



Membrane Filtration: Coliforms, Enterococci and Pseudomonas

**EPA-accepted Methods 8074 and 8367;
EPA-approved Method 10029**

For detection of: total coliforms, fecal coliforms, *E. coli*,
enterococci and pseudomonas

Media: m-Endo, m-FC, m-FC/RA, m-TEC, modified
m-TEC, m-EI, m-ColiBlue24[®], Pseudomonas Broth

Sample type: potable water, nonpotable water, recreational
water, and wastewater

TABLE OF CONTENTS

Introduction to Membrane Filtration Methods	5
Preparing Materials.....	5
Preparing Sample Containers	6
Using Field Filtration Apparatus	6
Using Autoclavable Apparatus.....	7
Preparing Autoclavable Filter Assembly.....	7
Collecting and Preserving Samples	8
Sample Size	9
Diluting Samples	10
Disposing of Completed Tests.....	11
Interpreting and Reporting Results.....	11
Table of Media for Membrane Filtration.....	13
Total Coliforms	17
m-ColiBlue24® Medium	17
Using m-ColiBlue24 Broth PourRite Ampules	17
Optional Verification of Total Coliforms	19
Using m-Endo Broth for Presumptive Total Coliform Test.....	21
Using Lauryl Tryptose Broth and Brilliant Green Bile Broth for Confirmation of Total Coliforms.....	23
Using EC Medium Broth for Confirmation of Fecal Coliforms.....	25
Using EC Medium with MUG (EC/MUG) Broth or Nutrient Agar with MUG for Confirmation of <i>E. coli</i>	26
Enrichment Technique for Stressed Total Coliforms.....	28
Fecal Coliforms	29
Using m-FC Broth or m-FC Broth with Rosolic Acid (m-FC/RA) for Fecal Coliform Test	29
Verifying Fecal Coliforms	31
<i>Escherichia coli</i>	33
Using m-TEC Agar for Presumptive <i>E. coli</i> Test with Confirmation Steps.....	33
Using Modified m-TEC Prepared Agar Plates for <i>E. coli</i> Test in Recreational Waters	35
Enterococci	37
Using m-EI Prepared Agar Plates for Enterococci Test in Recreational Waters	37
Optional Verification of Enterococci	38
Pseudomonas	39
Using Pseudomonas Broth for Detection of Pseudomonas Species.....	39
Media, Reagents, and Apparatus	41

Introduction to Membrane Filtration Methods

The Membrane Filtration (MF) method is a fast, simple way to estimate bacterial populations in water that is low in turbidity. This method is especially useful when evaluating large sample volumes or performing many tests daily.

In the initial step, an appropriate sample volume passes through a membrane filter with a pore size small enough (0.45 micron) to retain the bacteria present. The filter is placed on an absorbent pad (in a petri dish) saturated with a culture medium that is selective for the growth of a specific organism. The petri dish containing the filter and pad is incubated, inverted, for 24 hours at the appropriate temperature. After incubation, the colonies that have grown are identified and counted using a low-power microscope.

Convenient Packaging

Hach's PourRite™ Ampules contain prepared selective media. This eliminates the measuring, mixing, and autoclaving needed when preparing dehydrated media. The ampules are designed with a large, unrestrictive opening that allows media to pour out easily. Simply break off the top of the ampule and pour the medium onto an absorbent pad in an MF petri dish.

Each ampule contains enough medium for one test. Medium packaged in PourRite Ampules has a shelf-life of one year. Ampules are shipped with a Certificate of Analysis and have an expiration date printed on the label.

Pre-poured agar plates are also available for many of the media. Hach's Prepared Agar Plates contain prepared media, which eliminates the measuring, mixing, and autoclaving associated with preparing dehydrated medium. The plates are ready to use without the need for further preparation and contain enough medium for one test.

The shelf life of the prepared agar plates varies from 3 months to 1 year. (See *Table of Media for Membrane Filtration* on page 13.) Plates are shipped with a Certificate of Analysis and have an expiration date printed on the label.

Technique is Important

Good laboratory technique is essential when accuracy is important, particularly in microbiological procedures. To ensure reliable results, use care when collecting and preserving samples. Carefully clean your laboratory or work surface, use proper sterilization and inoculation practices. Maintain close temperature control.

Use high-quality laboratory equipment and ready-to-use media to save time and minimize errors. Hach's prepared media for MF testing helps eliminate contamination due to technique.

Preparing Materials

To save time, start the incubator while preparing other materials. Set the incubator to the temperature required by the specific procedure (usually 35 ± 0.5 °C for total coliforms and enterococci, and 44.5 ± 0.2 °C for fecal coliforms).

Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray, or dilute iodine solution. Wash your hands thoroughly with soap and water.

Mark each sample container with the sample number, dilution, date, and other necessary information. Avoid contaminating the inside of the sample container in any way.

Introduction to Membrane Filtration Methods, continued

Using Presterilized Equipment and Media

Use sterile equipment, a disinfected work area, and proper handling techniques to avoid contamination. To simplify the technique and minimize the possibility of contamination, Hach offers presterilized and disposable membrane filters, pipets, petri dishes, absorbent pads, inoculating loops, buffered dilution water, sampling bags, and prepared growth medium. MEL Portable Laboratories include presterilized consumables and a field filtration assembly.

Preparing Sample Containers

Note: Use dechlorinating agent with potable or chlorinated water samples. While dechlorinating agent is not necessary for unchlorinated or nonpotable water samples, it will not interfere. Therefore, plastic bags containing dechlorinating agent can be used for all samples.

Presterilized plastic bags and bottles

Plastic bags and bottles are available with or without dechlorinating agent.

Autoclavable glass or plastic bottles

To use glass or plastic bottles (at least 125-mL) instead of plastic bags, prepare the containers as follows:

1. Wash in hot water and detergent.
2. Thoroughly rinse with hot tap water, followed by deionized water to ensure that all detergent is removed.
3. If dechlorinating agent is needed (for chlorinated, potable water) add one Dechlorinating Reagent Powder Pillow to each 125-mL sample container. Add two powder pillows to a 250-mL sample container.
4. Steam sterilize glass and autoclavable plastic containers at 121 °C for 15 minutes. Glass sample containers can also be sterilized by dry heat at 170 °C for 1 hour.
5. Store sterile containers, tightly capped, in a clean environment until needed.

Using Field Filtration Apparatus

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, onto the center of the vacuum support.

Note: To sterilize forceps, dip forceps in alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.

4. Open a package of funnels (start at the bottom of the package). 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 onto the vacuum support.
6. Pour the sample into the funnel.
7. Pull on the syringe's plunger to draw the sample through the filter apparatus.

Note: See specific procedures for the sample volume required.

Introduction to Membrane Filtration Methods, continued

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 according to the appropriate procedure.
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.

Using Autoclavable Apparatus

When running numerous samples on a routine basis, you may prefer to use an autoclave and nondisposable materials.

1. Wash sample bottles, pipets, petri dishes, filter holder with stopper, and graduated cylinder (if needed) with hot water and detergent.
2. Rinse several times with tap water and then with deionized water. Dry thoroughly.
3. Prepare all equipment for autoclaving.
 - Loosely thread caps on sample bottles and cover caps and bottle necks with metal foil or paper.
 - Cover the openings of graduated cylinders with metal foil or paper.
 - Insert the filter funnel base into an autoclavable rubber stopper that fits the filter flask.
 - Wrap the two parts of the filter funnel assembly separately in heavy wrapping paper and seal with masking tape.
 - Wrap petri dishes (borosilicate glass) in paper or place in aluminum or stainless cans.
4. Sterilize equipment in an autoclave at 121 °C for 15 minutes. Borosilicate glass items can be sterilized with dry heat at 170 °C for a minimum of one hour.

Preparing Autoclavable Filter Assembly

Disinfect the work bench or work area with a germicidal cloth, dilute bleach solution, or dilute iodine solution. Wash hands thoroughly with soap and water.

1. After sterilization, remove the filter funnel assembly from the wrapping paper.
2. Do not touch the inner surfaces of the funnel.
3. Insert the funnel with rubber stopper into the filtering flask or filter funnel manifold. Connect the flask or manifold to the water trap and aspirator with rubber tubing or a vacuum pump.

Introduction to Membrane Filtration Methods, continued

4. Use sterile forceps to place a sterile membrane filter on the filter base and attach the filter funnel top.
5. Filter a small quantity of sterile Buffered Dilution Water through the funnel to ensure a good seal on the filter and connections before filtering the sample.

Collecting and Preserving Samples

Collect a sufficient volume of sample for analysis (usually a minimum of 100 mL of sample). World Health Organization guidelines prescribe 200 mL per sample while *Standard Methods for the Examination of Water and Wastewater* prescribes 100 mL per sample. Avoid sample contamination.

