Membrane Filtration Guidelines

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The Membrane Filtration (MF) method is used to estimate bacterial populations in water that is low in turbidity. This method is especially useful for large sample volumes or for many daily tests.

Overview

The following basic steps are necessary for a membrane filtration test.

1. Non-potable water samples are diluted.
2. The sample or dilution is filtered through a membrane filter that retains the bacteria.
3. The filter is put in a petri dish on an absorbent pad that contains a nutritional broth or agar that is selective for the growth of a specific organism.
4. The petri dish containing the filter and pad is incubated for 24 hours at a specific temperature.
5. After incubation, the colonies that have grown are identified and counted.

Sample collection and sterilization

Refer to *Bacteria Test Guidelines* for instructions on sample collection and equipment sterilization.

Sample size selection

Samples that contain a high level of bacteria must be diluted so that the bacteria that grows on the filter is at a density that can be measured. Table 420 and Table 421 list recommended sample volumes for various types of samples.

Select a sample size to give 20 to 200 colony-forming units (CFU) per filter. The ideal sample volume for non-potable water or wastewater yields 20–80 coliform colonies per filter. For finished, potable water, the volume to be filtered will be 100 mL.

When the sample volume is less than 20 mL (diluted or undiluted), add 10 mL of sterile dilution water to the filter funnel before vacuum is applied. The dilution water will help to distribute the bacteria uniformly across the membrane filter.

Table 420 Sample volume by sample type—total coliform test [1]

Sample type	100 mL	50 mL	10 mL	1 mL	0.1 mL	0.01 mL	0.001 mL	0.0001 mL
Drinking water	X							
Swimming pools	X							
Wells, springs	X	X	X					
Lakes, reservoirs	X	X	X					
Water supply intake			X	X	X			
Bathing beaches			X	X	X			
River water				X	X	X	X	
Chlorinated sewage				X	X	X		
Raw sewage					X	X	X	X

[1] *Standard Methods for the Examination of Water and Wastewater,* 19th ed., Table 9222:I, page 9–56.

Table 421 Sample volume by sample type—fecal coliform test[1]

	100 mL	50 mL	10 mL	1 mL	0.1 mL	0.01 mL	0.001 mL
Lakes, reservoirs	X	X					
Wells, springs	X	X					
Water supply intake		X	X	X			
Natural bathing waters		X	X	X			
Sewage treatment plant, secondary effluent			X	X	X		
Farm ponds, rivers				X	X	X	
Storm water run-off				X	X	X	
Raw municipal sewage					X	X	X
Feedlot run-off					X	X	X

[1] *Standard Methods for the Examination of Water and Wastewater*, 19th ed., Table 9222:III, pages 9–61.

Sample dilution

Non-potable water samples must be diluted to a level at which the bacteria can be measured. The ideal sample volume for total coliform testing yields approximately 20 to 80 coliform colonies and not more than 200 colonies of all types per filter. Ideal sample volumes for fecal coliform testing yield approximately 20 to 60 coliform colonies per filter. Analyze three different sample volumes when the coliform number is uncertain.

Procedure

1. Wash hands.
2. Open a bottle of sterile Buffered Dilution Water.
3. Invert the sample container in a waist-to-ear motion, approximately 25 times (for 30 seconds).
4. Use a sterile pipet to add 11 mL of sample into the dilution water bottle.
5. Put the cap on the dilution water bottle. Invert the bottle in a waist-to-ear motion, approximately 25 times (for 30 seconds). This is a 10-fold or 10x dilution (sample is diluted by a factor of 10).
6. Add 11 mL of the 10x dilution to another dilution bottle and mix well (100x dilution).
7. Add 11 mL of the 100x dilution to a third bottle and mix well (1000x dilution).
8. Continue to make dilutions until the necessary dilution level has been reached.

Dilution series

f. **10-mL sample:** Transfer 11 mL of sample into 99 mL of sterile Buffered Dilution Water.

g. **1-mL sample:** Transfer 11 mL of the 10-mL dilution from step f into 99 mL of sterile Buffered Dilution Water.

h. **0.1-mL sample:** Transfer 11 mL of the 1-mL dilution from step g into 99 mL of sterile Buffered Dilution Water.

i. **0.01-mL sample:** Transfer 11 mL of the 0.1-mL dilution from step h into 99 mL of sterile Buffered Dilution Water.

j. **0.001-mL sample:** Transfer 11 mL of the 0.01-mL dilution from step i into 99 mL of sterile Buffered Dilution Water.

k. **0.0001-mL sample:** Transfer 11 mL of the 0.001-mL dilution from step j into 99 mL of sterile Buffered Dilution Water.

Field filtration apparatus

The field filtration apparatus consists of disposable funnels, a portable funnel base (vacuum support) and hand pump for convenient filtration in the field. A portable incubator can be used for incubation or for transport to the laboratory.

1. Flame sterilize the top surface of the stainless steel field vacuum support.
2. Attach the syringe tip to the vacuum support tubing.
3. Using sterile forceps, place a membrane filter, grid side up, on the center of the vacuum support.

