Proposal Subject	Microbiology Checklist for m-Tec Procedure
Specific NSSP Guide Reference	NSSP Guide - Guidance Documents Chapter II. Growing Areas .11 Evaluation of Laboratories by State Shellfish Laboratory Evaluation Officers Including Laboratory Evaluation Checklists
Text of Proposal/ Requested Action	The Laboratory Evaluation Checklist – Pages 2, 10, 11, and 16 of the Microbiology of the Guidance Documents, Chapter II. Growing Areas, .11 Evaluation of Laboratories by State Shellfish Laboratory Evaluation Officers Including Laboratory Evaluation Checklists is attached as Attachment 1. It includes a section for the m-TEC procedure used to detect fecal coliforms. The recommended changes are included in the pages listed above.
Public Health Significance	The m-TEC procedure which determines the levels of fecal coliforms in shellfish growing waters was approved at the ISSC Meeting in 2003 in Portland, Oregon without the completion of a laboratory checklist. Laboratory Evaluation Officers need this document immediately to correctly evaluate any laboratory performing the m-TEC procedure.
Cost Information (if available)	None
Action by 2005 Laboratory Quality Assurance Committee	Recommended adoption of Proposal 05-112 as amended (see Attachment 2).
Action by 2005 Task Force I	Recommended adoption of Laboratory Quality Assurance Committee recommendation on Proposal 05-112.
Action by 2005 General Assembly	Adopted recommendation of 2005 Task Force I.
Action by USFDA	Concurred with Conference action.

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Check the applicable analytical methods:	
	Multiple Tube Fermentation Technique for Seawater (APHA)[PART II]
	Multiple Tube Fermentation Technique for Seawater Using MA-1 [PART II]
	Membrane Filtration Technique for Seawater using mTEC [PART II]
	Multiple Tube Fermentation Technique for Shellfish Meats (APHA)[PART III]
	Standard Plate Count for Shellfish Meats [Part III]
	Elevated Temperature Coliform Plate Method for Shellfish Meats [PART III]

PART 1 – QUALITY ASSURANCE			
CODE	**REF**		**ITEM**
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
C	8		2. QA Plan Implemented
K	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.
O	11		6. Pipet aid used, mouth pipetting not permitted.

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CODE	REF.	Bacteriological Examination of Seawater by Membrane Filtration using mTEC Agar	
		Equipment	
C	23, 24		1. When used for elevated temperature incubation, the temperature of the hot air incubator is maintained at 44.5+0.5°C under any loading capacity.
C	23		2. When using a waterbath for elevated temperature incubation, the level of the water completely covers the plates.
C	23		3. Pre-sterilized plastic or sterile glass culture plates that are clear, flat bottomed, free of bubbles and scratches with tight fitting lids are used.
K	11		4. Colonies are counted with the aid of magnification.
C	11, 23		5. Membrane filters are made from cellulose ester material, white, grid marked, 47 mm in diameter with a pore size of 0.45 µm and certified by the manufacturer for fecal coliform analyses.
O	2		6. Lot number, date of receipt and if provided the expiration date of the membrane filters are recorded.
K	2, 11		7. New lots of membrane filters are checked by comparing recovery of fecal coliform organisms against membrane filters from previously acceptable lots.
C	2		8. The sterility of each lot or autoclave batch of membrane filters are checked before use.
K	2		9. Membrane filters which are beyond their expiration date are not used.
O	11		10. Forceps tips are clean.
O	11		11.Forceps tips are smooth without pitting or corrugations to damage the filters being manipulated.
K	11		12. Forceps are dipped in alcohol and flame sterilized between sample filters.
K	11		13. If indelible graduation marks are used on clear glass or plastic funnels to measure sample volumes, their accuracy is checked with a Class A graduated cylinder before use and periodically rechecked. Funnels having a tolerance greater than 2.5% are not used. Checks are recorded and records maintained.
K	11		14. Membrane filtration units are made of stainless steel, glass or autoclavable plastic free of scratches, corrosion and leaks.
C	11		15. Membrane filter assemblies are autoclave sterilized for 15 minutes at 121°C prior to the start of a filtration series.
O	11, 23, 26		16. A UV sterilization unit is used to disinfect filter assemblies between sample and filtration runs.
K	11		17. If used, the effectiveness of the UV sterilization unit is determined by biological testing monthly. Results are recorded and records maintained.
		Media Preparation and Storage	
K	11		1. Phosphate buffered saline is used as the sample diluent.
C	11		2. Phosphate buffered saline is properly sterilized.
K	23		3. A sufficient amount of medium (4-5 ml) is used in each plate.
O	11		4. Refrigerated prepared plates are stored for no more than 2 weeks in sealed plastic bags or containers to minimize evaporation.
		Sample Analysis	
C	24		1. mTEC agar is used.