No dechlorination is necessary if the sample is added directly to the growth medium on site. Otherwise, treat the samples to destroy chlorine residual. Sodium thiosulfate that has been sterilized within the collection vessel is generally used to destroy chlorine residual. Transport for analysis immediately after collection.

Analyze as soon as possible after collection. Allow no more than 6 hours to elapse between collection and examination for non-potable water samples, and 30 hours for potable water samples. For best results, maintain the sample at or below 10 °C, but do not freeze. Failure to properly collect and transport samples will cause inaccurate results.

Collect at least 100 mL of sample in a presterilized plastic bag or bottle, or in a sterile glass or plastic bottle. Do not fill sample containers completely. Maintain at least 2.5 cm (approximately one inch) of air space to allow adequate space for mixing the sample prior to analysis.

Faucets, spigots, hydrants, or pumps

Collect representative samples by allowing the water to run from a faucet, hydrant, or pump at a moderate rate for 2 to 3 minutes before sampling. Adjust the flow rate before sample collection to avoid splashing during collection. Do not adjust the rate of flow while collecting the sample. Avoid valves, spigots, and faucets that swivel or leak, or those with attachments such as aerators and screens, or remove the attachments prior to sample collection.

Handle the sample containers carefully. Open the sample containers just prior to collection, and close immediately following collection. Do not lay the lid or cap down. Do not touch the lip or inside surface of the container. Do not rinse the containers before use. Label the sample containers immediately and analyze promptly.

Rivers, lakes, and reservoirs

When sampling a river, lake, or reservoir, do not sample near the edge or bank. Remove the cap, grasp the sample container near the bottom, and plunge the container, mouth down, into the water in order to exclude any surface scum. Fill the container entirely under water by positioning the mouth into the current or, in non-flowing water, by tilting the container slightly and allowing it to fill slowly. Do not rinse the container before use. Label the sample containers immediately, and analyze promptly.

Introduction to Membrane Filtration Methods, continued

Sample Size

Bacterial density governs the sample size. 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. *Table 1* and *Table 2* list recommended volumes for various types of samples.

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

Table 1 Suggested Sample Volumes for Membrane Filter Total Coliform Test*

Water Source	Volume to be Filtered (mL)							
	100	50	10	1	0.1	0.01	0.001	0.0001
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

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

Table 2 Suggested Sample Volumes for Membrane Filter Fecal Coliform Test*

Water Source	Volume to be Filtered (mL)						
	100	50	10	1	0.1	0.01	0.001
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

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

Introduction to Membrane Filtration Methods, continued

Diluting Samples

As indicated by *Table 1* and *Table 2*, samples high in coliform number require very small sample volumes. Because it is almost impossible to measure such small volumes accurately, make a series of dilutions. This procedure describes one method of preparing a dilution series:

Dilution Technique

1. Wash your hands.
2. Open a bottle of sterile Buffered Dilution Water.
3. Shake the sample collection container vigorously, approximately 25 times.
4. Use a sterile transfer pipet to pipet the required amount of sample into the sterile Buffered Dilution Water.
5. Recap the buffered dilution water bottle and shake vigorously 25 times.
6. If more dilutions are needed, repeat *step 3* through *step 5* using clean, sterile pipets and additional bottles of sterile Buffered Dilution Water.

Dilution Series

A. If 10-mL sample is required:

Transfer 11 mL of sample into 99 mL of sterile Buffered Dilution Water. Filter 100 mL of this dilution to obtain the 10-mL sample.

B. If 1-mL sample is required:

Transfer 11 mL of the 10-mL dilution from *step A* into 99 mL of sterile Buffered Dilution Water. Filter 100 mL of this dilution to obtain the 1-mL sample.

C. If 0.1-mL sample is required:

Transfer 11 mL of the 1-mL dilution from *step B* into 99 mL of sterile Buffered Dilution Water. Filter 100 mL of this dilution to obtain the 0.1-mL sample.

D. If 0.01-mL sample is required:

Transfer 11 mL of the 0.1-mL dilution from *step C* into 99 mL of sterile Buffered Dilution Water. Filter 100 mL of this dilution to obtain the 0.01-mL sample.

E. If 0.001-mL sample is required:

Transfer 11 mL of the 0.01-mL dilution from *step D* into 99 mL of sterile Buffered Dilution Water. Filter 100 mL of this dilution to obtain the 0.001-mL sample.

F. If 0.0001-mL sample is required:

Transfer 11 mL of the 0.001-mL dilution from *step E* into 99 mL of sterile Buffered Dilution Water. Filter 100 mL of this dilution to obtain the 0.0001-mL sample.

Introduction to Membrane Filtration Methods, continued

Disposing of Completed Tests

To safely dispose of active bacterial cultures grown during incubation, use either of the following methods:

Bleach

Sterilize used test containers with a 10% bleach solution. (Prepare this solution by mixing 10 parts commercial bleach with 90 parts water.) Add 5–10 mL of the prepared bleach solution to each test container. Allow 10 to 15 minutes contact time with the bleach. Pour the liquid down the drain, then dispose of the test containers in the normal waste.

Autoclave

Place used test containers in a contaminated items bag or a biohazard bag to prevent leakage into the autoclave. Autoclave the used test containers in the unsealed bag at 121 °C for 30 minutes at 15 pounds pressure. Once the test containers are cool, seal the bag, place it in another garbage bag, and tie tightly.

Interpreting and Reporting Results

Report bacterial density as the number of colonies per 100 mL of sample. For total coliforms, use samples that produce 20 to 80 coliform colonies, and not more than 200 colonies of all types, per membrane to compute coliform density. For fecal coliform and enterococcal testing, samples should produce 20 to 60 colonies.

Use *Equation A* to calculate density. Note that “mL sample” refers to actual sample volume, and not volume of the dilution.

Equation A—Bacterial density on a single membrane filter

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

- If growth covers the entire filtration area of the membrane, or a portion of it, and colonies are not discrete, report results as “Confluent Growth With or Without Coliforms.”
- 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 nonpotable water, if no filter meets the desired minimum colony count, calculate the average coliform density with Equation B.

Introduction to Membrane Filtration Methods, continued

Equation B—Average bacterial density for 1) duplicates, 2) multiple dilutions, or 3) more than one filter/sample

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

Controls:

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. Hach offers the AQUA QC-STIK™ for quality control procedures. Instructions for use come with each AQUA QC-STIK Device.

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

Table of Media for Membrane Filtration

Product	Platform	Cat. No.	Selectivity	Incubation	Shelf life	USEPA Approved	Standard Method Equivalent*	Sample
KF-Streptococcus	Broth in plastic ampules	28127-50	Enterococci	48 hours at 35 ± 0.5 °C	1 year at 2–8 °C	no	no	Drinking water Beverages Ground water Surface water Recreational water
m-ColiBlue24® (See page 17 for method)	Prepared Agar Plate	28052-15**	Total Coliform Bacteria and <i>Escherichia coli</i>	24 hours at 35 ± 0.5 °C	1 year at 2–8 °C	no (Agar Plate) yes (Broth ampules and 100-mL bottles)	no	Drinking water Beverages Ground water Surface water Recreational water Wastewater
	Broth in glass ampules	26084-20						
	Broth in 100 mL glass bottle	26084-42						
	Broth in plastic ampules	26084-50						
m-EI (See page 37 for method)	Prepared Agar Plate	28117-15**	Enterococci	24 hours at 41 ± 0.5 °C	3 months at 2–8 °C	no, but equivalent to USEPA-proposed Method 1600	no	Drinking water Ground water Surface water Recreational water
m-Endo (See page 21 for method)	Prepared Agar Plate	28116-15	Total Coliform Bacteria	24 hours at 35 ± 0.5 °C	1 year at 2–8 °C	equivalent	Standard Method 9222 B	Drinking water Beverages Ground water Surface water Recreational water
	Broth in glass ampules	23735-20						
	Broth in 100 mL glass bottle	23735-42						
	Broth in plastic ampules	23735-50						
m-FC (See page 29 for method)	Prepared Agar Plate	28115-15**	Fecal Coliform Bacteria	24 hours at 44.5 ± 0.2 °C	1 year at 2–8 °C	equivalent	Standard Method 9222 D	Ground water Surface water Recreational water Wastewater
	Broth in glass ampules	23732-20						
	Broth in plastic ampules	23732-50						
m-FC with Rosolic Acid (See page 29 for method)	Broth in glass ampules	24285-20	Fecal Coliform Bacteria	24 hours at 44.5 ± 0.2 °C	1 year at 2–8 °C	equivalent	Standard Method 9222 D	Ground water Surface water Recreational water Wastewater
	Broth in plastic ampules	24285-50						

Table of Media for Membrane Filtration (continued)

