COMMONWEALTH OF MASSACHUSETTS
EXECUTIVE OFFICE OF ENERGY & ENVIRONMENTAL AFFAIRS
DEPARTMENT OF ENVIRONMENTAL PROTECTION
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MEMORANDUM

TO: Directors of Massachusetts-Certified Environmental Microbiology Laboratories

FROM: Ann Marie Allen, Director, MassDEP Laboratory Certification Office/WES

RE: Rapid Determination of *E. coli* in Drinking Water under the Total Coliform Rule in Massachusetts (Revised from June 4, 2009 version)

DATE: October 21, 2009

1.0 SUMMARY

The Massachusetts Department of Environmental Protection (MassDEP) Laboratory Certification Office (LCO) will cease offering certification for certain microbiological methods used to analyze samples under the Total Coliform Rule that can require a lengthy time for completion. This memorandum lists methods that provide rapid determination of total coliform and *E. coli* in finished drinking water. It also describes a procedure for the rapid confirmation of the presence/absence of *E. coli* in potable water when using the membrane filtration method, SM 9222B.

Effective December 1, 2009, the MassDEP LCO will no longer offer certification for the following analytes/methods for the analysis of finished drinking water (indicated on the certified parameter lists as Water Treatment and Distribution [P/A]):

Total Coliform	MTF-SM 9221B
Total Coliform	P/A-SM 9221D
Fecal Coliform	EC-SM9221E
E. coli	EC-MUG-SM9221F

Note: Laboratory certification for these methods is still available for the analysis of source water (indicated on the certified parameter list as Source Water [Enumeration]).

2.0 BACKGROUND

The United States Environmental Protection Agency's (USEPA) Total Coliform Rule (TCR) requires drinking water suppliers to routinely test their finished water for the presence of total coliform. If a sample tests positive for total coliform, the water supplier must also determine if fecal coliforms or *E. coli* are present. Because *E. coli* is a more reliable bacterial indicator of drinking water quality than fecal coliform, the MassDEP Drinking Water Program is proposing to withdraw fecal coliform as a bacterial indicator.

Recent years have seen the development and USEPA approval of a number of new methods for the simultaneous detection of total coliform and *E. coli* within 24 hours. While not required by the TCR, many of these tests can also be used to enumerate total coliform and *E. coli*. The availability of critical information to the water supplier within a short period of time following sampling provides improved protection of public health.

Many MassDEP-certified microbiology laboratories are certified for the analysis of total coliforms in potable water using the standard membrane filtration procedure, SM 9222B, from *Standard Methods for the Examination of Water and Wastewater*. In this procedure, presumptive typical and atypical coliform colonies growing on m-Endo agar are counted and are then required to undergo confirmation as a coliform in lauryl tryptose broth (LTB) and brilliant green lactose bile broth (BGLBB). Confirmation as a fecal coliform in EC broth or as *E. coli* in EC-MUG broth is also required. These confirmation tests can take an additional 24 to 96 hours to complete. Given the public health urgency of quickly knowing if a water supply is contaminated with *E. coli*, this delay is not acceptable especially when there are rapid cost-effective tests available for confirming the presence of *E. coli* in a water sample.

3.0 RAPID DETERMINATION OF TOTAL COLIFORM AND *E. COLI*

The following methods provide Total coliform/*E. coli* determinations in 24 hours or less:

1. Total Coliform Membrane Filter Technique (EPA Method 1604 using MI agar)
2. ONPG-MUG Test (SM 9223) (Colilert)
3. Colisure Test
4. E*Colite® Test
5. Readycult® Coliforms 100 Presence/Absence Test
6. Membrane Filter Technique using Chromocult® Coliform Agar
7. Colitag® Test for the determination of the presence/absence of total coliforms and *E. coli*

4.0 RAPID CONFIRMATION OF *E. COLI* IN POTABLE WATER WHEN USING SM 9222B

Because most drinking water samples are free of coliforms (*i.e.*, no colonies grow on m-Endo agar), many laboratories continue to favor SM 9222B using m-Endo agar as a relatively inexpensive way to screen drinking water samples for total coliforms. This test also provides quantitation if coliforms are present in the water sample. The MassDEP Wall Experiment Station fully recognizes the value of SM 9222B as it pioneered the development of this method in water bacteriology. However, it also recognizes the need for rapid identification/confirmation of *E. coli* colonies on plates with presumptive typical and/or atypical coliform colonies.

