Bacteria

Prepared Agar Plates

Membrane Filtration

Scope and application: For low-turbidity water and wastewater.

⚠ Test preparation

Before starting

This method uses prepared agar plates for the analysis of different types of bacteria. Refer to Prepared agar plates on page 2 to select a prepared agar plate for the test procedure.

Set the incubator to the specified temperature. Refer to Table 1 on page 3. Let the incubator temperature become stable, then add the samples.

Wash hands thoroughly with soap and water.

Use a germicidal cloth, bactericidal spray, weak bleach solution or weak iodine solution to clean the work area.

Make sure that all of the materials that come in contact with samples are sterile.

During filtration, remove the vacuum as soon as the funnel is empty so that the membrane filter does not become dry.

As an alternative to the filter assembly with flask, use a sterile, disposable filter unit.

Items to collect

Description	Quantity
Prepared agar plate (refer to Consumables and replacement items on page 6)	1
Sterile buffered dilution water	1
Membrane filter, 0.45 micron	1
Filtration apparatus with aspirator or pump	1
Forceps, sterilized	1
Incubator	1
Microscope, low-power	1
Pipet(s) for dilution or for sample volumes less than 100 mL, if necessary	1

Refer to Consumables and replacement items on page 6 for order information.

Sample collection

- Use a sterile glass or plastic container such as a Whirl-Pak bag that contains sterilized sodium thiosulfate. The sodium thiosulfate is not necessary if the sample does not contain a residual disinfectant.
- Open the sample containers immediately before collection and close immediately after collection. Do not put the lid or cap down. Do not touch the lip or inner surfaces of the container. Do not rinse the containers before use.
- To collect a potable water sample from a faucet, spigot, hydrant or pump, let the water flow at a moderate rate for 2–3 minutes. Remove the screens or aerators. Do not use faucets or spigots that have a bad seal or that show a leak between components.
- To collect a non-potable sample from a river, lake or reservoir, hold the container below the water surface, then remove the cap. As an alternative, remove the cap and push the container, mouth down, below the water surface to prevent the collection of surface scum. Put the mouth of the container into the current. Fully fill the container below the water surface.
- Collect a minimum of 100 mL of sample. Keep a minimum of 2.5 cm (1 inch) of air space in the container.
- Write the sample information on the container and start the analysis as soon as possible.
- If immediate analysis is not possible, keep the sample at or below 10 °C (50 °F) for a maximum of 8 hours. Do not let the sample freeze.

Sample volumes

Use a sample volume that is applicable to the sample type. For samples with a low level of bacteria such as finished, potable water, use 100 mL of sample. Use less sample for non-potable water or water that contains more bacteria.

When the approximate bacteria level is unknown, analyze three different sample volumes. Use the results from the sample volume that shows approximately 20 to 200 colonies for each membrane filter.

When the sample volume is less than 20 mL (diluted or undiluted), add 10 mL of sterile buffered dilution water to the filter funnel before the vacuum is applied. The additional dilution water helps to apply the bacteria equally across the membrane filter.

Sample dilution

Dilute samples that contain a high level of bacteria so that approximately 20 to 200 bacteria colonies grow on the membrane filter. Use the steps that follow to make serial dilutions of the sample.

1. Wash hands thoroughly with soap and water.
2. Invert the sample container for 30 seconds (approximately 25 times).
3. Open a bottle of sterile buffered dilution water.
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 and invert for 30 seconds (25 times). This is a 10x dilution (sample is diluted by a factor of 10).
6. Add 11 mL of the 10-fold dilution to another dilution bottle (100x dilution). Mix well.
7. Add 11 mL of the 100-fold dilution to the third bottle (1000x dilution). Mix well.
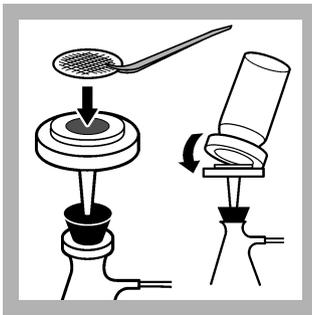8. If necessary, continue to dilute the sample.