   Note: *To sterilize forceps, dip forceps in alcohol and flame in an alcohol burner. Cool before use.*
4. Remove a funnel (base first) from the package.
5. Place the funnel onto the vacuum support. Do not touch the inside of the funnel. Push evenly on the funnel's upper rim to snap it on the vacuum support.
6. Pour the sample into the funnel.
7. Use the hand pump to draw the sample through the filter apparatus.

   Note: *See specific procedures for the sample volume required.*
8. Remove the funnel.
9. Press the lever on the vacuum support stem to lift the membrane filter from the vacuum support surface.
10. Use sterile forceps to remove the membrane filter.
11. Place the membrane filter into a prepared petri dish and incubate at the specified temperature.
12. Disconnect the syringe tip from the vacuum support tubing. Dispose of the liquid in the syringe.
13. Follow step 2 through step 12 to filter remaining samples.

Accuracy check

Positive and negative controls are important. Pseudomonas aeruginosa is recommended as a negative control, and a Escherichia coli is recommended as a positive control for total and fecal coliforms. Escherichia coli is recommended as a negative control and Enterococcus faecalis is recommended as a positive control for the enterococci. Escherichia coli is recommended as a negative control and Pseudomonas aeruginosa is recommended as a positive control for pseudomonas.

Note: *Potable water samples from municipal treatment facilities should be negative for total coliforms and fecal coliforms.*

Interpreting and reporting results

Report test results as the number of colonies per 100 mL of sample.

Single filter test
Use the following equation to calculate the result from a single membrane filter. Note that "mL sample" in the equation refers to the actual sample volume and not the diluted volume.

$$\text{Bacterial colonies per 100 mL} = \frac{\text{Bacterial colonies counted}}{\text{mL of sample}} \times 100$$

- **Indistinct colonies**—If growth covers the entire filtration area of the membrane or a portion of it, and colonies are not discrete, report the test results as "Confluent growth with or without coliforms."
- **High colony density**—If the total number of colonies exceeds 200 per membrane or the colonies are too indistinct for accurate counting, report the results as "Too numerous to count" (TNTC).

In either case, run a new sample using a dilution that will give about 50 to 200 colonies of all types.

When testing non-potable water, if no filter meets the desired minimum colony count, use the equation under *Multiple filter test* to calculate the test result.

Multiple filter test
Use the following equation to calculate the result from multiple membrane filters such as duplicate samples or multiple dilutions of the same sample.

$$\text{Bacterial colonies per 100 mL} = \frac{\text{Sum of colonies in all samples}}{\text{Sum of volumes (in mL) of all samples}} \times 100$$

Prepared broth and agar

Prepared broth or agar is ready to use and is available in broth ampules or agar plates. The ampules or agar plates contain enough medium for one test. Prepared media is shipped with a Certificate of Analysis and has an expiration date printed on the label.

The ampules are available in glass or plastic. Open the ampule and pour the broth on the absorbent pad in a petri dish. Open the plastic ampules by unscrewing the top of the ampule. Open the glass ampules with an ampule breaker.

Refer to *Prepared media for membrane filtration* for a list of prepared media that is available for microbiological tests.

Table 422 Prepared media for membrane filtration

Media	Description	Cat. No.	Selectivity	Incubation	Shelf life	Approval Citations	Sample
KF-Streptococcus	Broth in plastic ampules	2812750	Enterococci	48 hours at 35 ± 0.5 °C	1 year at 2–8 °C	—	Drinking water Ground water Surface water Recreational water
m-ColiBlue24®	Prepared agar plate	2805215	Total Coliform and Escherichia coli Bacteria	24 hours at 35 ± 0.5 °C	1 year at 2–8 °C	40CFR 141.21 (f) (3) footnote (11) and 40CFR 136.3 Does not apply to Agar plate	Drinking water Beverages Ground water Surface water Recreational water Wastewater
	Broth in glass ampules	2608420					
	Broth in plastic ampules	2608450					
	Broth in 100-mL glass bottle	2608442					
m-EI	Prepared agar plate	2811715	Enterococci	24 hours at 41 ± 0.5 °C	3 months at 2–8 °C	—	Drinking water Ground water Surface water Recreational water
m-Endo	Prepared agar plate	2811615	Total Coliform Bacteria	24 hours at 35 ± 0.5 °C	1 year at 2–8 °C	Standard Method 18th 9222 A, B and Federal Register V 68; #139 (7/21/2003)	Drinking water Beverages Ground water Surface water Recreational water
	Broth in glass ampules	2373520					
	Broth in plastic ampules	2373550					
	Broth in 100 mL glass bottle	2373542					
m-FC	Prepared agar plate	2811515	Fecal Coliform Bacteria	24 hours at 44.5 ± 0.2 °C	1 year at 2–8 °C	40CFR 141.21 (f) (5), Standard Method 18th 9222 D and Federal Register V 68; #139 (7/21/2003)	Ground water Surface water Recreational water Wastewater
	Broth in glass ampules	2373220					
	Broth in plastic ampules	2373250					
m-FC with Rosolic Acid	Broth in glass ampules	2428520	Fecal Coliform Bacteria	24 hours at 44.5 ± 0.2 °	1 year at 2–8 °C	40CFR 141.21 (f) (6) (i) and Standard Method 18th 9221 D	Ground water Surface water Recreational water Wastewater
	Broth in plastic ampules	2428550					
m-Green YM	Broth in glass ampules	2428320	Yeast and Mold	48 hours at 35 ± 0.5 °C	1 year at 2–8 °C	—	Beverages
	Broth in plastic ampules	2428350					
m-HPC	Prepared agar plate	2811415	Heterotrophic Bacteria	48 hours at 35 ± 0.5 °C	1 year at 2–8 °C	Standard Method 18th 9215 A, D	Drinking water Beverages Ground water Surface water Recreational water
	Broth in plastic ampules	2812450					

