CANADIAN SHELLFISH SANITATION PROGRAM

LABORATORY EVALUATION OFFICER

NAME:_____ **AFFILIATION:** _____

REGION: _____

ADDRESS: _____ **Phone:** _____

_____ **Fax:** _____

_____ **E-MAIL:** _____

SHELLFISH LABORATORY EVALUATION CHECKLIST

LABORATORY:		
ADDRESS:		
TELEPHONE:	FAX:	
DATE OF EVALUATION	DATE OF REPORT	LAST EVALUATION
LABORATORY REPRESENTED BY:	TITLE:	
OTHER OFFICIALS PRESENT:	TITLE:	

The CSSP Shellfish Laboratory Evaluation Checklist is based upon references cited in the References section at the end of this Annex. To facilitate the application of the Canada / United States Shellfish Agreement of 1948, this checklist incorporates material from the NSSP Form LAB-100 rev. 8-21-95 and NSSP Form LAB-100 rev. 2001-11-17 checklists with modifications to reflect differences between the CSSP and the NSSP.

The CSSP Laboratory Checklist specifies the operating requirements for laboratories conducting analyses within the confines of the Canadian Shellfish Sanitation Program for the classification of shellfish growing areas and the processing of shellfish for market.

Items which do not conform are noted by: **C - Critical** K - Key O - Other	NA - Not Applicable	Conformity is noted by a check " ✓ "

CSSP ver. 1-31-02

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]
	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 I -		**QUALITY ASSURANCE**	
CODE	**REF.**	**ITEM**	
K	8, 11	**Quality Assurance Plan**	
			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.
K	8		2. QA Plan Implemented.
O	11		3. Participates in a proficiency testing program annually. Specify Program(s) _____

CODE	**REF.**	**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.
K	11		6. Pipette aid used, mouth pipetting not permitted.

CODE	REF.	Equipment		
O	9		1.	To determine the pH of prepared media, the pH meter has a standard accuracy of 0.1 pH unit.
O	6		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 effect the accuracy of the pH reading).
K	6		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	6		5.	A minimum of two standard buffer solutions are used to calibrate the pH meter. The first must be near the electrode isopotential point (pH7). 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		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		8.	Balance calibrated monthly using NIST Class S or ASTM Class I or 2 weights or equivalent and records are maintained.
K	8		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.	A sufficient number of working thermometers are to be located throughout air incubators in areas of use.
C	**11**		**14.**	**Temperature of the waterbath is maintained at 44.5° ± 0.2°C under any loading capacity (if programmable waterbaths are used, must have capability of also maintaining 35° ± 0.5°C).**
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 (if programmable waterbaths are used, two high setting and one low setting readings shall be taken).
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 O°, 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	9		23.	Incubator and waterbath working thermometers are checked annually against the standards thermometer at the temperatures at which they are used. Records maintained.

CODE	REF.		Labware and Glassware Washing	
O	9		1.	Utensils and containers are clean borosilicate glass, stainless steel or other non-corroding material.
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 non-toxic 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 1 mL; nor, are pipettes larger than 1 mL used to deliver 0.1 mL.
K	9		7	Reusable sample containers are capable of being properly washed and sterilized.
K	9		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	**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 *Standard Methods for the Examination of Water and Wastewater.* Records are kept.** **Date of most recent testing _____** **Average difference between Groups A and B _____** **Average difference between Groups B and D _____** **Detergent brand_____ Lot _____**
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.

CODE	REF.		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 values, exhaust trap, chamber drain) and records maintained.
O	8		3.	Autoclave(s) and/or steam generators serviced annually or as needed by a 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	8		5.	An autoclave standards thermometer has been calibrated by the National Institute of Standards and Technology (NIST) or its equivalent at 121° C.
K	2		6.	The autoclave standards thermometer is checked every five years for accuracy at either 121°C or at the steampoint. Date of most recent determination_____

CODE	REF.		Sterilization and Decontamination
K	11		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 are recorded.
O	2		9. Heat sensitive tape is used with each autoclave batch.
K	8		10. Autoclave sterilization records including length of sterilization, total 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 materials, 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	8		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	8		15. Reusable sample containers are sterilized for 60 minutes at 170°C in a hot-air sterilizing oven or autoclaved for 15 minutes at 121 °C.
O	1		16 The sterility of reusable 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 pipettes is determined with each batch/lot. Results are recorded and maintained.
K	11		20. Hardwood applicator 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.