Prepared agar plates

Table 1 shows the available prepared agar plates for the analysis of different types of bacteria. The prepared agar plates include a certificate of analysis and expiration date. Most of the plates have a shelf life of 1 year when stored at 2–8 °C (35–46 °F). The m-EI

agar plates have a shelf life of 3 months. The sensitivity of all of the prepared agar plates is 1 CFU/100 mL.
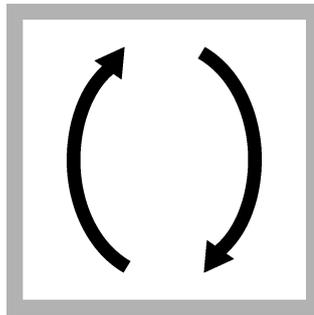
Table 1 Prepared agar plates

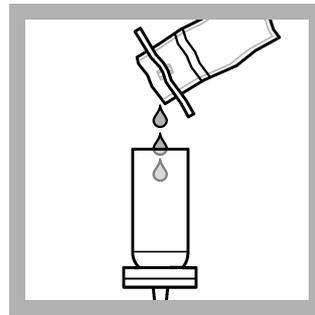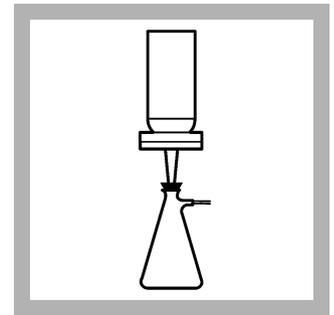| Bacteria	Prepared agar plate	Incubation time	Incubation temperature	
Heterotrophic bacteria	m-HPC	24 hours	35 (± 0.5) °C	95 (± 0.9) °F
Total coliform bacteria	m-Endo[1]	24 hours	35 (± 0.5) °C	95 (± 0.9) °F
Fecal coliform bacteria	m-FC[1]	24 hours	44.5 (± 0.2) °C	112.1 (± 0.7) °F
Total coliform and *E. coli* bacteria	m-ColiBlue24	24 hours	35 (± 0.5) °C	95 (± 0.9) °F
Enterococci bacteria	M-EI	24 hours	44.5 (± 0.5) °C	112.1 (± 0.9) °F
E. coli bacteria with confirmation	Nutrient agar with MUG	24 hours	35 (± 0.5) °C	95 (± 0.9) °F
E. coli bacteria	Modified m-TEC	2 hours at 35 (± 0.5) °C (95 (± 0.9) °F), then 22 hours at 44.5 °C (112.1 °F)		

Membrane filtration test procedure

1. Set up the membrane filtration apparatus. Use a sterile forceps to put a membrane filter in the assembly. Make sure that the grid side is up.
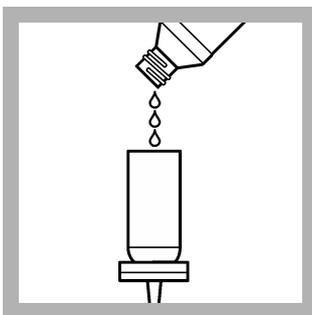

2. Invert the sample or the diluted sample for 30 seconds (25 times) to make sure that the sample is mixed well.
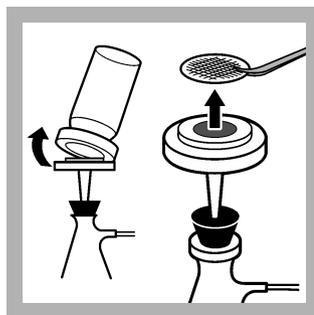

3. Pour or use a pipet to add the sample into the funnel. If the volume is less than 20 mL, add 10 mL of sterile buffered dilution water to the funnel.
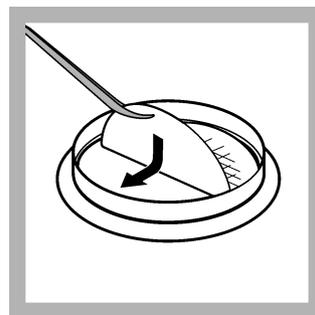

4. Apply the vacuum until the funnel is empty. Stop the vacuum.
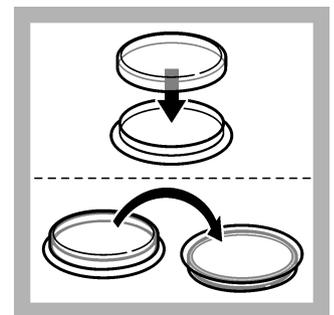

5. Rinse the funnel with 20 to 30-mL of sterile buffered dilution water. Apply the vacuum. Rinse the funnel two more times.

