PUBLIC HEALTH SERVICE
U.S. FOOD AND DRUG ADMINISTRATION
OFFICE OF FOOD SAFETY
SHELLFISH AND AQUACULTURE POLICY BRANCH
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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: **Conformity it noted by a "√"**

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

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	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, performance, and rejection criteria established.
		☐		e. Laboratory safety.
		☐		f. Internal performance assessment.
		☐		g. External performance assessment.
C	**8**	☐	**1.1.2**	**QA Plan Implemented.**
K	11	☐	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**	☐	**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	☐	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**	☐	**1.2.3**	**In commercial laboratories, the supervisor must have at least a bachelor's degree or equivalent in microbiology, biology, or equivalent discipline with at least two years of laboratory experience.**
K	USDA Microbiology & EELAP	☐	1.2.4	In commercial laboratories, the analyst(s) must have at least a high school diploma and shall have at least three months of experience in laboratory sciences.
		1.3 Work Area		
O	8,11	☐	1.3.1	Adequate for workload and storage.
K	11	☐	1.3.2	Clean, well-lighted.
K	11	☐	1.3.3	Adequate temperature control.
O	11	☐	1.3.4	All work surfaces are nonporous, easily cleaned and disinfected.
K	11	☐	1.3.5	Microbiological quality of the air is fewer than 15 colonies for a 15 minute exposure and determined monthly. The results are recorded and records maintained.
		1.4 Laboratory Equipment		
O	9	☐	1.4.1	To determine the pH of prepared media, the pH meter has a standard accuracy of 0.1 units.
O	14	☐	1.4.2	pH electrodes consisting of pH half-cell and reference half-cell or equivalent combination electrode free from (Ag/AgCl) or contains an ion exchange barrier preventing passage of Ag ions into the medium which may affect the accuracy of the pH reading.
K	11	☐	1.4.3	The effect of temperature on the pH is compensated for by an ATC probe or by manual adjustment.
K	8	☐	1.4.4	pH meter is calibrated daily or with each use Results are recorded and records maintained.
K	11	☐	1.4.5	A minimum of two standard buffer solutions is used to calibrate the pH meter. The first must be near the electrode isopotential point (pH 7). The second near the expected sample pH (i.e., pH 4 or pH 10). Standard buffer solutions are used once and discarded.
O	8,15	☐	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	☐	1.4.7	Balance provides a sensitivity of at least 0.1 g at weights of use.
K	11,13	☐	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	☐	1.4.9	Refrigerator temperature(s) are monitored at least once daily on workdays Results are recorded and records maintained.
K	1	☐	1.4.10	Refrigerator temperature is maintained at 0 to 4°C.
C	**9**	☐	**1.4.11**	**The temperature of the incubator is maintained at 35 ± 0.5°C.**
C	**11**	☐	**1.4.12**	**Thermometers used in the air incubator(s) are graduated in at least 0.1°C increments.**
K	9	☐	1.4.13	Working thermometers are located on top and bottom shelves or appropriately placed based on the results of spatial temperature checks.
C	**11**	☐	**1.4.14**	**Temperature of the waterbath is maintained at 44.5 ± 0.2°C under all loading conditions.**
C	**9**	☐	**1.4.15**	**The thermometers used in the waterbath are graduated in at least 0.1°C increments.**
C	**13**	☐	**1.4.16**	**The waterbath has adequate capacity for workload.**
K	9	☐	1.4.17	The level of water in the waterbath covers the level of liquid in the incubating tubes.
K	8, 11	☐	1.4.18	Air incubator/waterbath temperatures are taken twice daily on workdays. The results are recorded and records maintained.
C	**4**	☐	**1.4.19**	**All working thermometers are appropriately immersed.**
C	**29, 33**	☐	**1.4.20**	**Working thermometers are either: ~~calibrated~~ mercury-in-glass thermometers, ~~calibrated~~ non-mercury-in-glass thermometers having the accuracy and tolerance of mercury, or ~~appropriately calibrated~~low drift electronic devices, including Resistance Temperature Devises (RTDs) and Platinum Resistance Devices (PTDs)with an accurscy of less than or equal to ≤ ±0.05°C.**
C	**11**	☐	**1.4.21**	**A mercury-in-glass standards thermometer has been calibrated by NIST or a qualified calibration laboratory using a primary standard traceable to NIST or an equivalent authority at the points 0, 35 and 44.5°C (45.5°C for ETCP). These calibration records are maintained.**
K	9	☐	1.4.22	Standards thermometers are checked annually for accuracy by ice point determination. Results recorded and maintained. Date of most recent determination_____.
C	**29**	☐	**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	☐	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	☐	1.5.1	Utensils and containers are clean borosilicate glass, stainless steel or other noncorroding materials.
K	9	☐	1.5.2	Culture tubes are of a suitable size to accommodate the volume for nutritive ingredients and samples.
K	9	☐	1.5.3	Sample containers are made of glass or some other inert material.

O	9	☐	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	☐	1.5.5	Graduations are indelibly marked on dilution bottles and tubes or an acceptable alternative method is used to ensure appropriate volumes.
C	**9**	☐	**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 1.1mL used to deliver 0.1 mL aliquots.**
K	9	☐	1.5.7	Reusable sample containers are capable of being properly washed and sterilized.
K	9	☐	1.5.8	In washing reusable pipettes, a succession of at least three fresh water rinses plus a final rinse of distilled/deionized water is used to thoroughly rinse off all the detergent.
C	**2**	☐	**1.5.9**	**An alkaline or acidic detergent is used for washing glassware/labware.**
C	**11**	☐	**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		
K	9	☐	1.6.1	Autoclave(s) are of sufficient size to accommodate the workload.
O	8	☐	1.6.2	Routine autoclave maintenance is performed and the records are maintained.
C	**11, 30**	☐	**1.6.3**	**The autoclave provides a sterilizing temperature of 121± 2°C as determined for each load using a calibrated maximum registering thermometer. As an alternative, an appropriate temperature monitoring device is used in place of the maximum registering thermometer when these are unavailable due to the ban on mercury.**
K	11	☐	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	☐	1.6.5	The autoclave standards thermometer is checked every five (5) years for accuracy at 121°C by a qualified calibration laboratory; or, is checked in-house at the steam point (100°C) if it has been previously calibrated at both 100°C and 121°C. Any change in temperature at the steam point changes the calibrated temperature at 121°C by the same magnitude. Date of most recent determination_____
K	1	☐	1.6.6	Working autoclave thermometers are checked against the autoclave standards thermometer at 121°C yearly. Date of last check _____ Method _____
K	11	☐	1.6.7	Spore strips/suspensions appropriate for use in an autoclave media cycle are used monthly according to manufacturer's instructions to evaluate the effectiveness of the sterilization process. Results are recorded and the records maintained.
O	11	☐	1.6.8	Heat sensitive tape is used with each autoclave batch.
K	11, 13	☐	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	☐	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	☐	1.6.11	A thermometer capable of determining temperatures accurately in the range of 160 to 180°C is used to monitor the operation of the hot-air sterilizing oven
K	13	☐	1.6.12	Records of temperatures and exposure times are maintained for the operation of the hot-air sterilizing oven during use.