Product	Platform	Cat. No.	Selectivity	Incubation	Shelf life	USEPA Approved	Standard Method Equivalent*	Sample
m-Green YM	Broth in glass ampules	24283-20	Yeast and Mold	48 hours at 35 ± 0.5 °C	1 year at 2–8 °C	no	no	Drinking water Beverages Ground water Surface water Recreational water Wastewater
	Broth in plastic ampules	24283-50						
m-HPC	Prepared Agar Plate	28114-15	Heterotrophic Bacteria	48 hours at 35 ± 0.5 °C	1 year at 2–8 °C	no	Standard Method 9215 A	Drinking water Beverages Ground water Surface water Recreational water
	Broth in plastic ampules	28124-50						
m-TEC	Agar Tubes/2 tests per tube	25611-06	<i>Escherichia coli</i>	2 hours at 35 ± 0.5 °C then	1 year at 2–8 °C	no	Standard Method 9213 D (non-modified m-TEC only)	Recreational water
m-TEC, modified (See page 35 for method)	Prepared Agar Plate	28118-15**		22 hours at 44.5 ± 0.2 °C				
m-TGE	Broth in glass ampules	23738-20	Heterotrophic Bacteria	24 hours at 35 ± 0.5 °C	1 year at 2–8 °C	no	no	Drinking water Beverages Ground water Surface water Recreational water
	Broth in plastic ampules	23738-50						
m-TGE with TTC	Broth in glass ampules	24284-20	Heterotrophic Bacteria	24 hours at 35 ± 0.5 °C	1 year at 2–8 °C	no	no	Drinking water Beverages Ground water Surface water Recreational water
	Broth in plastic ampules	24284-50						
m-TSB/USP	Broth in plastic ampules	28126-50	Heterotrophic Bacteria	24 hours at 35 ± 0.5 °C	1 year at 2–8 °C	no	no	Drinking water Beverages Ground water Surface water Recreational water
Nutrient Agar/MUG (See page 27 for method)	Agar Tubes/2 tests per tube	24373-06	<i>Escherichia coli</i> (confirmation)	4 hours at 35 ± 0.5 °C	1 year at 2–8 °C	equivalent	Standard Method 9222 G	Drinking water Beverages Ground water Surface water Recreational water
	Prepared Agar Plate	28121-15						

Table of Media for Membrane Filtration (continued)

Product	Platform	Cat. No.	Selectivity	Incubation	Shelf life	USEPA Approved	Standard Method Equivalent*	Sample
Orange Serum	Broth in plastic ampules	28125-50	Acid-tolerant microorganisms associated with spoilage	At least 48 hours at 35 ± 0.5 °C	1 year at 2–8 °C	no	no	Beverages
Pseudomonas	Broth in plastic ampules	28122-50	Pseudomonas	24 hours at 35 ± 0.5 °C	1 year at 2–8 °C	no	no	Drinking water Beverages Ground water Surface water Recreational water
R2A	Agar Tubes/2 tests per tube	27241-06	Stressed Heterotrophic Bacteria	At least 72 hours at 35 ± 0.5 °C (7 days maximum)	1 year at 2–8 °C	no	Standard Method 9215 A	Drinking water Beverages
	Prepared Agar Plate	28142-15**						
	Broth in plastic ampules	28123-50		At least 72 hours at 35 ± 0.5 °C	1 year at 2–8 °C		no	
Rose Bengal Agar	Prepared Agar Plate	28119-15	Yeast and Mold	At least 48 hours at 25–30 °C (7 days maximum)	3 months at 2–8 °C	no	no	Drinking water Beverages Ground water Surface water Recreational water Wastewater

* Standard Methods are described in *Standard Methods for the Examination of Water and Wastewater*, 19th Edition.

** Available in bulk.

Total Coliforms

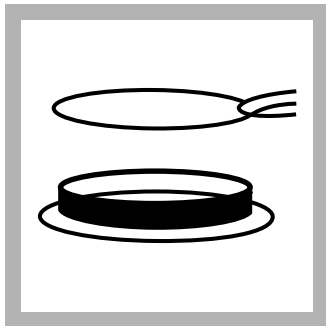
m-ColiBlue24[®] Medium

Hach's patented m-ColiBlue24[®]* medium is a nutritive membrane-filtration medium that simultaneously detects total coliforms and *Escherichia coli* (*E. coli*) within 24 hours. Red and blue colonies are total coliforms while blue colonies are specific to *E. coli*.

The highly selective enzymatic indicator for *E. coli* eliminates the confirmation steps needed when using traditional media. The nutritive medium maximizes the growth rate of coliform bacteria and provides for optimal recovery of stressed or injured organisms. Special inhibitors in the medium minimize the growth of non-coliform bacteria.

Using m-ColiBlue24 Broth PourRite Ampules

The m-ColiBlue24 Broth can be used to analyze drinking water, bottled water, beverages, surface, well, and groundwater, waste water, recreational waters, and process water for ultrapure, chemical processing and pharmaceutical applications.

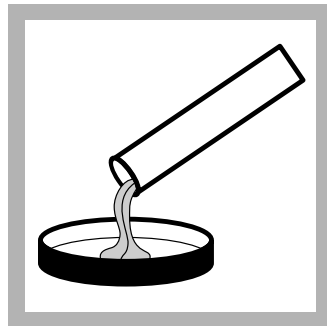


1. Use sterilized forceps to place a sterile, absorbent pad in a sterile petri dish. Replace the lid on the dish.

Note: Do not touch the pad or the inside of the petri dish.

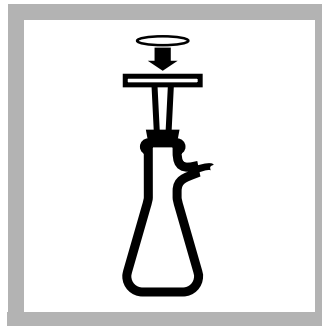
Note: To sterilize the forceps, dip them in alcohol and flame in an alcohol or Bunsen burner. Let the forceps cool before use.

Note: For ease of use, petri dishes with pads are available.

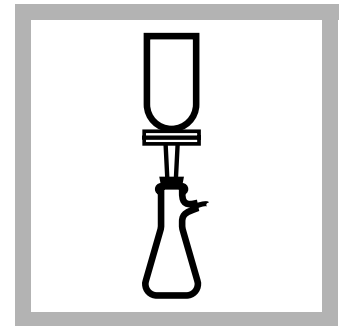


2. Invert ampules two or three times to mix broth. Break open an ampule of m-ColiBlue24 Broth. Pour the contents evenly over the absorbent pad. Replace the petri dish lid.

Note: If you do not require USEPA approval, you may use the pre-poured agar plates.



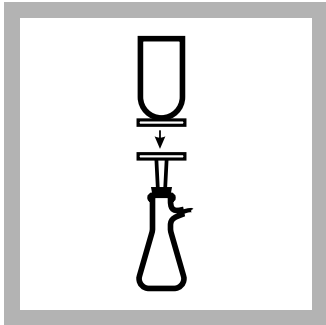
3. Set up the Membrane Filter Apparatus; see *Preparing Autoclavable Filter Assembly* on page 7. With sterile forceps, place a membrane filter, grid side up, into the assembly.



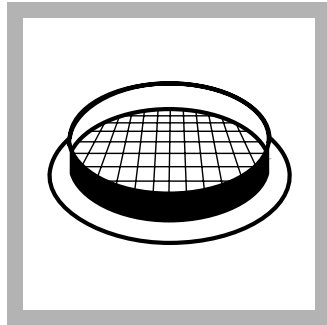
4. Shake the sample or diluted sample vigorously to mix. Pour 100 mL of the sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls three times with 20 to 30 mL of sterile buffered dilution water.

* m-ColiBlue24 is a registered trademark and patented product of Hach Company. U.S. Patents 5,650,290 and 5,849,515.

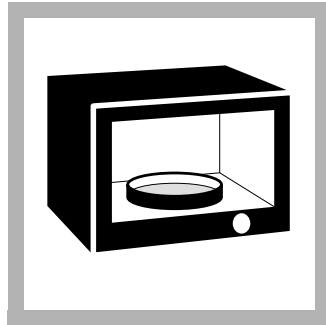
Total Coliforms, continued



5. Turn off the vacuum and lift off the funnel top. Using sterile forceps, transfer the filter to the previously prepared petri dish.

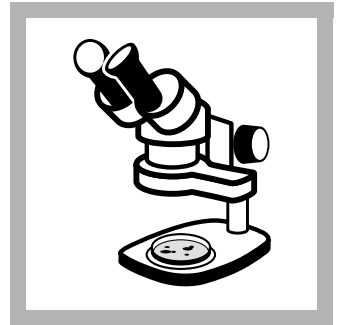


6. With a slight rolling motion, place the filter, grid side up, on the absorbent pad. Check for trapped air under the filter and make sure the filter touches the entire pad. Replace the petri dish lid.



