APPENDIX I

LABORATORY PROCEDURES

This Appendix provides Shellfish Program laboratories with information on: analytical methods and quality assurance procedures associated with the examination of seawater and shellfish; references and information necessary for conducting bacteriological, toxicological, chemical and physical tests; and guidance for development and implementation of quality assurance procedures. Adherence to the procedures identified in this Appendix will provide the uniformity necessary to produce reliable laboratory results upon which public health decisions can be made in determining whether shellfish are suitable for human consumption.

1. Bacteriological Procedures

American Public Health Association (APHA) *Laboratory Procedures for the Examination of Seawater and Shellfish* or equivalently Health Canada's Health Protection Branch Method MFHPB-19, *Enumeration of Coliforms, Faecal coliforms and of E. coli in foods using the MPN method* (Compendium of Analytical Methods, HPB Methods of Microbiological Analysis, Volume 2), shall be followed for the collection, transportation and examination of samples of shellfish and shellfish waters (Greenburg & Hunt 1984). The official reference for the examination of shellfish for *Vibrio parahaemolyticus* is Health Canada's Health Protection Branch Method MFLP-39a, *Detection of Vibrio Species,* (Compendium of Analytical Methods, HPB Methods of Microbiological Analysis, Volume 3) or equivalently, the U.S. Food and Drug Administration 2001 Bacteriological Analytical Manual Online. Available at: http://www.cfsan.fda.gov/~ebam-9.html [2001, June 15]. Laboratories should conduct the test for this organism when routine tests of marine foods suspected in foodborne outbreaks fail to demonstrate other enteric pathogens or bacterial toxins (Ratcliffe & Wilt 1971).

The multiple tube fermentation technique is most commonly used to estimate bacterial numbers in seawater and shellfish. This technique uses the principle of dilution to extinction to estimate the number of bacteria in a sample. Decimal dilutions of the sample are introduced into replicate tubes of a medium designed to select for growth of the particular organism being enumerated. Thus it reasonably can be assumed that the maximum dilution at which growth occurs represents a volume containing a single organism. The results of such an analysis are

expressed in terms of the Most Probable Number (MPN). This represents an estimate based on probability formulae.

The laboratory must be evaluated and approved triennially by a DOE or CFIA Laboratory Evaluation Officer (LEO) using the most recent CSSP Laboratory Evaluation Checklist (Annex 1). Quality assurance guidelines to be used are established below. In addition, the laboratory shall take part in an inter-laboratory analysis program (samples of unknown source) at least once per year.

Bacteriological water quality standards, based on fecal coliform levels, as determined by the MPN method, are presently in use for the classification of shellfish growing waters. Bacteriological shellstock count standards based on fecal coliform levels, as determined by the MPN method, are presently in use for the evaluation of depuration effectiveness and verification data to open areas closed under a management plan. Bacteriological shellstock count standards based on *E. coli* levels as determined by the MPN method, are presently in use for the evaluation of a facility's Quality Management Program (QMP).

Sample Condition

All water samples are to be held at a temperature below 10° C during a maximum transport time of 6 hours. Refrigerate these samples upon receipt in the laboratory and process within 2 h. When local conditions necessitate delays in delivery of samples longer than 6 h, consider making field examinations using field laboratory facilities located at the site of collection. No other method of sample preservation is acceptable. A minimum of 100 mL of water sample is required for this test, and only sterile glass or polypropylene bottles should be used. A complete list of sampling requirements can be found in the CSSP Water Sample Collection Checklist (Annex 2 - to be issued at a later date).

Shellstock samples should be collected in clean, waterproof and puncture resistant containers. Approximately 10-12 or more animals (sufficient to yield 150-250 g), free of open or cracked shells are required for each shellstock sample. Shellstock samples should be kept and transported in dry storage at 10° C or below but above 0° C until examined. Shellstock should not be allowed to come in direct contact with ice. Shellstock samples should be submitted to the laboratory as quickly as possible and analysed within 24 hours of collection.

Interference

Bacteriostatic or bactericidal agents, such as chlorine, silver, lead, and various organic complexes, can significantly reduce bacterial densities in a sample. Contaminating nutrients can cause unwanted growth of organisms in the sample which would result in an overestimation of bacterial densities.

Both of these problems can be greatly reduced by insuring that:

a) all glassware used in the analyses is free from such substances;

b) distilled/deionized water used in media preparation is not contaminated with bacterial, fungal or algal growth; and

c) samples are processed as quickly as possible after collection.