K	11	☐	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	☐	1.6.14	Reusable sample containers are sterilized for 60 minutes at 170°C in a hot-air oven or autoclaved for 15 minutes at 121°C.
C	**1**	☐	**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	☐	1.6.17	Reusable pipettes are stored and sterilized in aluminum or stainless steel canisters.
K	9	☐	1.6.18	Reusable pipettes (in canisters) are sterilized in a hot-air oven at 170°C for 2 hours.
C	**2**	☐	**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 pipettes is determined with each lot received. Results are recorded and the records maintained.**
K	18	☐	1.6.21	Hardwood applicator transfer sticks are properly sterilized. Method of sterilization _____
C	**2**	☐	**1.6.22**	**The sterility of the hardwood applicator transfer sticks is checked routinely. Results are recorded and the records maintained.**
O	13	☐	1.6.23	Spent broth cultures and agar plates are decontaminated by autoclaving for at least 30 minutes before conventional disposal.
		1.7 Media Preparation		
K	3, 5	☐	1.7.1	Media is commercially dehydrated except in the case of medium A-1 which must be prepared from the individual components and modified MacConkey agar which may be prepared from its components.
K	11	☐	1.7.2	Media is prepared according to manufacturer's instructions.
O	11	☐	1.7.3	Dehydrated media and media components are properly stored in a cool, clean, dry place.
O	11	☐	1.7.4	Dehydrated media are labeled with date of receipt and date opened.
C	**12**	☐	**1.7.5**	**Caked or expired media or media components are discarded.**
C	**11**	☐	**1.7.6**	**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. (C*ircle the appropriate water quality descriptor determined.*) Results are recorded and the records maintained.**
C	**11**	☐	**1.7.7**	**Reagent water is analyzed for residual chlorine monthly and is at a non-detectable level (< 0.1 mg/L). Results are recorded and the records maintained.** **Specify method of determination_____.**
K	11	☐	1.7.8	Reagent water contains <100 CFU/mL as determined monthly using the heterotrophic plate count method. Results are recorded and the records maintained.
K	11	☐	1.7.9	Media prepared from commercial dehydrated components are sterilized according to the manufacturer's instructions.
K	9	☐	1.7.10	The volume and concentration of media in the tube are suitable for the amount of sample inoculated.
C	**11**	☐	**1.7.11**	**Total time of exposure of sugar broths to autoclave temperatures does not exceed 45 minutes.**
C	**1**	☐	**1.7.12**	**Media sterility is determined for each load sterilized. Results are recorded and the records maintained.**
C	**1**	☐	**1.7.13**	**Media productivity is determined using media-appropriate, properly diluted positive and negative control cultures for each lot of dehydrated**

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

				Range of MPN_____ **Strength of media used_____**
K	9	☐	2.2.7	Inoculated tubes are incubated in air at 35 ± 0.5°C.
C	**2**	☐	**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	☐	2.2.9	Inoculated tubes are read after 24 ± 2 hours and 48 ± 3 hours of incubation and transferred at both time interval if positive for growth (the presence of turbidity) and gas or effervescence in the culture tube. These tubes are considered presumptive positive requiring further confirmatory testing.
		2.3 Confirmed Test for Seawater by APHA MPN		
C	**9**	☐	**2.3.1**	**Brilliant green bile 2% broth (BGB) is used as the confirmatory medium for total coliforms.**
C	**9**	☐	**2.3.2**	**EC medium is used as the confirmatory medium for fecal coliforms.**
C	**2**	☐	**2.3.3**	**The appropriate positive and negative productivity controls for the presumptive media are used. The results are recorded and the records maintained.** **Positive productivity control _____Negative productivity control _____**
K	9, 11	☐	2.3.4	Transfers are made to BGB/EC by either sterile loop or sterile hardwood transfer stick from positive presumptive tubes incubated for 24 and 48 hours as appropriate. *(Circle the method of transfer.)*
C	**9**	☐	**2.3.5**	**BGB tubes are incubated at 35 ± 0.5°C.**
K	9	☐	2.3.6	BGB tubes are read after 48 ± 3 hours of incubation.
C	**9**	☐	**2.3.7**	**EC tubes are incubated in a circulating waterbath maintained at 44.5 ± 0.2°C.**
C	**9**	☐	**2.3.8**	**EC tubes are read after 24 ± 2 hours of incubation.**
C	**9**	☐	**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	☐	2.4.1	Results of multiple dilution tests are read from tables in *Recommended Procedures for the Examination of Sea Water and Shellfish*, Fourth Edition.
K	7	☐	2.4.2	Results from single dilution series are calculated from Hoskins' equation or interpolated from Figure 1, Public Health Report 1621 entitled "Most Probable Numbers for Evaluation of Coli aerogenes Tests by Fermentation Tube Method".
C	**7, 9**	☐	**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 supports use of A-1medium without salicin. Study records are available.**
C	**5**	☐	**2.5.3**	**A-1 medium sterilized for 10 minutes at 121°C.**
C	**2**	☐	**2.5.4**	**The appropriate positive and negative productivity controls for the presumptive media are used. The results are recorded and the records maintained.** **Positive productivity control _____Negative productivity control _____**
C	**9**	☐	**2.5.5**	**Sample and dilutions of sample are shaken vigorously (25 times in a 12" arc in 7 seconds) before inoculation.**
C	**9**	☐	**2.5.6**	**In a multiple dilution series not less than 3 tubes per dilution are used (5 tubes are recommended).**
C	**6**	☐	**2.5.7**	**In a single dilution series at least 12 tubes are used.**