6. Stop the vacuum when the funnel is empty. Remove the funnel from the filter assembly. Use sterile forceps to lift the membrane filter.

7. Put the membrane filter on the prepared agar plate. Let the membrane filter bend and fall equally across the agar to make sure that air bubbles are not caught below the filter.
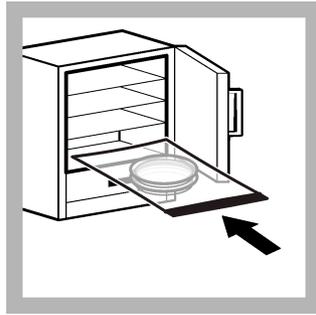

8. Put the lid on the petri dish and invert the petri dish.

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[1] Additional media is necessary for confirmation.

9. Put the inverted petri dish in a plastic bag and seal the bag.

10. Incubate the inverted petri dish at the specified temperature for the specified time. Refer to Table 1 on page 3.

11. Remove the petri dish from the incubator. Use a 10 to 15x microscope to count the number of bacteria colonies on the membrane filter.

Interpret and report the coliform results

Report the coliform density as the number of colonies in 100 mL of sample. For total coliforms, use a sample volume that gives 20–80 coliform colonies on the membrane filter. For fecal coliforms, use a sample volume that gives 20–60 fecal coliform colonies on the membrane filter.

If there are more than 200 colonies, dilute the sample and use the diluted sample in the test procedure. Use the sample volume before dilution in the coliform density determination.

1. Use the microscope to look at the colonies on the membrane filter. Count the number of isolated coliform colonies.

2. Determine the coliform density as follows:

Membrane filter(s)	Coliform density determination
One membrane filter	Coliform colonies in 100 mL = Coliform colonies counted ÷ mL sample × 100
	Example: 50 coliform colonies were counted. The sample volume was 20 mL. The coliform density is 50 ÷ 20 mL × 100 = 250 coliforms in 100 mL of sample.
Multiple filters, dilutions or duplicates for each sample	Average coliform colonies in 100 mL = Sum of coliform colonies in all samples ÷ sum of mL sample × 100
	Example: Two 50-mL samples gave 5 colonies on one filter and 9 colonies on another filter. The coliform density is (5 + 9) ÷ (50 + 50) × 100 = 14 coliforms in 100 mL of sample.

3. If colonies are not isolated or if there are more than 200 colonies of all types:

   a. Report the results as "Confluent growth with or without coliforms" when the bacteria grows together across some or all of the membrane filter.
   b. Do the test procedure again with half the sample volume. If the total number of colonies (coliforms plus non-coliforms) is more than 200 for each membrane or the colonies are not isolated, report the results as "Too numerous to count" (TNTC).
   c. Do the test procedure again with a dilution that gives approximately 50 coliform colonies and not more than 200 colonies of all types.

Interpret and report the HPC results

Use the steps that follow to determine the colony-forming units for each mL of sample (CFU/mL). A colony density of 20 to 200 colonies on the membrane filter is recommended for best results. If there are more than 200 colonies, dilute the sample and use the diluted sample in the test procedure. Use the sample volume before dilution in the CFU/mL determination.

1. Use the microscope to look at the colonies on the membrane filter. Estimate the number of colonies in each square of the membrane filter.

   Note: Make estimated counts only when there are isolated colonies without spreaders.

2. Determine the CFU/mL of sample for the estimated colony count as follows:

Estimated count	CFU/mL determination
1 to 2 colonies in each square	1. Count all of the colonies on the filter. 2. Divide the total number of colonies by the sample volume. *Example: 122 total colonies were counted. The sample volume was 100 mL. 122 colonies/100 mL = 1.2 CFU/mL*
3 to 10 colonies in each square	1. Count the colonies in 10 representative squares. 2. Divide by 10 to get the average number of colonies in each square. 3. Multiply the average number in each square by 100. 4. Divide by the sample volume. *Example: The average number of colonies was 8 colonies in each square. The sample volume was 10 mL. 8 colonies x 100/10 mL = 80 CFU/mL*
10 to 20 colonies in each square	1. Count the colonies in 5 representative squares 2. Divide by 5 to get the average number of colonies in each square. 3. Multiply the average number in each square by 100. 4. Divide by the sample volume. *Example: The average number of colonies was 17 colonies in each square. The sample volume was 10 mL. 17 colonies x 100/10 mL = 170 CFU/mL*
More than 20 colonies in each square	1. Divide 2000 by the volume of the original (undiluted) sample. 2. Report results as more than the result of step 1. *Example: The original sample volume was 0.1 mL. 2000/0.1 mL = > 20,000 CFU/mL*