CODE	REF		Media Preparation
K	9		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 (circle one) and exceeds 0.5 megohm resistance or is less than 2 mSiemens/cm conductivity at 25°C to be tested and recorded monthly for resistance or conductivity*(circle the appropriate).***

CODE	REF.		Media Preparation	
C	**11**		**6.**	**Make-up water is analyzed for residual chlorine monthly and is at a non-detectable level (< O.1 mg/L). Records are maintained. Specify method of determination_____**
K	11		7.	Make-up water is free from trace (< 0.05mg/L) dissolved metals specifically Cd, Cr, Cu, Ni, Pb, and Zn as determined annually with total heavy metal content ≤ 0.1 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 run with each batch of media prepared from its components as a check for media productivity. Results recorded and records maintained.**
O	9		13.	Sterile phosphate buffered dilution water or 0.5% peptone water is used as the sample diluent. *(circle appropriate choice)*
K	11		14.	pH is determined after sterilization to ensure that it is consistent with manufacturer's requirements and records are maintained.

CODE	REF.		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 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	9		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 head space 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 immediately placed in a cooler which is maintained between 0°C and 10°C until examined. Samples are to be delivered to the laboratory within 6 hours of collection of the first sample.**
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 to be tested if they are held beyond 8 hours of collection, regardless of refrigeration.**

CODE	REF.	Bacteriological Examination of Seawater by the APHA MPN		
C	**9**		**1.**	**Lactose broth or lauryl tryptose broth is used as the presumptive medium.** ***(circle appropriate one)***
C	**9**		**2.**	**Sample and dilutions of sample are mixed vigorously (25 times in a 30cm arc in 7 seconds) before inoculation.**
C	**9**		**3.**	**In a multiple dilution series 5 tubes per dilution are used.**
C	**6**		**4.**	**For depuration, a single dilution series of between 5 and 12 tubes may be 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.
C	**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.

CODE	REF.	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 are made to BGB/EC by either sterile loop or sterile hardwood applicator stick from positive presumptives incubated for 24 and 48 hours ***(circle the method of transfer).***
K	2		4.	When the inoculation of both EC and BGB broths is performed using the same loop or transfer stick, the order or 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.**

CODE	REF.	Computation of results		
K	9		1 .	Results of multiple dilution tests are read from tables in *Recommended Procedures,* 4th 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.

CODE	REF.	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 30 cm arc in 7 seconds) before inoculation.**
C	**9**		**3.**	**In a multiple dilution series 5 tubes per dilution are used.**
C	**6**		**4.**	**For depuration, a single dilution series of between 5 and 12 tubes may be 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_____ _____
C	**11**		**6.**	**Positive and negative control cultures accompany samples throughout the procedure. Records are maintained.** **Positive control _____ Negative control _____**
C	**5**		**7.**	**Inoculated media are incubated at 35° ± 0.5°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 ± 0.2°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.**

CODE	REF.	Computation of results		
K	9		1.	Results of multiple dilution tests are read from tables in Recommended Procedures, 4th 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 (minimum 10 - 12 live animals).**
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 O° 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.**

CODE	REF.		Preparation of Shellstock for Examination	
K	2		1.	Shucking knives, scrub brushes, and blender jar 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 100 grams of shellfish meat is used for analysis (based on a minimum of 10 - 12 live animals).
K	9		11.	The sample is weighed to the nearest gram and an equal amount by weight of (tempered for ETCP) diluent is added (to produce a 1 in 2 dilution).
O	9		12.	Sterile phosphate buffered dilution water or 0.5% peptone water is used as the sample diluent ***(circle appropriate choice)***
K	13		13.	Sterile phosphate buffered saline is used as a sample diluent for ETCP procedure
C	**9**		**14.**	**Samples are blended at high speed for 60 to 120 seconds.**
K	9		15.	For other than shellstock, APHA *Recommended Procedures* are followed for the examination of freshly shucked and frozen shellfish meats.

CODE	REF.		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.**	**From the initial 1 in 2 dilution, a 1 in 10 dilution is prepared (20 g of 1 in 2 dilution added to 80 g diluent). From the 1 in 10 dilution a 1 in 100 dilution is prepared (10 g of 1 in 10 dilution added to 90 g diluent). A 5 tube dilution series is inoculated using 10 mL and 1 mL from the 1 in 10 dilution and 1 mL from the 1 in 100 dilution.**
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	**11**		**6.**	**Positive and negative control cultures accompany samples throughout the procedure. Records 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.

CODE	REF.		Confirmed Test For Fecal Coliform - 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 ***(circle the method of transfer).***
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.**

CODE	REF.		Computation of results	
K	9		1.	Results of multiple dilution tests are read from tables in Recommended Procedures, 4th 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 g of sample.