C	**6**	☐	**2.5.8**	**In a single dilution series, the volumes analyzed are adequate to meet the needs of routine monitoring.** **Sample volume inoculated _____** **Range of MPN _____** **Strength of media used _____**
C	**2**	☐	**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**	☐	**2.5.10**	**Inoculated tubes are placed in an air incubator at 35 ± 0.5°C for 3 ± 0.5 hours of resuscitation.**
C	**5**	☐	**2.5.11**	**After 3 ± 0.5 hours resuscitation at 35°C, inoculated tubes are incubated at 44.5 ± 0.2°C in a circulating waterbath for the remainder of the 24 ± 2 hours.**
C	**5**	☐	**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 – APHA MPN		
K	9	☐	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	☐	2.6.2	Results from single dilution series are calculated from Hoskins' equation or interpolated from Figure 1, Public Health Report 1621 entitled "Most Probable Numbers for Evaluation of Coli aerogenes Tests by Fermentation Tube Method".
C	**7, 9**	☐	**2.6.3**	**Results are reported as MPN/100 mL of sample.**
		2.7 Bacteriological Analysis of Seawater by Membrane Filtration (MF) using mTEC Agar - Materials and Equipment		
C	**23, 24**	☐	**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**	☐	**2.7.2**	**When using a waterbath for elevated temperature incubation, the level of the water completely covers the plates.**
C	**23**	☐	**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	☐	2.7.5	Colonies are counted with the aid of magnification.
C	**11, 23**	☐	**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.**
C	**2**	☐	**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	☐	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**	☐	**2.7.10**	**The sterility of each lot or autoclave batch of membrane filters are checked before use.**
K	2	☐	2.7.11	Membrane filters which are beyond their expiration date are not used.
O	11	☐	2.7.12	Forceps tips are clean.
O	11	☐	2.7.13	Forceps tips are smooth without pitting or corrugations to damage the filters being manipulated.

K	11	☐	2.7.14	Forceps are dipped in alcohol and flame sterilized between sample filters.
K	11	☐	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	☐	2.7.16	Membrane filtration units are made of stainless steel, glass or autoclavable plastic free of scratches, corrosion and leaks.
C	**11**	☐	**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	☐	2.7.18	A UV sterilization unit is used to disinfect filter assemblies between sample and filtration runs.
K	11	☐	2.7.19	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	☐	2.8.1	Phosphate buffered saline is used as the sample diluent and filter funnel rinse.
C	**11**	☐	**2.8.2**	**The phosphate buffered saline is properly sterilized.**
K	23	☐	2.8.3	A sufficient amount of medium (4-5 mL) is used in each plate.
O	11	☐	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**	☐	**2.9.1**	**mTEC agar is used.**
C	**2**	☐	**2.9.2**	**The appropriate positive and negative productivity controls for the presumptive media are used. The results are recorded and the records maintained.** **Positive productivity control _____Negative productivity control _____**
C	**23**	☐	**2.9.3**	**The sample is shaken vigorously (25 times in a 12″ arc in 7 seconds) before filtration.**
C	**23**	☐	**2.9.4**	**The membrane is placed grid side up within the sterile filter apparatus.**
C	**23, 25**	☐	**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**	☐	**2.9.6**	**Sample volumes are filtered under vacuum.**
K	26	☐	2.9.7	The pressure of the vacuum pump does not exceed 15 psi.
C	**23, 26**	☐	**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**	☐	**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**	☐	**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).**
C	**2, 11**	☐	**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**	☐	**2.9.12**	**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**	☐	**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.**
			2.10 Computation of Results - MF using mTEC Agar	
C	**23**	☐	**2.10.1**	**All yellow, yellow-green or yellow-brown colonies are counted.**
C	**23**	☐	**2.10.2**	**Only plates having 80 or fewer colonies are counted. If it is unavoidable to use plates having more than 80 colonies, counts are given as >80 x 100/the volume of sample filtered.**
C	**2, 11, 23**	☐	**2.10.3**	**When multiple dilutions are filtered, the laboratory has developed a procedure for assessing the contribution of all positive dilutions to the final count.**
C	**23, 11**	☐	**2.10.4**	**The number of fecal coliforms is calculated by the following equation:** **Number of fecal coliforms per 100 mL = [number of colonies counted per plate used in the count / volume (s) of sample filtered in ml] x 100.**
C	**23, 11**	☐	**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**	☐	**3.1.1**	**A representative sample of shellstock is collected.**
K	9	☐	3.1.2	Shellstock samples are collected in clean, waterproof, puncture resistant containers loosely sealed.
K	9	☐	3.1.3	Shellstock samples are labeled with collector's name, type of shellstock, the source or harvest area, sampling station, time, date and place (if applicable) of collection.
C	**9**	☐	**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**	☐	**3.1.5**	**Analysis of the samples is initiated as soon as possible after collection. Shellfish samples are not tested if the time interval between collection and analysis exceeds 24 hours.**
			3.2 Preparation of Shellfish for Examination	
K	2,11	☐	3.2.1	Shucking knives, scrub brushes and blender jars are (autoclave) sterilized for 15 minutes prior to use.
O	2	☐	3.2.2	Blades of shucking knives are not corroded.
O	9	☐	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	☐	3.2.4	The faucet used for rinsing the shellstock does not contain an aerator.
K	9	☐	3.2.5	Shellstock are scrubbed with a stiff, sterile brush and rinsed under tap water of drinking water quality.
O	9	☐	3.2.6	Shellstock are allowed to drain in a clean container or on clean towels prior to opening.
K	9	☐	3.2.7	Immediately prior to shucking, the hands (or gloved hands) of the analyst are thoroughly washed with soap and water and rinsed in 70% alcohol.
C	**9**	☐	**3.2.8**	**Shellstock are not shucked directly through the hinge.**
C	**9**	☐	**3.2.9**	**Contents of shellstock (liquor and meat) are shucked into a sterile, tared blender jar or other sterile container.**
K	9	☐	3.2.10	At least 200 grams of shellfish meat or a quantity of meat sufficient to cover the blender blades is used for the analysis.
K	9	☐	3.2.11	A representative sample of at least 12 shellfish is used for the analysis.
K	2	☐	3.2.12	The sample is weighed to the nearest 0.1 gram and an equal amount by weight of diluent is added.
O	9	☐	3.2.13	Sterile phosphate buffered dilution water is used as the sample diluent.

