Proposal 03-115 QA/QC Checklist Modifications

Guidance Documents
Chapter II Growing Areas
11. Evaluation of Laboratories…Including…Checklists
NSSP Form Lab-100 rev. 2001- 11- 27

	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 Filter 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]

Laboratory Evaluation Checklist – Microbiology - 3

CODE	**REF.**		**Equipment**
K	23		18. The level of water in the water bath totally immerses the petri dishes.
K	2		25. Membrane filtration units are stainless steel, glass, or autoclavable plastic that are not scratched or corroded or do not leak.
K	23		26. 10 to 15 X stereomicroscope with a fluorescent light source is used to count colonies.
C	23		**27. Membrane filters are made from cellulose ester material, white, grid marked, 47mm diameter, 0.45um pore size and certified by the manufacturer for fecal coliform analyses.**
O	2		28. Lot number and date received are recorded for membrane filters.
K	2		29. New lots of membrane filters are checked by comparing recovery of coliform organisms against membrane filters for previously acceptable lots.
O	2		30. Forcep tips are clean and without pitting or corrugations.
K	2		31. If graduation marks on clear glass or plastic funnels are used to measure sample volume, their accuracy is checked with a Class A graduated cylinder before use and a record is maintained. It must be replaced if it has a tolerance greater than 2.5%.
C	2,23		**32. Presterilized plastic or sterilized glass culture dishes are used.**
K	2		33. Dishes are clear, flat bottom, free from bubbles and scratches.

Renumber current 18-23 to 19-24.

Laboratory Evaluation Checklist – Microbiology – 5

CODE	**REF**		**Sterilization and Decontamination**
K	2,11		20. Membrane filter assemblies are autoclaved for 15 minutes at 121º C at the start of the filtration series.
C	2		**21. Sterility of each lot number or autoclave batch of membranes is checked before use.**
K	2		22. UV light sterilization device effectiveness is determined by biological testing monthly.

Renumber current 20-21 to 23-24.

Laboratory Evaluation Checklist – Microbiology – 6

CODE	**REF**		**Media Preparation**
K	9		10. Volume and concentration of media in the tube or petri dish are suitable for the amount of sample inoculated.

Laboratory Evaluation Checklist – Microbiology

CODE	REF		Storage of Prepared Media
O	2		5. Refrigerated prepared plates are stored no more than 2 weeks in sealed plastic bags or containers to minimize evaporation.

Renumber current 5-7 to 6-8.

Laboratory Evaluation Checklist – Microbiology

CODE	REF.		Bacteriological Examination of Seawater by membrane filtration using mTEC Agar
C	23		**1. Sample is mixed vigorously (25 times in a 12" arc in 7 seconds) before filtration.**
C	23		**2. Membrane is placed grid side up within the sterile filter apparatus.**
C	23		**3. Sample volumes are consistent for each water classification area.**
C	23		**4. Sample volumes are filtered under vacuum with the sides of the funnel rinsed twice with 20 to 30ml of sterile buffered rinse water after sample filtration.**
C	23		**5. Membrane is removed from filtering apparatus with sterile forceps and rolled onto mTEC agar so no bubbles form between the membrane and agar.**
K	2		6. Membrane filter assemblies are sterilized by UV radiation between individual sample filtrations.
K	2		7. Blanks are run at the beginning of filtration, after every 10^{th} sample, and at the end of filtration.
K	2		8. Positive and negative control cultures treated like samples accompany the test sample throughout the procedure. Records are maintained. Positive Control _____ Negative Control _____
C	2,23		**9. Inoculated media are placed, inverted, in an air incubator at 35 + 0.5°C for 2 hours of resuscitation.**
C	23		**10. After 2 hours resuscitation at 35° C, inoculated media are transferred to a water tight container with the plate inverted, submerged and incubated at 44.5 + 0.2° C in a circulating water bath for the total incubation time of 22-24 hours.**

CODE	REF.		Computation of Results
C	23		**1. All yellow colonies are counted.**
K	23		2. The number of fecal coliforms are 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		3. Results are reported as CFU/100 ml of sample.

References:

23. United States Environmental Protection Agency, *Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and Escherichia coli*. EPA/821/R-97/004 EPA, Washington, D.C.