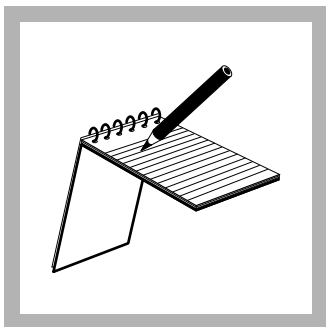
7. Invert the petri dish and incubate at 35 ± 0.5 °C for

- 24 hours when using m-ColiBlue24 Broth, or
- 16–20 hours when using m-ColiBlue24 Agar plates.



8. Remove the petri dish from the incubator and examine the filters for colony growth. Colonies are typically readily visible; however, a stereoscopic microscope or other 10–15X magnifier may be useful. Red and blue colonies indicate total coliforms and blue colonies specifically indicate *E. coli*.

Note: Sometimes only the center of a colony will be colored. Therefore, a colony with any amount of red color should be counted as red and a colony with any amount of blue should be counted as a blue colony. Red colonies may vary in color intensity. Blue colonies may appear blue to purple. Count all the red and blue colonies as total coliforms. Count all the blue to purple colonies as *E. coli*.



9. Record the results of the test. See *Interpreting and Reporting Results* on page 11

Total Coliforms, continued

Optional Verification of Total Coliforms

The m-ColiBlue24 medium is formulated so that coliforms other than *E. coli* grow as red colonies. The percentage of red colonies that are false positives (non-coliforms) is comparable to the percentage of sheen colonies grown on m-Endo Broth that are false positives (non-coliforms); therefore, confirmation is not required.

A few varieties of the non-coliform bacteria *Pseudomonas*, *Vibrio*, and *Aeromonas* spp. may grow on m-ColiBlue24 medium and form red colonies. Such bacteria can be readily distinguished from total coliforms by the oxidase test. *Pseudomonas*, *Vibrio*, and *Aeromonas* spp. are oxidase-positive. Total coliforms and *Escherichia coli* are oxidase-negative. If your sample contains high levels of interfering bacteria, you can perform an oxidase test to confirm which red colonies are total coliforms.

Note: In cases where unknown samples are being pressed for proficiency or certification testing, this optional verification step is recommended.

Two oxidase procedures are provided. Count the red and blue colonies on the membrane filter before starting the oxidase test.

Oxidase Method 1

This method enables you to conveniently and rapidly evaluate membrane filters that have numerous colonies. Use this method after 24 hours of incubation on m-ColiBlue24 Broth or after 16–20 hours of incubation on m-ColiBlue24 Agar.

Research* shows that the oxidase test cannot be performed on media that undergoes acidification during bacterial growth. The m-ColiBlue24 is formulated so that the medium does not undergo such acidification. Consequently, many colonies can be simultaneously tested for their oxidase reaction using the following procedure.

1. Remove the lid from the petri dish containing the m-ColiBlue24 membrane filter, invert the lid, and place it on the bench top.

Controls: Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended for positive controls and *Escherichia coli* for negative controls. Hach offers Aqua QC-Stiks™ for quality control procedures.

2. Drop approximately 1.0 mL of Difco BBL Oxidase Reagent into the center of the inverted lid. Use 2 vials if the reagent is supplied in 0.5-mL vials.
3. Using sterile forceps, transfer the membrane filter from the pad and place the filter upright in the inverted lid. Continuously monitor colony color throughout the reaction period.
4. Within 10 to 15 seconds, the oxidase reagent will soak into the filter and cause the oxidase-positive colonies to turn purple. This purple color may be visible in the colony itself or adjacent to the colony. Oxidase-negative colonies will retain the red color they developed when incubated on m-ColiBlue24 medium.

* A.H. Havelaar et al. 1980. False-negative oxidase reaction as a result of medium acidification. *Antonie van Leeuwenhoek*. 46, 301-312.

L.K. Hunt et al. 1981. Role of pH in oxidase variability of *Aeromonas hydrophila*. *Journal of Clinical Microbiology*. 13: 1054-1059.

Total Coliforms, continued

5. After the initial 10 to 15 second reaction time, start counting the red colonies that turn purple. Count individual colonies by using a microscope with 10X to 15X magnification

Note: To simplify colony counting place a spare lid on the lid containing the oxidase reagent and membrane filter. Use a felt-tip pen to mark the lid as you identify the purple colonies. After 30 seconds, you can count marks that indicate purple (oxidase-positive) colonies.

6. Stop counting 30 seconds after initial 10 to 15 second reaction time, because oxidase-negative colonies will start to develop a purple color, giving inaccurate results.

Note: Bacteria are not killed with this procedure, so colonies may be selected for streaking and for additional testing.

Colonies that are blue after the initial 24-hour incubation on m-ColiBlue24 medium are almost always *E. coli* and do not need confirmation with the oxidase procedure.

Oxidase Method 2

This method is the official oxidase test described in *Standard Methods for the Examination of Water and Wastewater*, 18th edition, 1992.

1. Select red colonies from an m-ColiBlue24 Broth membrane filter and streak onto Tryptic Soy Agar.
2. Incubate Tryptic Soy Agar plates at 35 °C for 18 to 24 hours or until isolated colonies are obtained.

Controls: Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended for positive and *Escherichia coli* for negative controls. Hach offers Aqua QC-Sticks for quality control procedures.

3. Saturate a piece of filter paper with Oxidase Reagent. (This reagent contains a stabilized solution of N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride.)

Note: Alternatively, oxidase reagent can be dropped directly onto colonies growing on Tryptic Soy Agar. Oxidase-positive colonies will turn pink to purple.

4. Using a sterile nichrome inoculating needle, transfer cellular material from an isolated Tryptic Soy Agar colony to the moist filter paper.

Note: Do not use iron or other reactive needles for inoculation, because they will cause false-positive results. Wooden applicator sticks work well.

5. Oxidase-negative colonies will not react with the reagent, but oxidase-positive colonies will cause the reagent to turn dark purple within 10 seconds.

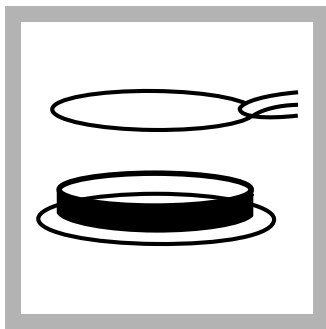
Oxidase-negative colonies should be counted as total coliform bacteria.

Total Coliforms, continued

Using m-Endo Broth for Presumptive Total Coliform Test

Method 8074

To test potable water with the MF Method using m-Endo Broth, examine a 100-mL sample for total coliforms by incubating a filter at 35 ± 0.5 °C for 22–24 hours. Coliforms ferment lactose in the medium and produce an acid-aldehyde complex. This complex combines with Schiff's Reagent (also in the medium) to form an iridescent green coating over the colonies. When magnified 10 to 15 times, coliforms appear as dark red colonies with a greenish-gold metallic sheen.

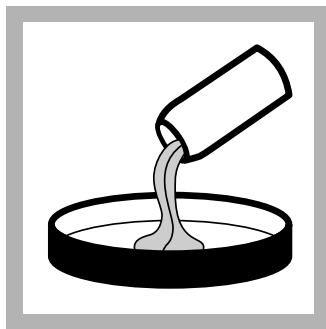


1. Place a sterile absorbent pad in a sterile petri dish using sterilized forceps. Replace the lid.

Note: Do not touch the pad or the inside of the petri dish.

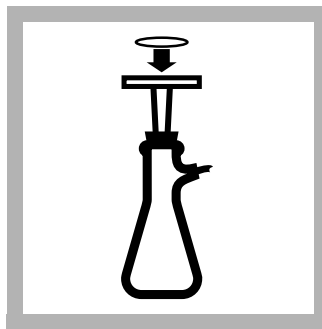
Note: To sterilize forceps, dip forceps in alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.

Note: For ease of use, petri dishes with pads are available.

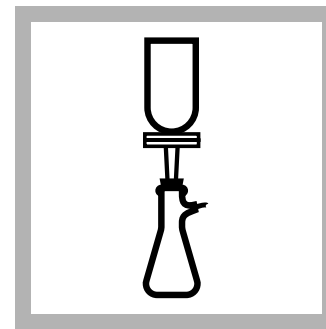


2. Invert an m-Endo Broth PourRite Ampule 2 to 3 times to mix the broth. Break open the ampule. Carefully pour the contents evenly over the absorbent pad. Replace the petri dish lid. Repeat *step 1* and *step 2* for each petri dish being prepared.

Note: Prepared m-Endo Agar plates may be used instead of m-Endo Broth.

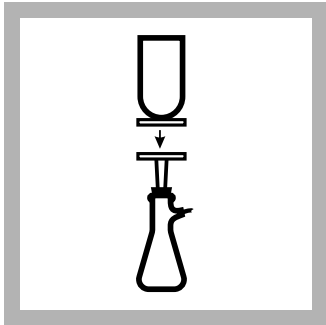


3. Set up the Membrane Filter Assembly; see *Preparing Autoclavable Filter Assembly* on page 7. Using sterilized forceps, place a membrane filter, grid side up, into the assembly.