C	**9**	☐	**3.2.14**	**Samples are blended at high speed for 60 to 120 seconds until homogenous.**
K	9	☐	3.2.15	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**	☐	**3.3.1**	**Appropriate strength lactose or lauryl tryptose broth is used as presumptive media in the analysis. *(Circle the medium used.)***
C	**2**	☐	**3.3.2**	**The appropriate positive and negative productivity controls for the presumptive media are used. The results are recorded and the records maintained.** **Positive productivity control _____Negative productivity control _____**
K	9	☐	3.3.3	Immediately (within 2 minutes) after blending, the ground sample is diluted and inoculated into tubes of presumptive media.
C	**9**	☐	**3.3.4**	**No fewer than 5 tubes per dilution are used in a multiple dilution MPN series.**
C	**9**	☐	**3.3.5**	**Allowing for the initial 1:1 dilution of the sample, appropriate portions are inoculated (i.e., 2 ml of original 1:1 dilution for the 1 g portion) and diluted for subsequent inoculation (i.e., 20 ml of 1:1 diluted sample to 80 ml of diluent or the equivalent for 0.1 g portion). All successive dilutions are prepared conventionally.**
K	6	☐	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**	☐	**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	☐	3.3.8	Inoculated media are incubated at 35 ± 0.5°C.
K	10	☐	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**	☐	**3.4.1**	**EC medium is used as the confirmatory medium.**
C	**2**	☐	**3.4.2**	**The appropriate positive and negative productivity controls for the presumptive media are used. The results are recorded and the records maintained.** **Positive productivity control _____Negative productivity control _____**
K	9, 11	☐	3.4.3	Transfers are made to EC medium by either sterile loop or hardwood sterile transfer sticks from positive presumptives. *(Circle the method of transfer.)*
C	**9**	☐	**3.4.4**	**EC tubes are incubated in a circulating waterbath at 44.5 ± 0.2°C**
K	9	☐	3.4.5	EC tubes are read for gas production after 24 ± 2 hours of incubation.
C	**9**	☐	**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	☐	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	☐	3.5.2	Results from single dilution series are calculated from Hoskins' equation or interpolated from Figure 1, Public Health Report 1621 entitled "Most Probable Numbers for Evaluation of Coli aerogenes Tests by Fermentation Tube Method".
C	**9**	☐	**3.5.3**	**Results are reported as MPN/100 grams of sample.**

		3.6 Standard Plate Count Method		
O	20	☐	3.6.1	A standard plate count (SPC) analysis may be performed in conjunction with the analysis for fecal coliform organisms.
K	9	☐	3.6.2	In the standard plate count procedure at least four plates are used, duplicates of two dilutions. One of the dilutions should produce colonies of 30 to 300 per plate.
K	2	☐	3.6.3	Fifteen to 20 mL of tempered sterile plate count agar is used per plate.
C	**9**	☐	**3.6.4**	**Agar tempering bath maintains the agar at 44-46°C.**
C	**9**	☐	**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	☐	3.6.6	Samples or sample dilutions to be plated are shaken vigorously (25 times in a 12" arc in 7 seconds) before plating.
C	**9**	☐	**3.6.7**	**Not more than 1 mL nor less than 0.1 mL of sample or sample dilution is plated.**
K	11	☐	3.6.8	Control plates are used to check air quality and the sterility of the agar and the diluent.
K	9,21	☐	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	☐	3.6.10	Quebec Colony Counter or its equivalent is used to provide the necessary magnification and visibility for counting plates.
K	1	☐	3.6.11	A hand tally or its equivalent is used for accuracy in counting.
		3.7 Computation of Results -SPC		
K	9	☐	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*, Fourth Edition.
C	**19**	☐	**3.7.2**	**Colony counts are reported as CFU/g of sample.**
		3.8 Bacteriological 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	3	☐	3.8.2	Double strength modified MacConkey agar is used.
C	**3**	☐	**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	☐	3.8.4	Twice boiled, double strength modified MacConkey agar and is maintained in a tempering bath at 45 to 50°C until used.
K	2, 3	☐	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**	☐	**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	☐	3.8.9	Sixty (60) mL of tempered, twice boiled double strength Modified MacConkey Agar is added.
K	2,3, 22	☐	3.8.10	The container is gently swirled or slowly inverted once to mix the contents, which are subsequently distributed uniformly over six plates.
C	**1**	☐	**3.8.11**	**Media and diluent sterility are determined with each use. Results are recorded and the records maintained.**
C	**1**	☐	**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**	☐	**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_____**
		3.9 Computation of Results - ETCP		
K	11	☐	3.9.1	Quebec Colony counter or its equivalent is used to provide the necessary magnification and visibility for counting.
O	1	☐	3.9.2	A hand tally or its equivalent is used to aid in counting.
C	**3, 6**	☐	**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.**
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	☐	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**	☐	**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.**
K	9	☐	3.10.3	The level of water in the tempering bath covers the level of liquid and agar in the container or culture tubes.
C	**27, 28**	☐	**3.10.4**	**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	☐	3.10.5	The sterility of each lot of pre-sterilized syringes and syringe filters is determined. Results are recorded and records maintained.
K	1	☐	3.10.6	The sterility of each batch of reusable glass syringes is determined. Results are recorded and records maintained.
C	**27, 28**	☐	**3.10.7**	**The balance used provides a sensitivity of at least mg (0.01g.).**
C	**27, 28**	☐	**3.10.8**	**The temperature of the incubator used is maintained at 36 ± 1°C.**
C	**28**	☐	**3.10.9**	**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	☐	3.11.1	Media preparation and sterilization is according to the validated method.
K	27, 28	☐	3.11.2	Bottom agar, double strength soft agar and growth broth are prepared from their individual components.
K	27, 28	☐	3.11.3	Soft agar is prepared double strength in volumes of 2.5 mL.
C	**27, 28**	☐	**3.11.4**	**The streptomycin and ampicillin solutions are added to tempered bottom agar and vortex for 2 minutes on stir plate.**
O	27, 28	☐	3.11.5	Storage of the bottom agar under refrigeration does not exceed 1 month.
K	27, 28	☐	3.11.6	Unsterilized soft agar is stored at -20 °C -15C for up to 3 months.
K	27, 28	☐	3.11.7	The soft agar is removed from the freezer and sterilized for 15 minutes at 121°C before use.
K	27, 28	☐	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	☐	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	☐	3.12.1	Shucking knives, scrub brushes and blender jars are autoclave sterilized for 15 minutes prior to use.
O	2	☐	3.12.2	The blades of shucking knives are not corroded.
O	9	☐	3.12.3	The hands of the analyst are thoroughly washed with soap and water immediately prior to cleaning the shells of debris.
O	2	☐	3.12.4	The faucet used for rinsing the shellfish does not contain an aerator.