Growth of certain organisms in the test media which are not of importance to the specific analysis performed can give false positive results, thereby overestimating the true bacterial density. However, the specificity of the test media normally eliminates most of these organisms. Incubation temperatures are critical, and slight changes can alter the kinds and numbers of bacteria growing in the test media.

Precision and Accuracy

The bacterial density calculated by the MPN method is a statistical estimation and should be treated as such. The 95 percent confidence limits for the 5-tube MPN test, range between 24% and 324% of the MPN; thus, the results of a single sample are by no means conclusive. Accuracy increases with increased sampling, and normally a minimum of five samples are required at each sample location to better approximate the true bacterial density.

Apparatus

- Sterile 10.0 mL and 1.0 mL serological pipettes.
- Sterile applicator sticks or 5 mm inoculating loops (platinum*).
- 35 ± 0.5° C air incubator.
- 44.5 ± 0.2° C or dual temperature programmable waterbath.
- Sterile 250 mL wide-mouth sample bottles*.
- 20 x 150 mm Pyrex test tubes and caps*.
- 16 x 150 mm Pyrex test tubes and caps*.

- 6 x 50 mm culture tubes (Durham tubes).
- Test tube racks.
- Autoclave.
- Sterile Pasteur pipettes.
- Milk dilution bottles*, 160 mL.
- Blender.
- 1.0 L (minimum size) blender jars*.
- sterile shucking knife and/or scalpel.
- sterile stiff brush

* Or suitable substitutes which meet or exceed CSSP Laboratory Evaluation requirements

Bacteriological Media and Reagents

With the exception of A-1 medium (which must be prepared from its individual components) and Modified MacConkey Agar (which may be prepared from its individual components), all other media listed are commercially available in a dehydrated form.

Lauryl Tryptose Broth (LTB)

This medium is commercially available.
Tryptose - 20.0 g
Lactose - 5.0 g
K_2HPO_4 - 2.75 g
KH_2PO_4 - 2.75 g
NaCl - 5.0 g
Sodium lauryl sulfate - 0.1 g
Distilled/deionized water - 1.0 L

Suspend 35.6 g in 1.0 L of distilled or deionized water and warm slightly to dissolve completely. Double strength media is prepared using the above amounts dissolved in 500 mL of water. Dispense 10 mL aliquots into tubes containing inverted fermentation vials. Autoclave at 121°C for 15 minutes. The pH of the medium should be 6.8 after sterilization.

Brilliant Green Bile 2% Broth (BGB)

This medium is commercially available.
Peptone - 10.0 g
Lactose - 10.0 g
Oxgall - 20.0 g
Brilliant Green - 0.0133 g
Distilled/deionized water - 1.0 L

Suspend 40 g in 1.0 L of distilled or deionized water and warm slightly to dissolve completely. Dispense 5 to 10 mL aliquots into tubes containing inverted fermentation vials. Autoclave at 121°C for 15 minutes. The pH of the medium should be 7.2 after sterilization.

EC Medium

This medium is commercially available.
Tryptose or trypticase - 20.0 g
Lactose - 5.0 g
Bile salts No. 3 - 1.5 g
K_2HPO_4 - 4.0 g
KH_2PO_4 - 1.5 g
NaCl - 5.0 g
Distilled/deionized water - 1.0 L

Suspend 37 g of the powder in 1.0 L of distilled or deionized water and warm slightly to dissolve completely. Dispense 5 to 10 mL aliquots into tubes containing inverted fermentation vials. Autoclave at 121°C for 15 minutes. The pH of the medium should be 6.9 after sterilization.

A-1 Medium

Lactose - 5.0 g
Tryptone - 20.0 g
NaCl - 5.0 g
Salicin - 0.5 g
Triton X-100 - 1.0 mL
Distilled/deionized Water - 1.0 L

Suspend the above ingredients in 1.0 L of distilled or deionized water. Mix thoroughly then add 1 mL of Triton X-100 and continue mixing until dissolved completely. Double strength media is prepared using the above amounts dissolved in 500 mL of water. Dispense 10 mL aliquots into tubes containing inverted fermentation vials. Autoclave at 121°C for 10 minutes. The pH of the medium should be 6.9 after sterilization.

Levine's Eosin Methylene Blue Agar

This medium is commercially available
Pancreatic Digest of Gelatin - 10.0 g
Lactose - 10.0 g
K_2HPO_4 - 2.0 g
Eosin Y - 0.4 g

Methylene Blue - 0.065 g
Agar - 15.0 g
Distilled/deionized Water - 1.0 L

Suspend 37.4 g of the powder in 1.0 L of distilled or deionized water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121°C for 15 minutes. The pH of the medium should be 7.0 after sterilization. Allow to cool to approximately 45°C and pour into petri dishes. Allow plates to cool to room temperature.