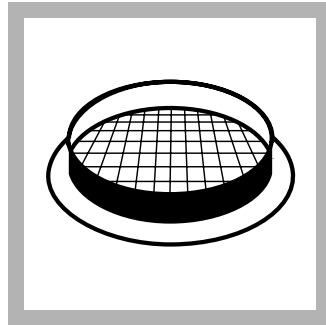


4. Shake the sample vigorously to mix. Pour 100 mL of sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls three times with 20 to 30 mL of sterile buffered dilution water.

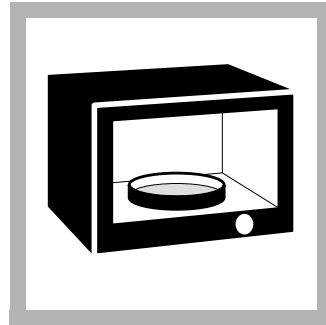
Total Coliforms, continued



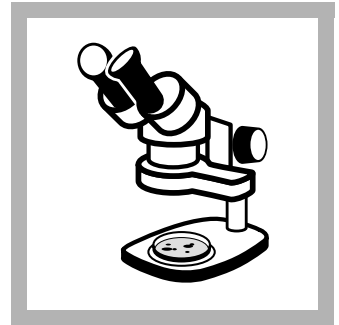
5. Turn off the vacuum and lift off the funnel top. Using sterilized forceps, transfer the filter immediately to the previously prepared petri dish.



6. With a slight rolling motion, center the filter, grid side up, on the absorbent pad. Check for air trapped under the filter and make sure the filter touches the entire pad. Replace the petri dish lid.

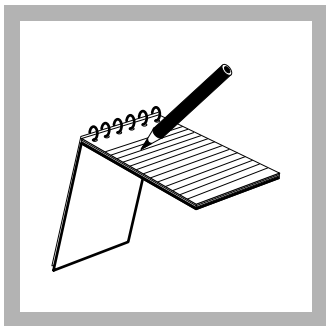


7. Invert the petri dish and incubate at 35 ± 0.5 ° C for 22–24 hours.



8. After incubating, use a 10 to 15X stereoscopic microscope or other type of illuminated magnifier to count the colonies having a greenish-gold metallic sheen.

Note: The sheen may extend over the entire colony, or it may be localized to the edge or to the center.



9. Record the results of the test. See *Interpreting and Reporting Results* on page 11



10. Depending on your test protocol, confirm positive results.

Note: To confirm total coliforms, follow *Using Lauryl Tryptose Broth and Brilliant Green Bile Broth for Confirmation of Total Coliforms* on page 23.

Note: To confirm fecal coliforms, follow *Using EC Medium Broth for Confirmation of Fecal Coliforms* on page 25.

Note: To confirm *E. coli*, follow *Using EC Medium with MUG (EC/MUG) Broth or Nutrient Agar with MUG for Confirmation of E. coli* on page 26.

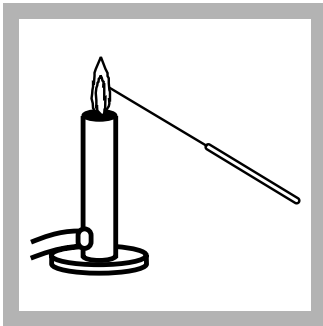
Total Coliforms, continued

Using Lauryl Tryptose Broth and Brilliant Green Bile Broth for Confirmation of Total Coliforms

Method 8074

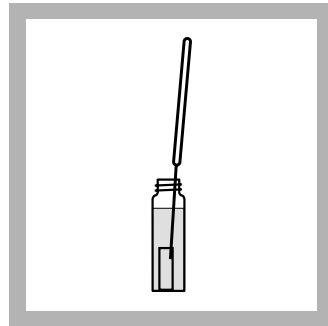
For potable water samples, verify (confirm) typical colonies to ensure they are coliforms. (Confirm sheen colonies, up to a maximum of five.) Inoculate parallel tubes of Lauryl Tryptose (LT) Broth and Brilliant Green Bile (BGB) Broth by transferring growth from each colony. Growth and gas production in both tubes verifies that the suspect organisms are coliforms. Hach's Most Probable Number (MPN) coliform tubes are ideal for this purpose.

Note: Follow the swabbing technique for fecal coliforms or *E. coli* if inoculating EC or EC/MUG media. Inoculate in this order: EC or EC/MUG, LTB, BGB. Also use this swabbing technique when determining the presence or absence of total coliforms.

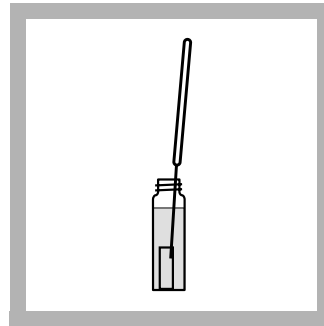


1. Sterilize an inoculating needle. Or, use a sterile, disposable inoculating needle.

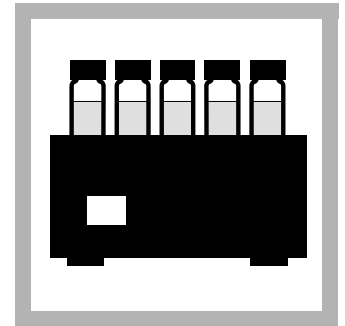
Note: To sterilize an inoculating needle, heat to red hot in an alcohol or Bunsen burner. Let the needle cool before use.



2. Touch the needle to the coliform (sheen) colony grown on m-Endo Broth. Transfer to a single-strength Lauryl Tryptose (LT) Broth tube.

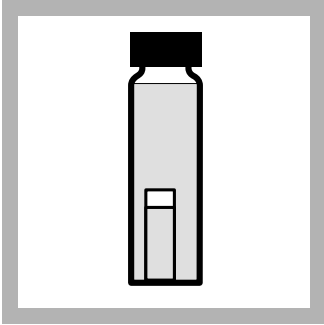


3. Again touch the same coliform colony with the needle. Transfer to a Brilliant Green Bile (BGB) Broth tube.

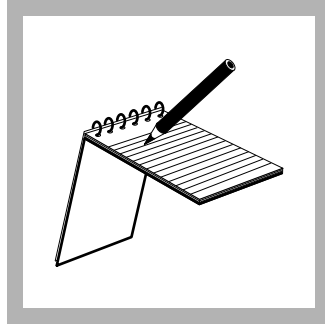


4. Invert both tubes to eliminate any air bubbles trapped in the inner vials. Incubate the tubes at 35 ± 0.5 °C. After one hour, invert the tubes to remove trapped air in the inner vial, then continue incubation.

Total Coliforms, continued



5. After 24 ± 2 hours, check the inner vials for growth and gas bubbles. Growth (turbidity) and gas bubbles in both the LT and BGB Broth tubes verify that the colonies are coliforms. If one or both tubes do not show gas, continue incubating both tubes for an additional 24 hours.



6. If no gas is present in the LT Broth tube after 48 hours, the colony is not a coliform and additional testing is unnecessary.

Record the results of the test. See *Interpreting and Reporting Results* on page 11.



7. Confirm positive results. If growth and gas are produced in the LT Broth tube but not in the BGB Broth tube, inoculate another BGB tube from the gas-positive LT Broth tube. Incubate this BGB Broth tube and check for growth and gas after 24 hours and/or after 48 hours. If growth and gas are produced within 48 ± 3 hours, the colony is confirmed as coliform.

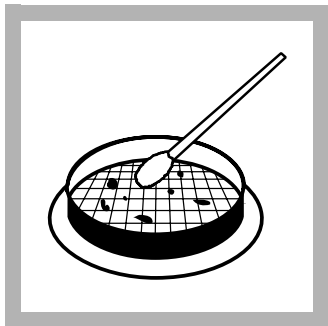
Record the results of the test. See *Interpreting and Reporting Results* on page 11.

Total Coliforms, continued

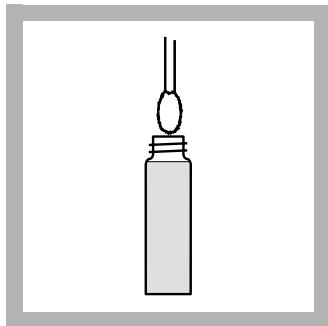
Using EC Medium Broth for Confirmation of Fecal Coliforms

Method 8074

Analyze total-coliform-positive potable water samples for the presence of fecal coliform or *E. coli*. Confirm fecal coliforms from a membrane filter positive for total coliforms by swabbing the membrane with a sterile cotton swab and inoculating a tube of EC Medium Broth. Growth and gas production in the EC Medium confirms the presence of fecal coliforms.