CODE	REF.		Standard Plate Count Method	
K	9		1.	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	9		2.	15 to 20 mL of tempered sterile plate count agar is used.
K	9		3.	Agar tempering bath maintains the agar at 44° to 46°C.
O	9		4.	Temperature control of the plate count agar is used in the tempering bath.
K	11		5.	Not more than 1 mL nor less than 0.1 mL of sample or sample dilution is plated.
C	**9**		**6.**	**Samples or sample dilutions to be plated are mixed vigorously (25 times in a 30 cm arc in 7 seconds) before plating.**
K	9		7.	Control plates are used to check the sterility of the air, agar and the diluent.
K	9		8.	Solidified plates are incubated at 35° ± 0.5°C for 48 ± 3 hours inverted and stacked not more than 4 high.
K	9		9.	Quebec Colony Counter or its equivalent is used to provide the necessary magnification and visibility for counting plates.
K	13		10.	A hand tally or its equivalent is used for accuracy in counting.

CODE	REF.		Computation of Results	
K	9		1.	Colony counts determined in accordance with Part III, A, Sections 4.31 through 4.33 *Recommended Procedures,* 4th Edition.
O	9		2.	Colony counts reported as APC/g of sample.

CODE	REF.		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	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	**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	3		7.	Container is gently swirled or rotated to mix contents which are then distributed uniformly over 6 to 8 petri plates.
C	**1**		**8.**	**Media and diluent sterility is determined with each use. Results 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**		**10.**	**Plates are incubated inverted within 3 hours of plating in air at 45.5° ± O.5°C for 18 to 30 hours. Plates are stacked not more than four high.**
C	**3**		**11.**	**Incubator temperature maintained at 45.5° ± 0.5°C.**

CODE	REF.		Expression of Results	
K	11		1.	Quebec Colony Counter or its equivalent is used to provide the necessary magnification and visibility.
O	13		2.	A hand tally or its equivalent is used to aid in counting.
C	**3**		**3.**	**All brick red colonies greater than 0.5 mm in diameter are totaled over all the plates and multiplied by a factor of 16.7 to report results as CFU/100 grams of sample.**

REFERENCES

1 Compendium of Methods for the Microbiological Examination of Foods, 2nd Edition, APHA, 1984

2 Good Laboratory Practice.

3 Interim Guides for the Depuration of the Northern Quahog *Mercenaria mercenaria,* Northeast Marine Health Sciences Laboratory, North Kingstown, RI, 1968.

4 NBS Monograph 150, U.S. Department of Commerce, Washington, D.C., 1976.

5 Official Methods of Analyses of the Association of Official Analytical Chemists, 15th Edition, 1990.

6 Proceeding 8th National Shellfish Sanitation Workshop, 1984.

7 Public Health Service, Public Health Report, Reprint # 1621, 1947.

8 Quality Assurance Principles for Analytical Laboratories, Association of Official Analytical Chemists, 1991.

9 Recommended Procedures for the Examination of Sea Water and Shellfish, 4th Edition, American Public Health Association, 1970.

10 Shellfish Sanitation Interpretation #SS-39, Interstate Shellfish Sanitation Conference, 1986.

11 Standard Methods for the Examination of Water and Wastewater, 18th Edition, APHA/WEF/AWWA, 1992.

12 Title 21, Code of Federal Regulations, Part 58, Good Laboratory Practice for Non-clinical Laboratory Study, Washington, D.C.

13 Standard Methods for the Examination of Dairy Products, 16th Edition, APHA, 1992.

LABORATORY:			DATE OF EVALUATION
SHELLFISH LABORATORY EVALUATION CHECKLIST SUMMARY OF NON-CONFORMITIES			
Page	**Item**	**Observation**	**Documentation Required**

LABORATORY STATUS	
LABORATORY:	DATE:
LABORATORY REPRESENTATIVE:	

MICROBIOLOGICAL COMPONENT: (PART I - III)

A. Results

Total # of Critical (C) Non-conformities in Parts I through III _____

Total # of Key (K) Non-conformities in Parts I through III _____

Total # of Critical, Key & Other (O) Non-conformities in Parts I through 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 non-conformities is ≥4 or

b) The total # of Key non-conformities is ≥13 or

c) The total # of Critical, Key, and Other is ≥18 (not to exceed the Critical and Key Criteria)

2. Provisionally Conforms Status; The microbiological component of this laboratory is determined to be provisionally conforming to NSSP requirements if the number of critical non-conformities is ,≥1 but ≤ 3 (not to exceed Key and Total criteria.)

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 _____

CSSP ver. 1-31-02