Plate Count Agar (or Standards Methods Agar)

This medium is commercially available
Pancreatic Digest of Casein - 5.0 g
Yeast extract - 2.5 g
Dextrose - 1.0 g
Agar - 15.0 g
Distilled/deionized water - 1.0 L

Suspend 23.5 g of the powder in 1.0 L of distilled or deionized water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121°C for 15 minutes. The pH of the medium should be 7.0 after sterilization.

Modified MacConkey Agar (Double strength)

Peptone - 34.0 g
Polypeptone - 6.0 g
Lactose - 20.0 g
Bile Salts No. 3 - 1.5 g
Agar - 27.0 g
Neutral Red - 0.06 g
Crystal Violet - 0.02 g
Distilled/deionized Water - 1.0 L

Suspend the above ingredients in 1.0 L of distilled/deionized water. Mix thoroughly. Heat with frequent agitation until boiling. Remove from heat and boil again (do not autoclave). Temper in waterbath at 45 - 50°C for up to six hours.

Phosphate Buffer

This buffer is prepared from 2 stock buffer solutions:

Stock phosphate buffer solution: dissolve 34.0 g of potassium dihydrogen phosphate (KH_2PO_4) in 500 mL distilled water, adjust to pH 7.2 with 1 N NaOH (approximately 150 to 175 mL of 1 N NaOH may be required to adjust to pH 7.2), and dilute to 1.0 L with distilled water.

Magnesium Chloride solution:
Dissolve 81.1 g $MgSO_4 \cdot 6H_2O$ in 1.0 L distilled/deionized water

Final Phosphate buffer dilution water:
1.25 mL Stock phosphate buffer solution
5.0 mL Magnesium Chloride solution
1.0 L distilled/deionized water

Fill dilution bottles or tubes with dilution water so that after sterilization (autoclave at 121°C for 15 minutes) they will contain the quantity desired with a tolerance of ± 2%.

0.5% Peptone Water

Peptone or gelysate - 5.0 g
Distilled/deionized water - 1.0 L

Dissolve peptone in distilled/deionized water and fill dilution bottles or tubes with dilution water so that after sterilization (autoclave at 121°C for 15 minutes) they will contain the quantity desired with a tolerance of ± 2%.

Procedure

Water Analysis for Coliform and Fecal Coliform

Generally, five 10 mL aliquots, five 1.0 mL aliquots, and five 0.1 mL aliquots of the sample are aseptically inoculated into test tubes containing Lauryl Tryptose Broth (LTB). The 10 mL aliquots are inoculated into double strength LTB. It is necessary to perform serial 1/10 dilutions on some samples to prevent indeterminate results. Dilutions are made in phosphate buffered distilled water and should be chosen such that approximately half the tubes give positive results. The tubes are incubated at 35 ± 0.5 °C and examined for the presence of growth accompanied by gas production at 24 (± 2) and 48 (± 4) hours. Growth and gas production are both necessary for a positive result. The MPN is calculated and results are expressed as "Presumptive Coliform MPN/100 mL".

To confirm the presence of coliforms, inocula from 24- and 48-hour positive presumptive tubes are aseptically transferred to tubes of Brilliant Green Bile (2%) Broth. Transfers are done at both 24 and 48 hours after the initial inoculation into Lauryl Tryptose Broth, dependent on time of gas formation in Lauryl Tryptose Broth. The tubes are incubated at 35 ± 0.5° C and examined for growth with gas production at 24 (± 2) and 48 (± 4) hours. Results are expressed as "Confirmed Coliform MPN/100 mL".

To enumerate fecal coliforms, inocula from 24- and 48-hour positive presumptive tubes are aseptically transferred to tubes of EC medium. These tubes are incubated at 44.5 ± 0.2° C for 24 ± 2 hours and examined for the presence of growth with gas production. Results are expressed as "Fecal Coliform MPN/100 mL".

Rapid Fecal Coliform MPN Test (Modified A-1 Method)

Inoculation and dilution procedures for this technique are identical to those described for lauryl tryptose broth in the preceding section except the medium used is A-1 medium. The tubes are incubated for 3 ± 0.5 hours at 35 ± 0.5 °C and then transferred to a waterbath maintained at 44.5 ± 0.2 °C for an additional 21 ± 2 hours incubation. As an alternative, laboratories can use programmable waterbaths to incubate the samples for the full 24 hours. At the completion of the 24 hour incubation period tubes are examined for the presence of both growth and gas. The MPN is calculated and results are expressed as "Fecal Coliform MPN/100 mL". The use of the A-1 medium for the rapid determination of fecal coliforms is presently restricted to fecal coliform enumeration in marine shellfish growing waters and is not applicable to other types of waters or effluents.