K	9	☐	3.12.5	The shellfish are scrubbed with a stiff, sterile brush and rinsed under tap water of drinking water quality.
O	9	☐	3.12.6	The shellfish are allowed to drain in a clean container or on clean towels unlayered prior to shucking.
K	9	☐	3.12.7	Immediately prior to shucking, the hands (or gloved hands) of the analyst are thoroughly washed with soap and water and rinsed in 70% alcohol.
C	**9**	☐	**3.12.8**	**Shellfish are not shucked through the hinge.**
C	**9**	☐	**3.12.9**	**The contents of shellfish (liquor and meat) are shucked into a sterile, tared blender jar or other sterile container.**
K	9	☐	3.12.10	A representative sample of at least 12 shellfish is used for the analysis.
K	2, 19	☐	3.12.11	The sample is weighed to the nearest 0.1 gram.
		3.13 MSC Sample Analysis		
C	**28**	☐	**3.13.1**	**E.coli *Famp* ATCC 700891 is the bacterial host strain used in this procedure.**
K	27, 28	☐	3.13.2	Host cell growth broth is tempered at 36 ± 1°C and vortexed (or shaken) to aerate prior to inoculation with host cells.
K	27, 28	☐	3.13.3	Several host cell colonies are transferred to a tube of tempered, aerated growth broth and incubated at 36 ± 1°C for 4-6 hours to provide host cells in log phase growth for sample analysis.
C	**27, 28**	☐	**3.13.4**	**After inoculation, the host cell growth broth culture is not shaken.**
C	**28**	☐	**3.13.5**	**A 2:1 mixture of sterile growth broth to shellfish tissue is used for eluting the MSC.**
C	**28**	☐	**3.13.6**	**The elution mixture is prepared w/v by weighing the sample and adding two equal portions of sterile growth broth by volume to the shellfish tissue.**
C	**28**	☐	**3.13.7**	**The elution mixture is homogenized at high speed for 180 seconds.**
C	**28**	☐	**3.13.8**	**Immediately after blending, 33 grams of the homogenized elution mixture are weighed into centrifuge tubes.**
C	**28**	☐	**3.13.9**	**The homogenized elution mixture is centrifuged for 15 minutes at 9000 x g at 4°C.**
C	**27, 28**	☐	**3.13.10**	**The supernatant is pipetted off, weighed and the weight recorded.**
C	**27, 28**	☐	**3.13.11**	**The supernatant is allowed to warm to room temperature about 20 to 30 minutes.**
K	27, 28	☐	3.13.12	The autoclaved soft agar is tempered and held at 51 ± 1°C throughout the period of sample analysis.
K	27, 28	☐	3.13.13	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	☐	3.13.14	The sample supernatant is shaken or vortexed before being added to the tempering soft agar.
C	**27, 28**	☐	**3.13.15**	**2.5 mL of sample supernatant is added to each tube of tempering soft agar.**
C	**27, 28**	☐	**3.13.16**	**The soft agar/sample supernatant/host cell mixture is gently rolled between the palms of the hands to mix.**
C	**27, 28**	☐	**3.13.17**	**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**	☐	**3.13.18**	**Ten (10) plates are used, 2.5 mL per plate for a total of 25 mL of supernatant analyzed per sample.**
K	27, 28	☐	3.13.19	Negative and positive control plates are prepared and accompany each set of samples analyzed. The results are recorded and records maintained. Positive control _____
K	27, 28	☐	3.13.20	Growth broth is used as the negative control or blank.
K	27, 28	☐	3.13.21	Type strain MS2 (ATCC 15597) male specific bacteriophage appropriately diluted to provide countable low levels of phage is used as the positive control.
K	2	☐	3.13.22	A negative control plate is plated at the beginning and end of each set of samples analyzed.

K	27, 28	☐	3.13.23	The positive control is plated after all the samples are inoculated and immediately prior to the final negative control.
C	**27, 28**	☐	**3.13.24**	**All plates are incubated at 36 ± 1°C for 18 ± 2 hours.**
		3.14 Computation of Results - MSC		
C	**27**	☐	**3.14.1**	**Circular zones of clearing or plaques of any diameter in the lawn of host bacteria are counted.**
C	**28, 32**	☐	**3.14.2**	**The working range of the method is 1 to 200 PFU per plate. When there are no plaques on all ten plates, the count is <6 PFU/100 grams for soft-shelled clams, <7 PFU/ 100 grams for American oysters, and <5 PFU/ 100 grams for quahog (hard) clams. If the density exceeds 200 PFU per plate on all plates, the count is given as > 20,000 PFU/100 grams.**
K	28	☐	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	☐	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. October 2015

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U.S. FOOD AND DRUG ADMINISTRATION
OFFICE OF FOOD SAFETY
SHELLFISH AND AQUACULTURE POLICY BRANCH
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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:**

OTHER OFFICIALS PRESENT:	**TITLE:**

Items which do not conform are noted by: Conformity is noted by a "√"

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

Check the applicable analytical methods:

	MPN Real-time PCR method for *Vibrio vulnificus* detection in Oysters [PART III] SmartCycler II
	MPN Real-time PCR method for *Vibrio parahaemolyticus* detection in Oysters [PART III] SmartCycler II and AB 7500 Fast

PART I – Quality Assurance			
ITEM			
CODE	**REF**		
		1.1 Quality Assurance (QA) Plan	
K	4, 6	1.1.1 Written Plan (Check √ those items which apply).	
			a. Organization of the Laboratory.
			b. Staff training requirements.
			c. Standard operating procedures (SOPs).
			d. Internal quality control measures for equipment, their calibration maintenance, repair, performance and rejection criteria established.
			e. Laboratory safety.
			f. Internal performance assessment.
			g. External performance assessment.
C	**4**		**1.1.2 The QA plan is implemented.**
K	6		1.1.3 The Laboratory participates in a proficiency testing program annually. Specify the program(s): _____
		1.2 Educational/Experience Requirements	
C	**State's Human Resources Department**		**1.2.1 In state/county 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	State's Human Resources Department		1.2.2 In state/county laboratories, the analysts meet the state/county educational and experience requirements for processing samples in a public health laboratory.
C	**USDA Microbiology & EELAP**		**1.2.3 In 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		1.2.4 In commercial laboratories, the analysts must have at least a high school diploma and at least three months of experience in laboratory sciences.
		1.3 Work Area	
O	4, 6		1.3.1 Adequate for workload and storage.
K	6		1.3.2 Clean, well lighted.
K	6		1.3.3 Adequate temperature control.
O	6		1.3.4 All work surfaces are nonporous, easily cleaned and disinfected.
K	6		1.3.5 Microbiological quality of the air contains fewer than 15 colonies/plate for a 15 minute exposure determined monthly. The results are recorded and records maintained.
		1.4 Laboratory Equipment	
K	5		1.4.1 To determine the pH of prepared media and reagents, the pH meter has a standard accuracy of 0.1 pH units.
K	9		1.4.2 pH electrodes consisting of pH half-cell and reference half-cell or equivalent combination electrode free from (Ag/AgCl) or contains an ion exchange barrier preventing passage of Ag ions into the medium which may affect the accuracy of the pH reading.
K	6		1.4.3 The effect of temperature on the pH is compensated for by an internal/external ATC probe or by manual adjustment (*Circle the appropriate type of adjustment*).
K	4		1.4.4 The pH meter is calibrated daily or with each use as per product literature. Results are recorded and records maintained.
K	6		1.4.5 A minimum of two standard buffer solutions are used to calibrate the pH meter. The first is near the electrode isopotential point (pH 7). The second is near the expected sample pH (i.e. pH 4 or pH 10). Standard buffer solutions are used once and discarded.
O	4		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	5		1.4.7 The balances used provide a sensitivity of at least 0.1 g at the weights of use.