3. Report the test results as colony forming units for each mL (CFU/mL). Report averaged counts as estimated CFU/mL. Include in the report the method used, the incubation temperature, time and the nutritional medium. *Example: 98 CFU/mL, membrane filter method, 35 °C, 24 hours, m-TGE Broth.*

Summary of method

The membrane filtration procedure is used for samples that are low in turbidity and have low bacteria counts. The sample is poured through a membrane filter. The bacteria in the sample stays on the membrane filter. The membrane filter is moved to a petri dish that contains a nutritional broth or agar. During incubation, the bacteria grow and form colonies on the membrane filter. After incubation, the filter is examined with a microscope for bacteria colonies.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Item no.
m-ColiBlue24® prepared agar plates	1	15/pkg	2805215
m-EI prepared agar plates	1	15/pkg	2811715
m-Endo prepared agar plates	1	15/pkg	2811615
m-FC prepared agar plates	1	15/pkg	2811515
m-HPC prepared agar plates	1	15/pkg	2811415
m-TEC, modified, prepared agar plates	1	15/pkg	2811815
Nutrient agar with MUG prepared agar plates	1	15/pkg	2812115
Dilution water, buffered, 99 mL, sterile[2]	1	25/pkg	1430598

Required apparatus

Description	Unit	Item no.
Membrane filter holder, magnetic, 300-mL funnel	each	1352900
Filter pump, aspirator	each	213100
Flask, filtering, glass, 1000 mL	each	54653
Forceps, stainless steel	each	2141100
Membrane filter, 0.45 micron, 47 mm diameter, sterile	200/pkg	1353001
Membrane filter, 0.45 micron, 47 mm diameter, sterile EO (ethylene oxide)	150/pkg	2936100
Microscope, compound	each	2947050
Pipet, serological, 10–11 mL, sterile, disposable	25/pkg	209798
Pipet filler, safety bulb	each	1465100
Pipet, TenSette®, 1.0–10.0 mL	each	1970010
Pipet tips, TenSette, 1.0–10.0 mL, sterile, individually wrapped	50/pkg	2558996
Stopper, rubber, size 8, for filtration assembly	6/pkg	211908
Tubing, rubber, 7.9 mm (5/16-in.) inside diameter	3.66 m (12 ft)	56019

Incubators

Description	Unit	Item no.
Laboratory incubator, culture, 110 VAC	each	2619200
Laboratory incubator, culture, 230 VAC	each	2619202
Portable incubator with 12 VDC power socket	each	2569900
AC power supply for portable incubator, 110–240 VAC	each	2968100
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Portable incubator rack, general purpose/petri dish	each	2580502

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[2] Buffered dilution water is prepared with magnesium chloride and potassium dihydrogen phosphate.

Sample collection

Description	Unit	Item no.
Sampling bags, Whirl-Pak® with dechlorinating reagent, 177 mL	100/pkg	2075333
Sampling bags, Whirl-Pak without dechlorinating reagent, 207 mL	100/pkg	2233199
Sampling bottles, sterilized, with dechlorinating agent, 100-mL sample	100/pkg	8888006
Sampling bottles, sterilized, without dechlorinating reagent, 100-mL sample	12/pkg	2495012
Sampling bottles, sterilized, without dechlorinating reagent, 100-mL sample	50/pkg	2495050
Sample transport kit, includes 100 sample bags with dechlorinating agent, refrigerant pack, rack and 9-L cooler	each	2568700

Optional reagents and apparatus

Description	Unit	Item no.
Disposable filter funnels with membrane filters, sterile	150/pkg	2586300
Pipet, serological, 10–11 mL, sterile, disposable	25/pkg	209798
Pipet, serological, 2 mL, sterile, glass	35/pkg	2093136
Pipet filler, safety bulb	each	1465100
Support base for disposable filter funnels	each	2586201
Vacuum pump, hand-operated	each	1428300

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