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C	23		2. The sample is mixed vigorously (25 times in a 12" arc in 7 seconds) before filtration.
C	23		3. The membrane is placed grid side up within the sterile filter apparatus.
C	23, 25		4. Sample volumes tested are consistent with the sampling regime employed (i.e. half log or other appropriate dilutions are used with systematic random sampling).
C	23		5. Sample volumes are filtered under vacuum.
K	26		6. The pressure of the vacuum pump does not exceed 15 psi.
C	23, 26		7. The sides of the filter funnel are rinsed at least twice with 20-30 ml of sterile phosphate buffered saline after sample filtration.
C	23		8. The membrane filter is removed from the filtering apparatus with sterile forceps and rolled onto mTEC agar so that no bubbles form between the filter and the agar.
C	11		9. Blanks are run at the beginning of filtration, after every 10th sample and at the end of the filtration run to check the sterility of the testing system (phosphate buffered saline, filter funnel, forceps, membrane filter, media and culture plate).
K	2, 11		10. Positive and negative control cultures treated like samples accompany test samples throughout the procedure. Positive control _____ Negative control _____ Results are recorded and records maintained.
C	11, 23, 24		11. Inoculated plates are placed inverted either directly in an air incubator or in a watertight, tightly sealed containers at 35 + 0.5°C for 2 hours of resuscitation prior to waterbath incubation or in Ethyfoam for incubation in air at 44.5 +0.5°C.
C	11, 23, 24		12. After 2 hours of resuscitation at 35°C watertight sealed containers are transferred to a circulating waterbath at 44.5 + 0.2°C, submerged completely and incubated for 22-24 hours. Individual plates are transferred inverted to a watertight container, tightly sealed and submerged completely in a circulating waterbath at 44.5 + 0.2°C for 22-24 hours of incubation.
		Computation of Results	
C	23		1. All yellow, yellow-green or yellow-brown colonies are counted.
C	23		2. Only plates having 80 or fewer colonies are counted. If it is necessary to use plates having more than 80 colonies, counts are given as >80 x 100/the volume filtered.
K	23, 11		3. The number of fecal coliforms is calculated by the following equation: Number of fecal coliforms per 100 ml =[number of colonies counted/volume of sample filtered in ml] x 100.
K	23, 11		4. Results are reported as CFU/100 ml of sample.

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25.	**FDA Manual of Interpretations, National Shellfish Sanitation Program *Guide for the Control of Molluscan Shellfish*, 2003 Revision, Interpretation Number 03-IV-@.02-102.**
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			Equipment
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C	**23**		**2. When using a waterbath for elevated temperature incubation, the level of the water completely covers the plates.**
C	**23**		**3. Pre-sterilized plastic or sterile glass culture plates that are clear, flat bottomed, free of bubbles and scratches ~~with tight fitting lids~~ are used.**
K	11		4. Colonies are counted with the aid of magnification.
C	**11, 23**		**5. Membrane filters are made from cellulose ester material, white, grid marked, 47 mm in diameter with a pore size of 0.45 µm and certified by the manufacturer for fecal coliform analyses.**
O	2		6. Lot number, date of receipt and if provided the expiration date of the membrane filters are recorded.
K	2, 11		7. New lots of membrane filters are checked by comparing recovery of fecal coliform organisms against membrane filters from previously acceptable lots.
C	**2**		**8. The sterility of each lot or autoclave batch of membrane filters are checked before use.**
K	2		9. Membrane filters which are beyond their expiration date are not used.
O	11		10. Forceps tips are clean.
O	11		11.Forceps tips are smooth without pitting or corrugations to damage the filters being manipulated.
K	11		12. Forceps are dipped in alcohol and flame sterilized between sample filters.
K	11		13. If indelible graduation marks are used on clear glass or plastic funnels to measure sample volumes, their accuracy is checked with a Class A graduated cylinder before use and periodically rechecked. Funnels having a tolerance greater than 2.5% are not used. Checks are recorded and records maintained.
K	11		14. Membrane filtration units are made of stainless steel, glass, pre-sterilized or autoclavable plastic free of scratches, corrosion and leaks.
C	**11**		**15. Membrane filter assemblies are autoclave sterilized for 15 minutes at 121°C prior to the start of a filtration series unless pre-sterilized by the manufacturer.**
O	11, 23, 26		16. A UV sterilization unit is used to disinfect filter assemblies between sample and filtration runs.
K	11		17. If used, the effectiveness of the UV sterilization unit is determined by biological testing monthly. Results are recorded and records maintained.
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K	11		1. Phosphate buffered saline is used as the sample diluent.
C	**11**		**2. Phosphate buffered saline is properly sterilized.**
K	23		3. A sufficient amount of medium (4-5 ml) is used in each plate.
O	11		4. Refrigerated prepared plates are stored for no more than 2 weeks in sealed plastic bags or containers to minimize evaporation.
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C	**24**		**1. mTEC agar is used.**

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C	**23**		**2. The sample is mixed vigorously (25 times in a 12" arc in 7 seconds) before filtration.**
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C	**23, 25**		**4. Sample volumes tested are consistent with the sampling regime employed (i.e. half log or other appropriate dilutions are used with systematic random sampling).**
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C	**23, 26**		**7. The sides of the filter funnel are rinsed at least twice with 20-30 ml of sterile phosphate buffered saline after sample filtration.**
C	**23**		**8. The membrane filter is removed from the filtering apparatus with sterile forceps and rolled onto mTEC agar so that no bubbles form between the filter and the agar.**
C	**11**		**9. Blanks are run at the beginning of filtration, after every 10th ~~sample~~aliquot and at the end of the filtration run to check the sterility of the testing system (phosphate buffered saline, filter funnel, forceps, membrane filter, media and culture plate).**
K	2, 11		10. Positive and negative control cultures treated like samples accompany test samples throughout the procedure. Positive control _____ Negative control _____ Results are recorded and records maintained.
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