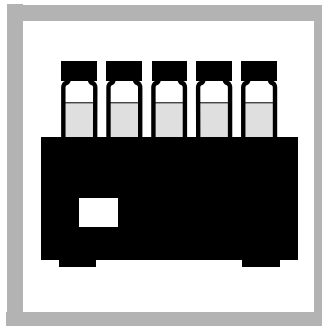


1. Use a sterile cotton swab to swab the entire surface of the total coliform-positive membrane filter (colonies grown on m-Endo Broth).

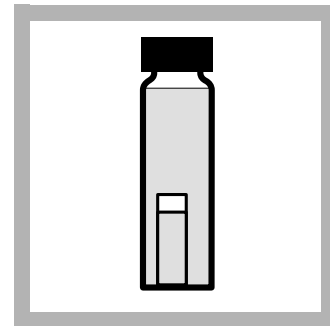


2. Swirl the cotton swab in an EC Medium Broth tube to transfer the colonies collected from the filter. Remove the cotton swab from the medium.

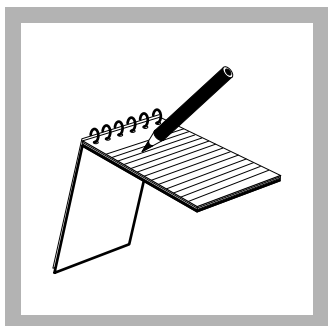
Use the same cotton swab to transfer colonies from the same petri dish to other broth media if desired.



3. Invert the tubes to eliminate any air bubbles trapped in the inner vial. Incubate the tube at 44.5 ± 0.2 °C. After one hour, invert the tubes to remove trapped air in the inner vial and continue incubation.



4. After 24 ± 2 hours, check the inner vial for gas bubbles. Growth and gas bubbles in the EC Medium Broth tube confirm the presence of fecal coliforms.



Record the results of the test.
See *Interpreting and Reporting Results* on page 11.

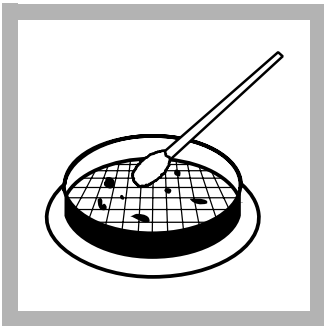
Total Coliforms, continued

Using EC Medium with MUG (EC/MUG) Broth or Nutrient Agar with MUG for Confirmation of *E. coli*.

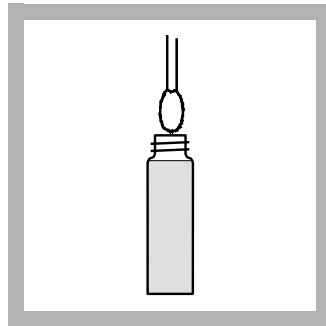
Method 8074

Potable water samples that test positive for total coliforms may be analyzed for the presence of *E. coli* in lieu of fecal coliforms. Use either of these methods to confirm the presence of *E. coli* on a membrane filter positive for total coliforms.

EC/MUG Broth Method

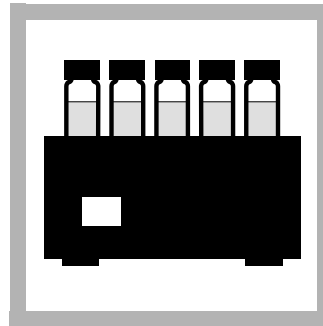


1. Use a sterile cotton swab to swab the entire surface of the membrane filter that is positive for total coliforms (colonies grown on m-Endo Broth).

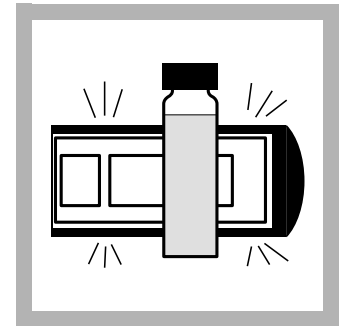


2. Swirl the cotton swab in an EC/MUG Broth tube to transfer the colonies collected from the filter. Remove the cotton swab from the medium.

Use the same cotton swab to transfer colonies from the same petri dish to other broth media if desired.



3. Invert the tubes to eliminate any air bubbles trapped in the inner vial. Incubate the tube at 44.5 ± 0.2 °C. After one hour, invert the tubes to remove trapped air in the inner vial and continue incubation.



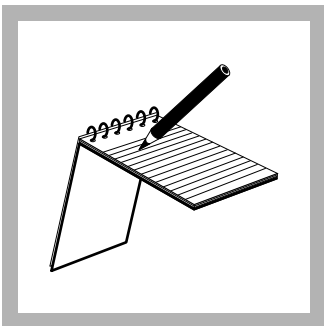
4. After 24 ± 2 hours, use a long-wave UV lamp to check the tube for fluorescence. Growth and fluorescence indicate the presence of *E. coli*.

Note: **Do not** look directly through the MPN tube into the UV lamp. UV light can damage eyesight. View the tube at 90° from the lamp. Examine the tubes in a darkened area.

Note: Some glass will auto-fluoresce. Always use Hach MPN tubes for best results.

Note: Some UV lamps are not the correct wattage and can give false results. Always use Hach UV lamps and replacement bulbs. (See REQUIRED APPARATUS on page 41).

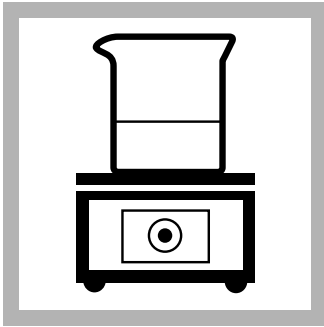
Note: Have a fluorescent-positive and a fluorescent-negative tube, both with turbidity, to compare with the sample tube.



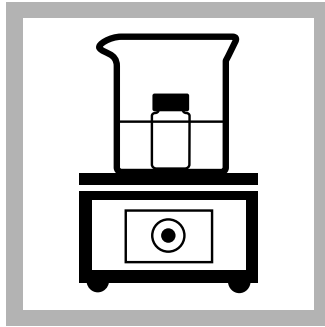
5. Record the results of the test. See *Interpreting and Reporting Results* on page 11.

Total Coliforms, continued

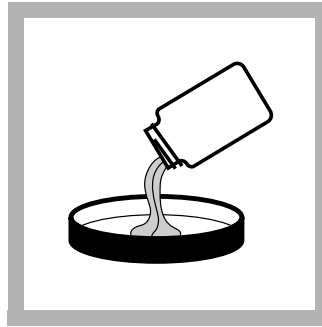
Nutrient Agar with MUG (NA/MUG) Method



1. Heat a beaker of water, or a water bath, but do not allow it to boil.

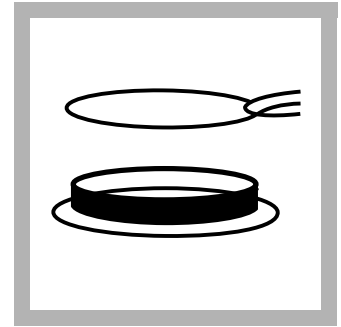


2. Loosen the cap on one or more NA/MUG nutrient agar tubes. Place the tubes into hot water. When the agar melts, carefully remove the tubes from the water with tongs.



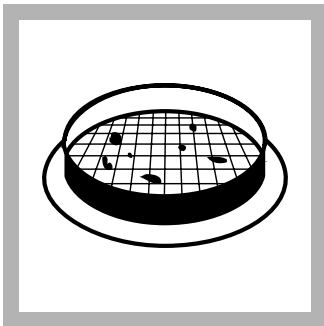
3. Using sterile technique, pour half of the contents of the tube into a sterile 50-mm petri dish. Immediately replace petri dish lid and allow agar to solidify.

Note: Hach Company also offers prepoured agar plates for this procedure. (See Media, Reagents, and Apparatus on page 41.)

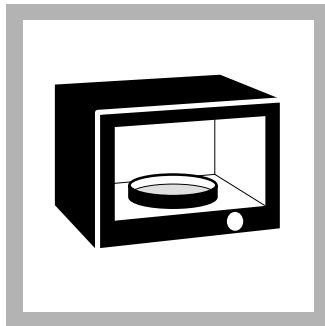


4. Use sterilized forceps to lift the membrane filter with total coliform colonies off the m-Endo absorbent pad.

Note: To sterilize forceps, dip forceps in alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.



5. Immediately transfer the membrane filter to the petri dish containing NA/MUG. With a slight rolling motion, center the filter, grid side up, on the agar. Check for air trapped under the filter and make sure the entire filter touches the agar. Replace the petri dish lid.



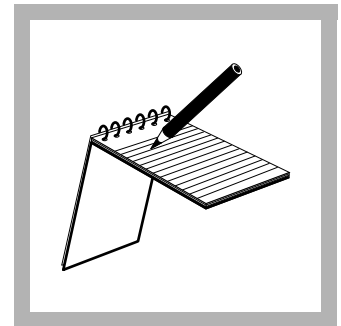
6. Invert the petri dish. Incubate for 4 hours at 35 ± 0.5 °C.