Shellfish Analysis

Prior to performing the standard MPN procedure on shellstock, the following sample preparation is required. Shellstock to be used is cleaned prior to shucking. Sterile shucking knives, brushes, and blender jars are used. Prior to shucking, shellstock are scrubbed with a stiff, sterile brush and rinsed under water of drinking water quality. Shellstock are allowed to drain in a clean area prior to shucking. A minimum of 100 g (minimum of 10-12 animals) of shellstock sample (meat and liquor) is aseptically shucked into a sterile, tared blender jar using

sterile shucking equipment. An equal weight of sterile phosphate-buffered dilution water is added to the blender jar, and the contents are blended at high speed for 90-120 seconds. Immediately after blending, 20 grams of this mixture is aseptically added to 80 mL of dilution water resulting in a 1/10 dilution of the original sample. A 1/100 dilution is prepared by aseptically adding 10 mL of the 1/10 dilution into 90 mL of dilution water. The standard MPN procedure (using LTB/EC) is performed using these dilutions with 10 and 1 mL aliquots inoculated from the 1/10 dilution and 1 mL aliquots from the 1/100 dilution.

Calculations

MPN values, expressed as MPN/100 mL, for those tube codes which normally occur are presented in the following Table for 5-tube MPN procedures. If dilutions are performed on the sample, the MPN value appearing in the table is multiplied by the appropriate dilution factor.

MOST PROBABLE NUMBERS (MPN)
per 100 mL of sample
planting 5 portions in each of 3 dilutions in geometric series

No. of Positive tubes 10 1 .1 (mL)	MPN	No. of Positive tubes 10 1 .1 (mL)	MPN	No. of Positive tubes 10 1 .1 (mL)	MPN	No. of Positive tubes 10 1 .1 (mL)	MPN	No. of Positive tubes 10 1 .1 (mL)	MPN	No. of Positive tubes 10 1 .1 (mL)	MPN
0 0 0		1 0 0	2.0	2 0 0	4.5	3 0 0	7.8	4 0 0	13	5 0 0	23
0 0 1	1.8	1 0 1	4.0	2 0 1	6.8	3 0 1	11	4 0 1	17	5 0 1	31
0 0 2	3.6	1 0 2	6.0	2 0 2	9.1	3 0 2	13	4 0 2	21	5 0 2	43
0 0 3	5.4	1 0 3	8.0	2 0 3	12	3 0 3	16	4 0 3	25	5 0 3	58
0 0 4	7.2	1 0 4	10	2 0 4	14	3 0 4	20	4 0 4	30	5 0 4	76
0 0 5	9.0	1 0 5	12	2 0 5	16	3 0 5	23	4 0 5	36	5 0 5	95
0 1 0	1.8	1 1 0	4	2 1 0	6.8	3 1 0	11	4 1 0	17	5 1 0	33
0 1 1	3.6	1 1 1	6.1	2 1 1	9.2	3 1 1	14	4 1 1	21	5 1 1	46
0 1 2	5.5	1 1 2	8.1	2 1 2	12	3 1 2	17	4 1 2	26	5 1 2	64
0 1 3	7.3	1 1 3	10	2 1 3	14	3 1 3	20	4 1 3	31	5 1 3	84
0 1 4	9.1	1 1 4	12	2 1 4	17	3 1 4	23	4 1 4	36	5 1 4	110
0 1 5	11	1 1 5	14	2 1 5	19	3 1 5	27	4 1 5	42	5 1 5	130
0 2 0	3.7	1 2 0	6.1	2 2 0	9.3	3 2 0	14	4 2 0	22	5 2 0	49
0 2 1	5.5	1 2 1	8.2	2 2 1	12	3 2 1	17	4 2 1	26	5 2 1	70
0 2 2	7.4	1 2 2	10	2 2 2	14	3 2 2	20	4 2 2	32	5 2 2	95
0 2 3	9.2	1 2 3	12	2 2 3	17	3 2 3	24	4 2 3	38	5 2 3	120
0 2 4	11	1 2 4	15	2 2 4	19	3 2 4	27	4 2 4	44	5 2 4	150
0 2 5	13	1 2 5	17	2 2 5	22	3 2 5	31	4 2 5	50	5 2 5	180
0 3 0	5.6	1 3 0	8.3	2 3 0	12	3 3 0	17	4 3 0	27	5 3 0	79
0 3 1	7.4	1 3 1	10	2 3 1	14	3 3 1	21	4 3 1	33	5 3 1	110
0 3 2	9.3	1 3 2	13	2 3 2	17	3 3 2	24	4 3 2	39	5 3 2	140
0 3 3	11	1 3 3	15	2 3 3	20	3 3 3	28	4 3 3	45	5 3 3	180
0 3 4	13	1 3 4	17	2 3 4	22	3 3 4	31	4 3 4	52	5 3 4	210
0 3 5	15	1 3 5	19	2 3 5	25	3 3 5	35	4 3 5	59	5 3 5	250
0 4 0	7.5	1 4 0	11	2 4 0	15	3 4 0	21	4 4 0	34	5 4 0	130
0 4 1	9.4	1 4 1	13	2 4 1	17	3 4 1	24	4 4 1	40	5 4 1	170
0 4 2	11	1 4 2	15	2 4 2	20	3 4 2	28	4 4 2	47	5 4 2	220
0 4 3	13	1 4 3	17	2 4 3	23	3 4 3	32	4 4 3	54	5 4 3	280
0 4 4	15	1 4 4	19	2 4 4	25	3 4 4	36	4 4 4	62	5 4 4	350
0 4 5	17	1 4 5	22	2 4 5	28	3 4 5	40	4 4 5	69	5 4 5	430
0 5 0	9.4	1 5 0	13	2 5 0	17	3 5 0	25	4 5 0	41	5 5 0	240
0 5 1	11	1 5 1	15	2 5 1	20	3 5 1	29	4 5 1	48	5 5 1	350
0 5 2	13	1 5 2	17	2 5 2	23	3 5 2	32	4 5 2	56	5 5 2	540
0 5 3	15	1 5 3	19	2 5 3	26	3 5 3	37	4 5 3	64	5 5 3	920
0 5 4	17	1 5 4	22	2 5 4	29	3 5 4	41	4 5 4	72	5 5 4	1600
0 5 5	19	1 5 5	24	2 5 5	32	3 5 5	45	4 5 5	81	5 5 5	>1600