K	6		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	6		1.4.9 Refrigerator temperatures are monitored at least once daily on workdays. Results are recorded and records maintained.
K	1		1.4.10 Refrigerator temperatures are maintained between 0 and 4 °C, except for reagent refrigerators which are maintained between 2 and 8 °C.
C	**7**		**1.4.11 Freezer temperature is maintained at -15 °C or below.**
O	7		1.4.12 Freezer temperature is monitored at least once daily on workdays. Results are recorded and records maintained.
C	**5**		**1.4.13 The temperature of the incubator is maintained at 35 +/- 2.0 °C.**
K	6		1.4.14 Thermometers used in the air incubators are graduated at no greater than 0.5 °C increments.
K	5		1.4.15 Working thermometers are located on top and bottom shelves of use in the air incubator or appropriately placed based on the results of spatial temperature checks.
K	4, 6		1.4.16 Air incubator temperatures are taken twice daily on workdays. Results are recorded and records maintained.
C	**3**		**1.4.17 All working thermometers are appropriately immersed.**
C	**~~2,~~ 20, 23**		**1.4.18 Working thermometers are either: ~~calibrated~~ mercury-in-glass thermometers, ~~calibrated~~ non-mercury-in-glass thermometers having the accuracy and tolerance of mercury, or ~~appropriately calibrated~~low drift electronic devices, including Resistance Temperature Devises (RTDs) and Platinum Resistance Devices (PTDs) with an accuracy of less than or equal to ≤ ±0.05°C.**
C	**6, 20**		**1.4.19 A standards thermometer has been calibrated by NIST or a qualified calibration laboratory using a primary standard traceable to NIST or an equivalent authority at the points 0 and 35. These calibration records are maintained.**
K	3, 5		1.4.20 Standard thermometers are checked annually for accuracy by ice point determination. Results are recorded and maintained. Date of most recent determination:_____
C	**2, 20**		**1.4.21 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	3, 8		1.4.22 All working thermometers are checked annually against the standards thermometer at temperature(s) of use. Results are recorded and records maintained.
O	6		1.4.23 Appropriate pipet aids are available and used to inoculate samples.
K	2		1.4.24 Micropipettors are calibrated annually at appropriate volumes used and checked for accuracy quarterly. Results are recorded and records maintained.
		1.5 Labware and Glassware Washing	
K	5		1.5.1 Utensils, containers, glassware and plasticware are clean borosilicate glass, stainless steel or other noncorroding material.
K	5		1.5.2 Culture tubes are new and of a suitable size to accommodate the volume for nutritive ingredients and sample.
K	5		1.5.3 Dilution bottles and tubes are made of borosilicate glass or plastic and closed with secure caps or screw caps with nontoxic liners.
K	5		1.5.4 Graduations are indelibly marked on dilution bottles and tubes or an acceptable alternative method is used to ensure appropriate volumes.
K	5		1.5.5 In washing reusable pipets, glassware and labware, a succession of at least three fresh water rinses plus a final rinse of deionized water is used to thoroughly rinse off all detergent.
C	**2**		**1.5.6 An alkaline or acidic detergent is used for washing glassware/labware.**
C	**6**		**1.5.7 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 as appropriate) with aqueous 0.04% bromothymol blue (BTB) solution. Results are recorded and records maintained.**

		1.6 Sterilization and Decontamination	
K	5		1.6.1 The autoclave is of sufficient size to accommodate the workload.
K	4		1.6.2 Routine autoclave maintenance is performed and the records maintained.
C	**6, 20**		**1.6.3 The autoclave provides a sterilizing temperature of 121 ± 2 °C as determined for each load using a calibrated maximum registering thermometer. As an alternative, an appropriate temperature monitoring device is used in place of the maximum registering thermometer when these are unavailable due to the ban on mercury.**
K	6		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	10		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		1.6.6 Working autoclave thermometers are checked against the autoclave standards thermometer at 121 °C yearly. Date of last check:_____
K	6		1.6.7 Spore strips/suspensions appropriate for use in an autoclave media cycle are used monthly according to manufacturer's instructions to evaluate the effectiveness of the sterilization process. Results are recorded and the records maintained.
O	6		1.6.8 Heat sensitive tape is used with each autoclave batch.
K	6		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 the appropriate type or types*).
K	6		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	5		1.6.11 A thermometer capable of determining temperatures accurately in the range of 160 to 180 °C is used to monitor the operation of the hot air sterilizing oven.
K	8		1.6.12 Records of temperature and exposure times are maintained for the operation of the hot-air sterilizing oven.
K	6		1.6.13 Spore strips/suspensions appropriate for use in dry heat are used quarterly to evaluate the effectiveness of the sterilization process in the hot-air oven. Results are recorded and records maintained.
K	5		1.6.14 Reusable pipets are stored and sterilized in aluminum or stainless steel containers.
K	5		1.6.15 Reusable pipets (in canisters) are sterilized in a hot-air oven at 170 °C for 2 hours.
C	**2**		**1.6.16 The sterility of reusable pipets is determined with each load sterilized. Results are recorded and records maintained.**
C	**2**		**1.6.17 The sterility of autoclave sterilized disposable pipet tips and microcentrifuge tubes is determined with each load sterilized. Results are recorded and records maintained.** **If presterilized pipet tips and microcentrifuge tubes are purchased certificate should be maintained and sterility confirmed as in 1.6.18.**
C	**2**		**1.6.18 The sterility of presterilized disposable pipets, pipet tips and microcentrifuge tubes is determined with each lot received. Results are recorded and records maintained.**
K	8		1.6.19 Spent broth cultures and agar plates are properly decontaminated before disposal.
		1.7 Media Preparation	
K	13, 14		1.7.1 Alkaline peptone water (APW) is prepared from the individual components and pH adjusted appropriately.
K	6		1.7.2 Media components are properly stored in a cool dry place.
O	6		1.7.3 Media components are labeled with the analyst's initials, date of receipt and date opened.
O	6		1.7.4 Dehydrated media are labeled with date of receipt and date opened.