SM 9222G [MF Partition Procedure using nutrient agar + MUG medium (NA-MUG)] is a rapid (4-hour) USEPA-approved test for the confirmation of *E. coli* colonies on m-Endo agar plates from method SM 9222B. The test involves the transfer of the membrane filter containing presumptive colonies from m-Endo medium, after incubation for 22-24 hours, to the surface of NA-MUG medium followed by incubation for four hours at 35 ± 0.5˚C. Any *E. coli* colonies present fluoresce under ultra-violet light. An outline of the procedure follows:

1. Aseptically transfer a membrane filter containing typical and/or atypical presumptive coliform colonies on m-Endo medium following analysis by SM 9222B to a plate containing NA-MUG medium.
2. Mark each presumptive positive colony (typical and atypical, sheen and non-sheen) with a permanent marker on the lid of the plate. Do not mark pink, blue, white, or colorless colonies without sheen. Mark the lid and the base of the plate with a line so that the lid of the plate can be realigned with the base if it is removed. Marking the presumptive positive colonies on m-Endo medium is critical as there are other bacteria (e.g., *Pseudomonas* spp.) that fluoresce on NA-MUG medium.
3. Incubate the inoculated NA-MUG plate at 35 ± 0.5˚C for four hours.
4. Examine the plate for fluorescence using an ultraviolet lamp (366-nm) with a 6-watt bulb in a darkened area. Any blue fluorescence observed on the outer edge of a colony or from the back side of the plate indicates the presence of *E. coli*. (See attached Figure 1).
5. If *E. coli* colonies are confirmed, no further verification is required—the sample is total coliform and *E. coli* positive.

6. If no *E. coli* colonies are detected after 4-hour incubation on NA-MUG medium, the entire membrane filter surface is then swabbed with a sterile cotton swab and transferred to lauryl tryptose broth (LTB) and brilliant green lactose bile broth (BGLBB) for total coliform verification. Although these tests require at least an additional 24 hours for completion, there is less urgency as the absence of *E. coli* has already been determined.

NOTE: The laboratory may check the m-Endo plates for the presence of presumptive positive colonies after only 18 hours of incubation. If colonies are absent, the laboratory must continue incubation of the m-Endo plates for the full 22-24 hours. If colonies are present, the laboratory may then transfer the membrane filter onto the NA-MUG medium and follow steps 2-6 above. This procedure is useful for saving time only when performing the test to determine presence/absence; enumeration requires the full 22-24 hours for incubation of the m-Endo plates. There are several reasons that the shortened incubation time for m-Endo plates is not desirable for some laboratories to employ:

1. The laboratory may need to read plates twice, once after 18 hours of incubation and, if no colonies were present at 18 hours, a second time at 22-24 hours.
2. Laboratories certified or accredited in other states may not receive approval from those states for this approach. (Note that EPA-New England and EPA-Cincinnati have reviewed this approach and find it acceptable).
3. Colonies that fluoresce on the NA-MUG medium but that were not present on the m-Endo medium after 18 hours of incubation cannot be identified definitively as *E. coli* because other bacteria, such as *Pseudomonas* spp., can also fluoresce on NA-MUG. If such colonies are found, further testing to confirm or rule out *E. coli* would be required.

5.0 COST COMPARISON

The comparative 2008 costs of media used for the analysis of total coliforms and *E. coli* by several USEPA-approved methods are provided in Table 1. The cost of NA-MUG is lower than that of EC-MUG and quite comparable to that of EC. The cost of dehydrated MI agar (for confirmed detection of both total coliforms and *E. coli* in 24 hours by EPA Method 1604) is very comparable to the total cost of confirmed analysis of total coliforms and *E. coli* by SM 9222B (using m-Endo medium) with EC-MUG or NA-MUG.

Note that laboratories may prepare NA-MUG medium and store it at 4˚C in tightly closed screw cap tubes, flasks, bottles, or other sealed containers for up to three months before pouring plates. Poured plates with loose-fitting covers must be stored at 4˚C for no more than one week.

6.0 LABORATORY CERTIFICATION PROCEDURE

On December 1, 2009, the MassDEP LCO will discontinue certification for the analytes/methods listed above in section 1.0. Certification for the listed analytes/methods will be withdrawn and laboratories wishing to continue analyzing drinking water samples under the TCR will be required to use other methods.

6.1 Laboratories seeking certification for the methods listed in Section 3.0 above must submit an application and successfully perform two out of the three most recent proficiency tests for the desired analytes/methods.

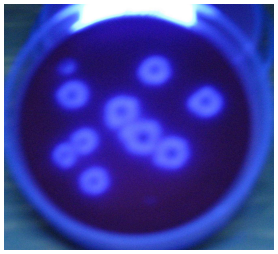
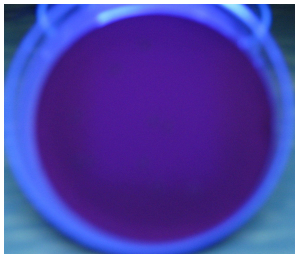
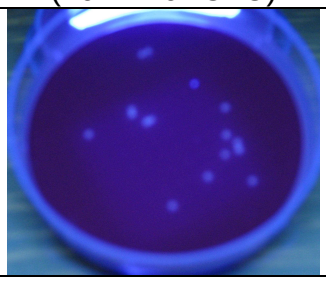
6.2 Laboratories currently certified for the analysis of total coliform by SM 9222B that wish to continue using this procedure must become certified for and use NA-MUG-SM 9222G for the determination of *E. coli.* In order to obtain certification for SM 9222G, laboratories must submit to the MassDEP LCO:

1) Letter requesting certification for *E. coli* by NA-MUG-SM9222G
2) Standard operating procedure for the use of SM9222B and SM9222G
3) Revised quality assurance plan

4) During calendar year 2009 **only**, one successful proficiency test (PT) study for SM 9222B and SM 9222G; if the laboratory fails the first PT study, it must then pass two out of the three most recent PT studies

6.3 Laboratories not currently certified for the analysis of total coliform by SM 9222B that wish to become certified for this procedure must follow the regular application procedure including the successful performance of two of the three most recent proficiency test studies. Laboratories must use the NA-MUG-SM 9222G method to determine the presence of *E. coli* in samples that are presumptive positive for total coliform.

Figure 1. Analysis of Spiked Samples by Membrane Filtration Using a Combination of SM9222B and SM9222G – Membranes were incubated at 35ºC for 24 hr on m-Endo agar plate and then transferred to NA-MUG plate for 4 hr.

Escherichia coli	*Enterobacter aerogenes*	*Pseudomonas aeruginosa* *
Low conc. (10^0 – 10^1 CFU)	Low conc. (10^0 – 10^1 CFU)	High conc. (10^1 – 10^2 CFU)
		

* *Pseudomonas aeruginosa* colonies fluoresce under UV light. However, viewed under ambient light, *P. aeruginosa* colonies on m-Endo agar are pink to colorless, lack a metallic surface sheen, and have a rough surface. Pink, blue, white, or colorless colonies lacking sheen are considered non-coliforms.

Table 1. Comparative Cost of Media Used for the Analysis of Total Coliforms and *E. coli* in Drinking Water

Medium[a]	Amount	Unit	Cost	Recipe	Unit	# plates/tubes	mL (plate/tube)	Price/sample	Total cost/ sample[b]	
m-Endo agar dehydrated	100	g	$55	51	g/L	327	6	$0.22		[g]
	500	g	$118	51	g/L	1634	6	$0.12		[g]
m-Endo broth dehydrated	100	g	$44	48	g/L	833	2.5	$0.10		[g]
	500	g	$92	48	g/L	4167	2.5	$0.07		[g]
LTB dehydrated	100	g	$31	35.6	g/L	234	12	$0.13		
	500	g	$69	35.6	g/L	1170	12	$0.06		
LTB prepared tubes	15	pk	$22			15	15	$1.45		
BGLBB dehydrated	100	g	$44	40	g/L	208	12	$0.21		
	500	g	$93	40	g/L	1042	12	$0.09		
BGLBB prepared tubes	15	pk	$22			15		$1.45		
EC dehydrated	100	g	$38	37	g/L	225	12	$0.17	$0.73	
	500	g	$86	37	g/L	1126	12	$0.08	$0.34	
EC prepared tubes	15	pk	$21			15		$1.38	$4.50	
EC-MUG dehydrated	100	g	$136	37.1	g/L	225	12	$0.61	$1.17	
	500	g	$297	37.1	g/L	1123	12	$0.26	$0.63	
EC-MUG prepared tubes	15	pk	$21			15		$1.42	$4.54	
NA-MUG dehydrated	100	g	$154	23	g/L	725	6	$0.21	$0.78	$3.33 [d]
	500	g	$416	23	g/L	3623	6	$0.11	$0.48	$5.67 [e]
NA-MUG 6-tube pk (HACH)	6	pk	$31			12	7	$2.55	$3.11	$7.49 [f]
NA-MUG prepared plates	15	pk	$66			15		$4.37	$4.93	
MI agar dehydrated	100	g	$347	36.5	g/L	457	6	$0.76	$0.76	
	500	g	$1,589	36.5	g/L	2283	6	$0.70	$0.70	
Colilert	20	pk	$110	$5.50/sample + bottle[c]				$5.92	$5.92	
	200	pk	$700	$3.50/sample + bottle[c]				$3.92	$3.92	

NOTES:

[a]Dehydrated Media -- Fisher Sci. 2008-2009 Online Catalog List Prices

Prepared Media -- HACH 2008 Online Catalog List Prices

Colilert Media -- IDEXX 2008 Catalog List Prices

[b]For dehydrated media, the total cost per sample is calculated from either a 100-g or 500-g bottle for each of m-Endo, LTB, and BGLBB

[c]Cost of bottles is $41.76/100 bottles

[d]Dehydrated m-Endo, prepared LTB & BGLBB tubes & dehydrated NA-MUG

[e]Dehydrated m-Endo, prepared LTB & BGLBB tubes & prepared NA-MUG tubes

[f]Dehydrated m-Endo, prepared LTB & BGLBB tubes & prepared NA-MUG plates

[g] Cost of ethanol (ETOH) may vary depending on supplier, taxes, and shipping charges