7. Using a long-wave UV lamp, examine the colonies for fluorescence. Fluorescence indicates the presence of *E. coli*.

Note: Make sure to examine petri dish in a darkened area.

Note: Some UV lamps are not the correct wattage and can give false results. Always use Hach UV lamps and replacement bulbs. (See REQUIRED APPARATUS on page 41.)



8. Record the results of the test. See *Interpreting and Reporting Results* on page 11.

Total Coliforms, continued

Enrichment Technique for Stressed Total Coliforms

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

Enrichment Technique

1. Place a sterile absorbent pad in the lid of a sterile petri dish.
2. Saturate the pad with the contents of 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%.
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 those colonies with a greenish-gold metallic sheen.

To confirm total coliforms, follow *Using Lauryl Tryptose Broth and Brilliant Green Bile Broth for Confirmation of Total Coliforms* on page 23.

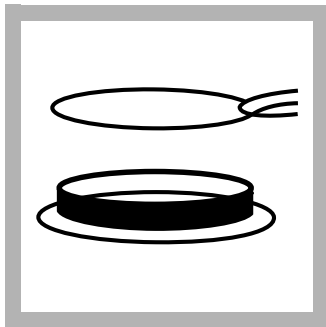
To confirm fecal coliforms, follow *Using EC Medium Broth for Confirmation of Fecal Coliforms* on page 25.

To confirm *E. coli*, follow *Using EC Medium with MUG (EC/MUG) Broth or Nutrient Agar with MUG for Confirmation of E. coli* on page 26.

Fecal Coliforms

Wastewater, river water, bathing water, and other nonpotable waters are usually tested for fecal coliforms. In testing for fecal coliforms, a special medium (m-FC Broth) and an elevated incubation temperature inhibit growth of nonfecal coliforms. Rosolic Acid (1%) added to m-FC Broth inhibits the growth of nonfecal coliform bacteria. Fecal coliforms growing on a membrane in m-FC Broth form an acid that reacts with an aniline dye in the medium, producing a blue color. Sample sizes are selected from *Table 2* on page 9.

Using m-FC Broth or m-FC Broth with Rosolic Acid (m-FC/RA) for Fecal Coliform Test Method 8074

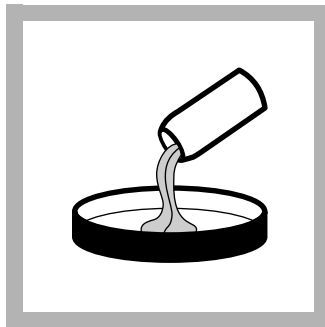


1. Place a sterile absorbent pad in a sterile petri dish using sterilized forceps. Replace the petri dish lid.

Note: Do not touch the pad or the inside of the petri dish.

Note: To sterilize forceps, dip forceps in alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.

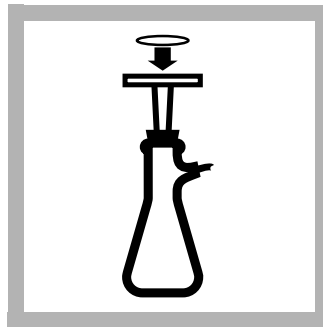
Note: Petri dishes with pads are available from Hach.



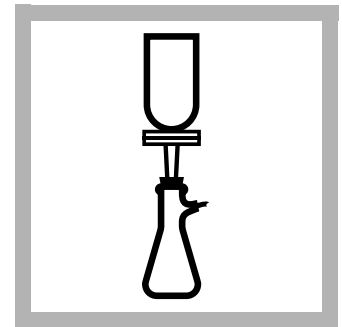
2. Invert an m-FC Broth PourRite™ Ampule 2 to 3 times to mix the broth. Use the ampule breaker to open an ampule. Carefully pour the contents evenly onto the absorbent pad. Replace the petri dish lid

Note: Prepared m-FC Agar plates may be used instead of m-FC Broth.

Note: Use m-FC Broth with Rosolic Acid to increase specificity when high levels of non-coliform bacteria may be present, unless the organisms are stressed or injured.



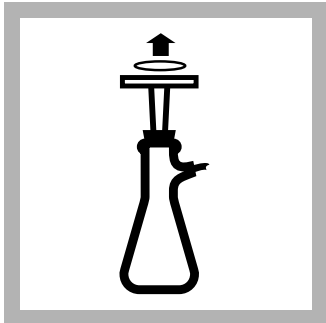
3. Set up the Membrane Filter Assembly; see *Preparing Autoclavable Filter Assembly* on page 7. Use sterilized forceps to place a membrane filter, grid side up, into the assembly.



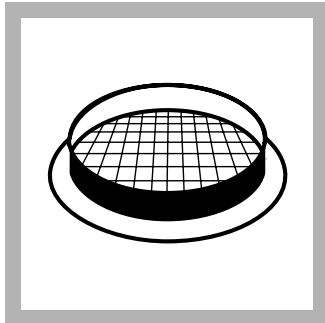
4. Prepare the necessary dilutions to obtain the proper sample size. Shake the sample vigorously to mix. Pour sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls 3 times with 20 to 30 mL of sterile buffered dilution water.

Note: See *Sample Size* on page 9 and *Diluting Samples* on page 10.

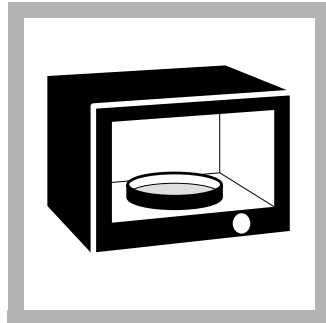
Fecal Coliforms, continued



5. Turn off the vacuum and lift off the funnel top. Use sterile forceps to transfer the membrane filter to the previously prepared petri dish.

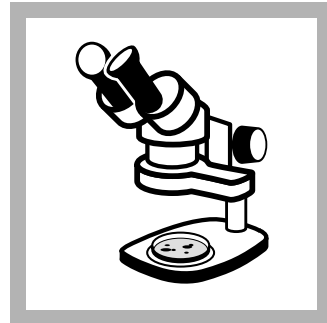


6. With a slight rolling motion, center the filter, grid side up, on the absorbent pad. Check for air trapped under the filter and make sure the entire filter touches the pad. Replace the petri dish lid.

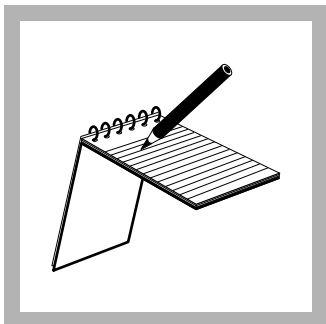


7. Invert the petri dish and incubate at 44.5 ± 0.2 °C for 24 ± 2 hours.

Note: To eliminate environmental *Klebsiella* from the fecal coliform population elevate the temperature to 45.0 ± 0.2 °C.



8. After incubating, count the blue colonies using an illuminated magnifier or a 10 to 15X stereoscopic microscope.



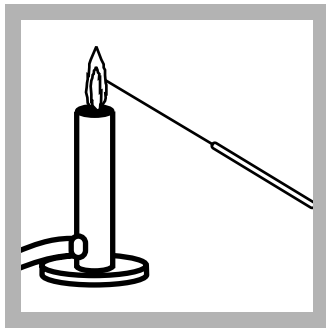
9. Record the results of the test. See *Interpreting and Reporting Results* on page 11.



10. To verify results, follow the *Verifying Fecal Coliforms* procedure.

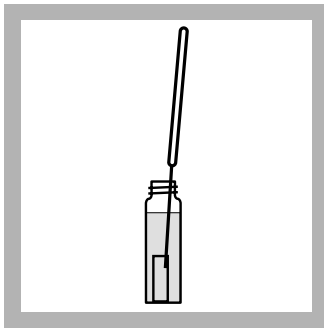
Fecal Coliforms, continued

Verifying Fecal Coliforms



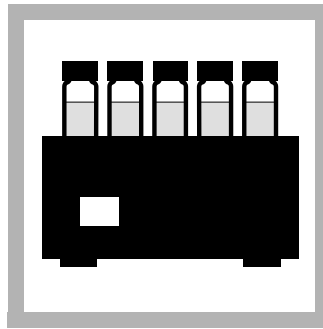
1. Sterilize an inoculating needle. Or, use a sterile, disposable inoculating needle.

Note: To sterilize an inoculating needle, heat to red hot in an alcohol or Bunsen burner flame. Let the needle cool before use.



2. Touch the needle to a typical blue colony and transfer to a Lauryl Tryptose (LT) Broth tube. Repeat *step 1* and *step 2* for each test being verified.

Note: Simultaneous inoculation of LT and EC medium is acceptable. Inoculate EC medium with a portion of the same colony used to inoculate LT Broth and follow *Step 4*.