C	**6**		**1.7.5 Caked or expired media or media components are discarded.**
C	**6**		**1.7.6 Reagent water for media and diluent preparation is analyzed for residual chlorine monthly and is at a non-detectable level (≤0.1 ppm). Results are recorded and records maintained**
K	6		1.7.7 Reagent water for media and diluent preparation contains <100 CFU/mL as determined monthly using the heterotropic plate count method. Results are recorded and records maintained.
K	5		1.7.8 The volume and concentration of media in the tube is suitable for the amount of sample inoculated.
C	**6**		**1.7.9 Media broths are not in the autoclave for more than 60 minutes.**
C	**1**		**1.7.10 Media and diluent sterility is determined for each load sterilized. Results are recorded and records maintained.**
C	**1**		**1.7.11 Media productivity is determined using media-appropriate positive and negative control cultures for each lot of dehydrated media received or with each batch of media prepared when the medium is made from its individual components.**
C	**6**		**1.7.12 The pH of the prepared media is determined after sterilization to ensure that it is consistent with manufacturer requirements and/or method tolerance. Results are recorded and records are maintained.**
		1.8 Storage of Prepared Culture Media	
K	5		1.8.1 Prepared culture media are stored in a cool, clean, dry place where excessive evaporation and the danger of contamination is minimized.
K	8		1.8.2 Stored media are labeled with the storage expiration date or sterilization date.
K	5		1.8.3 Storage of prepared culture media at room temperature does not exceed 7 days.
K	2		1.8.4 Storage under refrigeration of prepared broth media with loose fitting closures does not exceed 1 month.
K	6		1.8.5 Storage under refrigeration of prepared culture media with screw- cap closures does not exceed 3 months.
K	11		1.8.6 All prepared broth media stored under refrigeration is warmed to room temperature prior to use, without exceeding incubation temperature.
PART II –Samples			
		2.1 Sample Collection, Transportation and Receipt	
C	**2, 6**		**2.1.1 A representative sample is collected and a chain of custody documenting the history of the sample(s) from collection to final disposal has been established.**
K	5		2.1.2 Shellfish samples as received are collected in clean, waterproof, puncture resistant containers loosely sealed or are rejected for regulatory analysis.
K	5		2.1.3 Shellfish samples as received are labeled with the collector's (or if PHP, company/processor and collector's) name, the source, the time and date of collection or are rejected for regulatory analysis.
C	**5**		**2.1.4 Immediately after collection, shellfish samples are placed in dry storage (ice chest or equivalent) which is maintained between 2 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**		**2.1.5 Analysis of the samples is initiated as soon as possible after collection, but not to exceed 36 h. If processing IQF samples, samples are defrosted under refrigeration for no longer than 36 h once removed from the freezer.**
		2.2 Preparation of Samples for Analysis	
K	2, 6		2.2.1 Shucking knives, scrub brushes and blender jars are autoclave sterilized for 15 minutes.
O	2		2.2.2 Blades of shucking knives are not corroded.
K	5		2.2.3 The hands of the analyst are thoroughly washed with soap and water or new gloves are donned, immediately prior to cleaning the shells of debris.
O	2		2.2.4 The faucet used for rinsing the shellfish does not contain an aerator.
K	5		2.2.5 Shellfish are scrubbed with a stiff, sterile brush and rinsed under tap water of drinking water quality.
K	5		2.2.6 Samples are allowed to drain in a clean container or on clean towels prior to opening
K	5, 15		2.2.7 Immediately prior to shucking, the hands or gloved hands of the analyst are thoroughly washed with soap and water and rinsed in 70% alcohol. The gloves if worn are latex, nitrile and/or stainless steel mesh to protect analyst's hands from injury.

C	**5**		**2.2.8 Shellfish are not shucked through the hinge.**
C	**5**		**2.2.9 The contents of the sample (liquor and meat) are shucked into a sterile, tared blender jar or other sterile container.**
C	**5**		**2.2.10 A representative sample of at least 12 shellfish is used for analysis**
C	**2, 5**		**2.2.11 A quantity of meat and liquor is sufficient to cover the blender blades or additional oysters are used in order to ensure sample homogeneity.**
K	2, 13		2.2.12 The sample can be processed directly or a 1:1 dilution of shellfish:diluent made. If a dilution is made, the sample is weighed to the nearest 0.1 g and an equal amount, by weight, of diluent is added.
K	13		2.2.13 Sterile phosphate buffered saline (pH 7.4) is used as the sample diluent.
C	**5**		**2.2.14 Samples are blended for 60 to 120 seconds until homogenous.**
PART III- PCR method for *Vibrio vulnificus* and *Vibrio parahaemolyticus* detection in Oysters			
		3.1 APW Enrichment	
K	5		3.1.1 Sterile phosphate buffered saline (PBS) is used as the sample diluent.
C	**5, 15**		**3.1.2 The 1:10 dilution is prepared gravimetrically with PBS. All successive dilutions are prepared volumetrically.** **For example, if an initial 1:1 dilution of the sample was used for blending, the 1:10 dilution is prepared by adding 20 g of sample homogenate to 80 ml of PBS. If the homogenate was not diluted, the 1:10 dilution is prepared by adding 10 g of sample homogenate to 90 ml of PBS.**
C	**17**		**3.1.3 Appropriate sample dilutions are inoculated into APW.** **Specify dilution(s) used _____ Specify number of tubes per dilution _____**
C	**2, 15**		**3.1.4 For *V. parahaemolyticus* analysis, a tdh+, trh+ *V. parahaemolyticus* culture diluted to $<10^3$ per ml is used as a positive process control. A non *V. parahaemolyticus* culture is used as a negative process control.** **For *V. vulnificus* analysis, a *V. vulnificus* culture diluted to $<10^3$ per ml is used as a positive process control. A non *V. vulnificus* culture is used as a negative process control.** **The process control cultures accompany the samples throughout incubation, isolation, and confirmation. Records are maintained.**
C	**13**		**3.1.5 Inoculated APW enrichment tubes are incubated at 35 +/- 2 °C.**
C	**13**		**3.1.6 Tubes are read after 18 – 24 hours of incubation. Clear tubes are negative. Turbid tubes are positive and shall be further processed.**
		3.2 PCR Reagents	
C	**14, 15**		**3.2.1 Lyophilized primers and probes are stored according to manufacturer's instructions.**
K	14, 15		3.2.2 Fluorescent probes are stored in light occluding tubes or containers.
C	**14, 15, 18, 19**		**3.2.3 The PCR forward and reverse primers and probes are appropriate for the platform.** **<u>For Total and Pathogenic Vp Real-time PCR Method</u>** tdh_269-20: 6FAM-5'-TGACATCCTACATGACTGTG-3'-MGBNFQ trh_133-23: NED/TET-5'-AGAAATACAACAATCAAAACTGA-3'-MGBNFQ tlh_1043: JOE/TEXAS RED-5'- CGCTCGCGTTCACGAAACCGT -3'-BHQ2 IAC_109: CY5-5'- TCTCATGCGTCTCCCTGGTGAATGTG -3'- BHQ2 trh_20F: 5'-TTGCTTTCAGTTTGCTATTGGCT-3' trh_292R: 5'-TGTTTACCGTCATATAGGCGCTT-3' tdh_89F:5'-TCCCTTTTCCTGCCCCC-3' tdh_321R: 5'-CGCTGCCATTGTATAGTCTTTATC-3' tlh_884F: 5'-ACTCAACACAAGAAGAGATCGACAA-3' tlh_1091R: 5'-GATGAGCGGTTGATGTCCAAA-3' IAC_46F: 5'-GACATCGATATGGGTGCCG-3'