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Table 422 Prepared media for membrane filtration

Media	Description	Cat. No.	Selectivity	Incubation	Shelf life	Approval Citations	Sample
m-TEC	Agar Tubes/2 tests per tube	2561106	Escherichia coli	2 hours at 35 ± 0.5 °C then 22 hours at 44.5 ± 0.2 °C	1 year at 2–8 °C	Federal Register V 68; #139 (7/21/2003) (applies to m-TEC and Modified m-TEC)	Recreational water
m-TEC, modified	Prepared agar plate	2811815					
m-TGE	Broth in glass ampules	2373820	Heterotrophic Bacteria	24 hours at 35 ± 0.5 °C	1 year at 2–8 °C	—	Drinking water Beverages Ground water Surface water Recreational water
	Broth in plastic ampules	2373850					
m-TGE with TTC	Broth in glass ampules	2428420	Heterotrophic Bacteria	24 hours at 35 ± 0.5 °C	1 year at 2–8 °C	—	Drinking water Beverages Ground water Surface water Recreational water
	Broth in plastic ampules	2428450					
m-TSB/USP	Broth in plastic ampules	2812650	Heterotrophic Bacteria	24 hours at 35 ± 0.5 °C	1 year at 2–8 °C	—	Drinking water Beverages Ground water Surface water Recreational water
Nutrient Agar/MUG	Agar Tubes/2 tests per tube	2437306	Escherichia coli (confirmation)	4 hours at 35 ± 0.5 °C	1 year at 2–8 °C	—	Drinking water Beverages Ground water Surface water Recreational water
	Prepared agar plate	2812115					
Pseudomonas	Broth in plastic ampules	2812250	Pseudomonas	24 hours at 35 ± 0.5 °C	1 year at 2–8 °C	—	Drinking water Beverages Ground water Surface water Recreational water
R2A	Agar Tubes/2 tests per tube	2724106	Stressed Heterotrophic Bacteria	At least 72 hours at 35 ± 0.5 °C (7 days maximum)	1 year at 2–8 °C	Standard Method 18th 9215 A, D	Drinking water Beverages
	Prepared agar plate	2814215		At least 72 hours at 35 ± 0.5 °C	1 year at 2–8 °C	—	
	Broth in plastic ampules	2812350					

Dehydrated media

Refer to the *Dehydrated media and reagents* table for a list of dehydrated media that can be prepared. The media must be measured, mixed with water and sterilized before use.

Dehydrated media and reagents

Description	Unit	Catalog number
Brain Heart Infusion Agar	500 g	2405634
Brain Heart Infusion Broth	500 g	2815534
Magenta GIcA (5-bromo-6-chloro-3indoyl-beta-D-glucuronide)	2 g	2815035
M-E Agar	100 g	2281226
M-E Agar	500 g	2281234
m-TEC Agar, dehydrated	100 g	2281126
Nalidixic Acid	25 g	2407124
Oxidase Reagent	0.5 mL	2622500
Tryptic Soy Agar	100 g	2565926
Tryptic Soy Broth, ampules	50/pkg	2812650

Enrichment technique for total coliforms

Stressed coliforms require an enrichment technique, such as the one using Lauryl Tryptose (LT) Broth Ampules described here, to get complete recovery with the MF Method. Consult your local or state authorities about approved test methods for your application.

Procedure

1. Place a sterile absorbent pad in the lid of a sterile petri dish.
2. Saturate the pad with one LT Broth Ampule. Pour off any excess liquid.
3. Filter the sample through a membrane filter.
4. Place the membrane filter onto the saturated pad.
5. Incubate the filter without inverting the petri dish for 1.5 to 2 hours at 35 ± 0.5 °C in a relative humidity of at least 90 percent.
6. Remove the petri dish from the incubator and open it.
7. Place a sterile absorbent pad in the bottom half of the petri dish.
8. Pour the contents of one m-Endo Broth Ampule into the bottom half of the petri dish.
9. Carefully remove the filter from the lid and roll it onto the new pad to avoid trapping air between the filter and the nutrient pad.
10. Discard the old pad (the enrichment pad saturated with LT Broth). Replace the culture dish lid.
11. Invert the culture dish and incubate at 35 ±0.5 °C for 20 to 22 hours.
12. After incubation, use an illuminated magnifier or a 10 to 15X microscope to count the colonies with a greenish-gold metallic sheen.

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