical Chemists (AOAC). 1999. <i>AOAC Methods Validation and Technical Programs - Criteria for Laboratories Performing Food Testing</i> . AOAC, Arlington, VA.
17.	U.S. Environmental Protection Agency (EPA). 1975. <i>Handbook for Evaluating Water Bacteriological Laboratories</i> . EPA-670/9-75-006. U.S. EPA, Cincinnati, OH
18.	Adams, W.N. 1974. NETSU. Personal communication to Dr. Wallace Andrews, FDA.
19.	U.S. Food and Drug Administration (FDA). 1995. <i>Bacteriological Analytical Manual</i> . U.S. FDA, 8 th Edition, AOAC, Arlington, VA.
20.	U.S. Food and Drug Administration (FDA) and Interstate Shellfish Sanitation Conference (ISSC). 1997. <i>NSSP Guide to the Control of Molluscan Shellfish</i> . FDA/ISSC, Washington, D.C. and Columbia, S.C.
21.	U.S. Environmental Protection Agency. 1978. <i>Microbiological Methods for Monitoring the Environment, Water and Wastes</i> . EPA/600/8/78/017. EPA, Washington, D.C.
22.	Furfari, Santo. March 21, 1972. Personal Communication to Dan Hunt, FDA.
23.	United States Environmental Protection Agency, <i>Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and Escherichia coli</i> . EPA/821/R-97-004, EPA, Washington, DC
24.	Rippey, Scott, R, Adams, Willard, N, and Watkins, William, D. Enumeration of fecal coliforms and <i>E. coli</i> in marine and estuarine waters: an alternative to the APHA-MPN approach, <i>Journal WPCF</i> , 59, 8 (1987).
25.	FDA Manual of Interpretations, National Shellfish Sanitation Program <i>Guide for the Control of Molluscan Shellfish</i> , 2003 Revision, Interpretation Number 03-IV-@.02-102.
26.	<i>Membrane filtration: A Users Guide and Reference Manual</i> , Thomas D. Brock, Science Tech Inc., Madison, WI, 1983.

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: <ul style="list-style-type: none"> 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 (<i>circle appropriate</i>) <div style="display: flex; justify-content: space-around; font-weight: bold;"> Does Not Conform Provisionally Conforms Conforms </div>		
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: _____		