			IAC_186R: 5'-CGAGACGATGCAGCCATTC-3' **<u>For Vv Real-time PCR Method</u>** vvhF 5'-TGTTTATGGTGAGAACGGTGACA-3' vvhR 5'-TTCTTTATCTAGGCCCCAAACTTG-3
C	**14, 18**		**3.2.4 Lyophilized forward and reverse primers, and probes, are hydrated with TE buffer to produce a 0.1 mM stock solution.**
C	**14, 18**		**3.2.5 Using molecular grade, nuclease free water, primer and probe stock solutions are diluted to produce a 0.01 mM working solution.**
C	**14, 18**		**3.2.6 Reconstituted primers and probes are stored in a -20 °C manual defrost freezer for up to 5 freeze thaw cycles, not to exceed two years.**
C	**21, 22**		**3.2.7 Platinum *Taq* DNA is stored in -20 °C manual defrost freezer until first use. After first use, can be stored between 2-8 °C.**
C	**21, 22**		**3.2.8 PCR reagents (dNTPs, buffer, MgCl2, fluorescent dyes) are stored in -20 °C manual defrost freezer until first use. After first use, they can be stored between 2-8 °C.**
		3.3 DNA Extraction	
C	**14, 18**		**3.3.1 All microcentrifuge tubes and pipet tips are sterile.**
C	**14, 18**		**3.3.2 Pipet tips have aerosol barriers.**
K	14, 18		3.3.3 Latex or nitrile gloves are worn throughout the extraction and PCR preparation process.
K	14, 18		3.3.4 All work surfaces, centrifuge racks and equipment used in PCR analysis are disinfected immediately prior to DNA extraction, Master Mix preparation and PCR analysis.
C	**14, 18**		**3.3.5 Aseptic technique is observed throughout the extraction and PCR analysis.**
C	**14, 18**		**3.3.6 One thousand (1000) µL aliquots from each positive APW enrichment tube, including the process controls, are extracted.**
C	**14, 18**		**3.3.7 Positive APW aliquots are placed in sterile microcentrifuge tubes and heated at 95-100 °C for 10 minutes.**
K	14, 18		3.3.8 A set of positive and negative process controls are included with each batch of samples in a heating block/boiling bath.
C	**14, 18**		**3.3.9 After boiling, tubes are chilled in ice or immediately frozen in a manual defrost freezer for future analysis. Boil preps may be refrigerated not to exceed 72 hours.**
K	14, 18		3.3.10 Frozen extracts are analyzed within 6 months of frozen storage.
		3.4 Preparation of the Master Mix for PCR	
C	**14, 16, 18**		**3.4.1 Nuclease-free microcentrifuge tubes and pipette tips, with filters, are used in Master Mix preparation.**
C	**14, 16, 18**		**3.4.2 For each reaction, add the specified amount of water, buffer, MgCl2, dNTPs, specific primers, nuclease probes, *Taq*, and internal control DNA is added.**
K	14, 21, 18		3.4.3 The Master Mix is gently vortexed to mix constituents and then briefly spun.
C	**14, 16, 18**		**3.4.4 Twenty-three (23) µL of Master Mix is used for each PCR reaction.**
C	**14, 16, 18**		**3.4.5 Master Mix must be used on the day of preparation or stored at –20 °C until time of use.**
		3.5 PCR	
C	**14, 19**		**3.5.1 If previously frozen, the DNA extracts are completely thawed at temperatures no warmer than room temperature. Immediately prior to use, DNA extracts are centrifuged at >5,000 x g for 2 minutes to remove particulate matter and cell debris.**
C	**14, 19**		**3.5.2 Two (2) µL of DNA template is added to each reaction tube or plate well containing 23 µL of Master Mix for a total PCR reaction volume of 25 µL.**
K	14, 19		3.5.3 Two (2) µL of molecular grade, nuclease free water is added to a reaction tube or plate well containing 23 µL of Master Mix for each batch of Master Mix prepared as a no template control.
C	**14, 19**		**3.5.4 Two (2) µL of DNA template extracted from the negative process control culture is added to a reaction tube or plate well containing 23 µL of Master Mix.**
C	**14, 19**		**3.5.5 Two (2) µL of DNA template extracted from the positive process control culture is added to a reaction tube or plate well containing 23 µL of Master Mix**.

O	14, 19		3.5.6 Two (2) µL of DNA template extracted from the positive control culture (prepared separately from the positive process control) is added to a reaction tube or plate well containing 23 µL of Master Mix as the positive PCR control.
K	14, 19		3.5.7 Immediately prior to loading the reaction tubes or plates into the instrument they are centrifuged for 3-5 seconds to ensure that all reagents and the DNA template are in the bottom of the tube to optimize the PCR amplification process.
C	16		**3.5.8 After centrifugation, tubes or plates are inserted into the instrument.**
		3.6 PCR Amplification	
C	**14, 19**		**3.6.1 The appropriate instrument platform is used for the protocol.**
K	16		3.6.2 Manufacturer's instructions are followed in operating the instrument.
C	**14, 19**		**3.6.3 The PCR cycle parameters used are appropriate for the protocol.**
K	14, 19		3.6.4 Optical calibrations for the dyes being used are current, per the instrument manufacturer's recommendations.
C	**14, 19**		**3.6.5 The analysis settings are adjusted as specified in the protocol.**
		3.7 Computation of Results	
K	14, 19		3.7.1 All runs in which the positive control generates a Ct value for the target(s) of interest and the negative control reaction generates no Ct value for the target(s), but a Ct value for the internal control are considered valid.
C	**2**		**3.7.2 Data is quality checked by the analyst.**
C	**14, 19**		**3.7.3 All reactions in a valid run which generate a Ct value for the target(s) of interest with a sigmoidal amplification curve are considered to be positive.**
C	**16**		**3.7.4 Any sample which does not demonstrate a sigmoidal amplification curve may have a reported positive/negative determination that is discrepant from the instrument if appropriately justified using the raw fluorescent data.**
K	16		3.7.5 All reactions in a valid run which do not generate a Ct value for the target(s) of interest, but do generate a Ct value for the internal control are considered negative.
C	**16**		**3.7.6 Any reaction in which no Ct value is generated for the target(s) of interest or the internal control is considered invalid and should be re-tested.**
C	**13**		**3.7.7 Upon determination of positive reactions, refer to the original positive dilutions of APW and record MPN values as derived from the calculator in Appendix 2 of the FDA Bacteriological Analytical Manual (BAM).**
K	13		3.7.8 For APW enrichment, results are reported as MPN/g of sample.

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LABORATORY:	**DATE of EVALUATION:**

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**

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:_____

LABORATORY:

Page	Item	Observation