Recommended Procedures for the Examination of Sea Water and Shellfish, 4th edition, 1970

2. Toxicological

Current Association of Official Analytical Chemists (AOAC) and APHA official methods shall be followed in the bioassay for PSP (Greenburg & Hunt 1984, AOAC 1995). Methods validated by Canadian Food Inspection Agency laboratories shall be followed for the determination of Domoic Acid.

3. Chemical and Physical

a) Current AOAC and APHA official methods shall be followed in making chemical and physical determinations.

b) Results of all chemical and physical determinations shall be expressed in standard units. (For example, salinity should be expressed in parts per thousand rather than hydrometer readings).

4. Quality Assurance

The CSSP laboratory (government or private) shall ensure that all samples are collected, preserved, transported and analysed in a manner that assures the validity of the analytical results. To ensure this, the CSSP laboratory shall:

a) Develop a quality assurance plan specific to the laboratory. The QA plan shall:

- describe the organization of the laboratory;
- describe staff training requirements and maintain records of training;
- include written Standard Operating Procedures (SOP’s) for all procedures conducted by the laboratory;
- describe and maintain records for internal quality control measures for equipment calibration, maintenance, repair and performance checks;
- describe laboratory safety issues and maintain applicable records (training, MSDS’s);
- describe and maintain records of internal laboratory performance assessment;
- describe and maintain records of external laboratory performance assessment.

b) Participate in annual proficiency testing programs. For example, each March, the FDA Laboratory Quality Assurance Branch (Summit Argo, Illinois) sponsors an annual shellfish split sample program whereby samples of a mashed potato matrix containing unknown amounts of various bacteria are

shipped to all participating shellfish laboratories. Participating laboratories must comply with biocontainment level 2. The service is free and international in scope.

c) Participate in triennial onsite laboratory evaluations. Continued acceptance of microbiological data in support of the CSSP from any operating CSSP laboratory (government or private) is contingent upon being found to conform or provisionally conform to CSSP requirements as determined during the most recent laboratory evaluation using the most recent version of the CSSP Shellfish Laboratory Evaluation Checklist (see last page of checklist for laboratory approval criteria) This checklist is used during triennial laboratory evaluations conducted by an FDA Laboratory Evaluation Officer (LEO) or a CSSP LEO.

ANNEX IA

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.**

CSSP ver. 1-31-02

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.

CSSP ver. 1-31-02

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.**

CSSP ver. 1-31-02

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