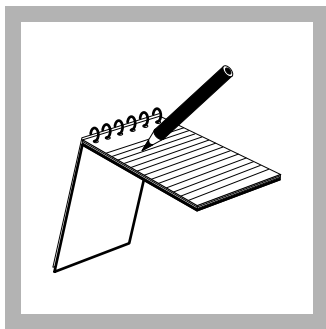


3. Invert the tubes to eliminate air trapped inside the inner vials. Incubate the tubes at 35 ± 0.5 °C. After one hour, invert the tubes to remove trapped air in the inner vial and continue incubation. Check tubes for growth and gas production at 24 hours. If no change has occurred, continue incubation for another 24 hours.

Note: If growth and gas are not produced in 48 ± 3 hours, the colony was not coliform. If growth and gas are produced in 48 ± 3 hours, use a sterile loop to inoculate one EC Medium Broth tube from each gas-positive LT Broth tube.



4. Invert the tubes to eliminate air trapped inside the inner vials. Incubate the EC Medium tubes at 44.5 ± 0.2 °C for 24 ± 2 hours. After one hour, invert the tubes to remove trapped air in the inner vial.



5. Growth and gas production at 44.5 °C within 24 ± 2 hours confirms the presence of fecal coliforms.

Record the results of the test. See *Interpreting and Reporting Results* on page 11.

Other Methods for Detecting Fecal Coliforms

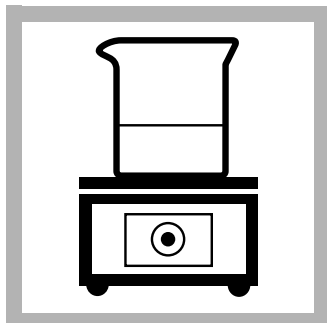
See *Using EC Medium Broth for Confirmation of Fecal Coliforms* on page 25 for an additional test for fecal coliforms.

Escherichia coli

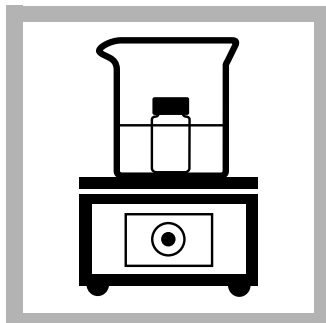
Using m-TEC Agar for Presumptive *E. coli* Test with Confirmation Steps

The m-TEC method detects *E. coli* in recreational fresh water samples with a two step process. First, membrane filters are incubated on m-TEC Agar for 2 hours at 35 °C to resuscitate injured organisms. The thermotolerant organisms are then selected by fermentation of lactose at an elevated temperature of 44.5 °C. The second step uses a substrate medium containing urea to distinguish urease-negative *E.coli* from other thermotolerant coliforms that hydrolyze urea. Yellow or yellow-brown urease-negative colonies are positive for *E. coli*.

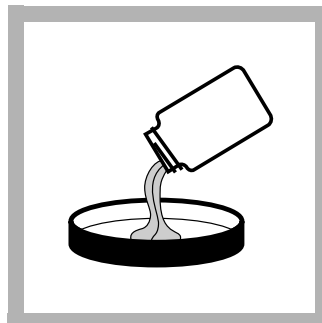
Method 8367



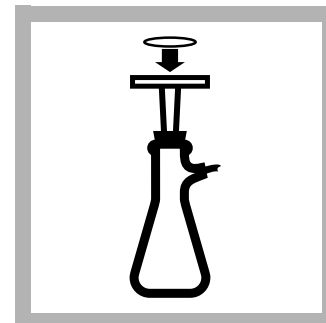
1. Heat a beaker of water or water bath but do not allow it to boil.



2. Place m-TEC Agar tubes into the hot water. When agar melts, carefully remove tubes from hot water with tongs.



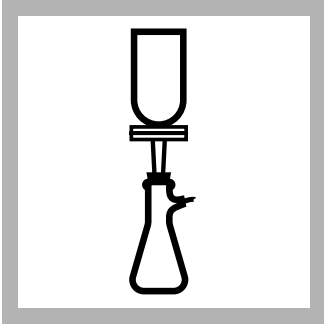
3. Using sterile technique, pour half of the contents of the tube into a sterile, 50-mm petri dish. Immediately replace the petri dish lid and allow agar to solidify.



4. Set up the Membrane Filter Assembly; see *Preparing Autoclavable Filter Assembly* on page 7. Using sterilized forceps, place a membrane filter, grid side up, into the assembly.

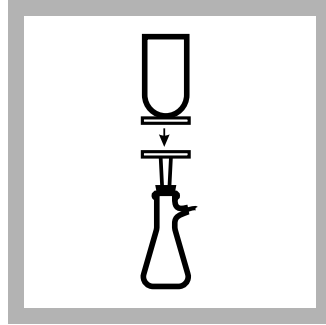
Note: To sterilize forceps, dip forceps into alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.

Escherichia coli, continued

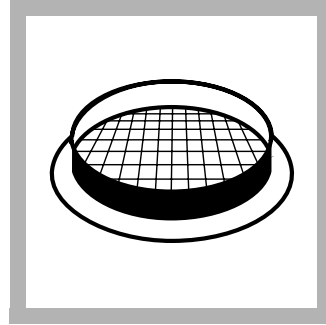


5. Prepare the necessary dilutions to obtain the proper sample size. Shake the sample vigorously to mix. Pour sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls 3 times with 20 to 30 mL of sterile buffered dilution water.

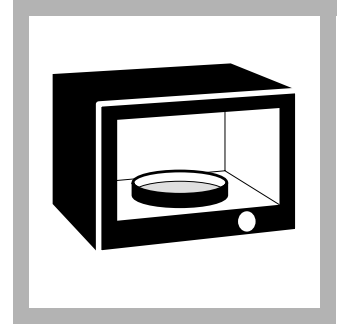
Note: See *Sample Size* on page 9 and *Diluting Samples* on page 10.



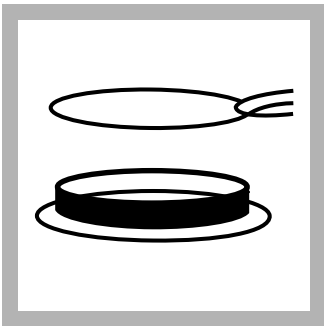
6. Turn off the vacuum and lift off the funnel top. Using sterilized forceps, transfer the membrane filter to the prepared agar plate.



7. With a slight rolling motion, place the filter, grid side up, on the agar. Check for air trapped under the filter and make sure the entire filter touches the agar. Replace the petri dish lid.



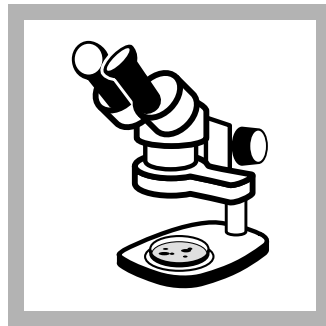
8. Invert the petri dish. Incubate at 35 ± 0.5 °C for 2 hours and then at 44.5 ± 0.2 °C for 22 hours.



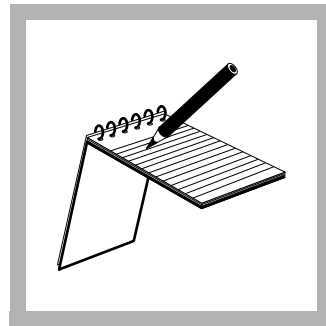
9. To confirm the presence of *E. coli*, use sterilized forceps to transfer the filter to a pad saturated with at least 2 mL of urea substrate.

Preparing urea substrate:

- a.** Dissolve 2.0 g of urea in 100 mL of deionized water.
- b.** Add 10 mg of phenol red sodium indicator to the urea solution.
- c.** Use 0.02 N sulfuric acid to adjust the pH to between 3 and 4. The solution will turn yellow.
- d.** Store solution at 2 to 8 °C. Use within one week.



10. After 15 minutes, count yellow or yellow-brown colonies by using an illuminated magnifier or a 10 to 15X microscope.



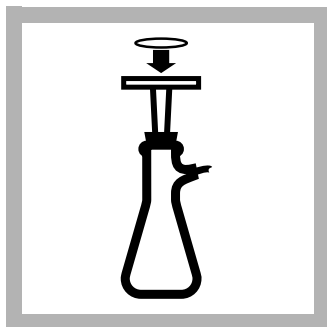
11. The presence of yellow or yellow-brown colonies confirms *E. coli*.

Record the results of the test. See *Interpreting and Reporting Results* on page 11.

Escherichia coli, continued

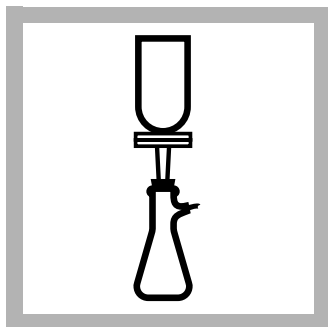
Using Modified m-TEC Prepared Agar Plates for *E. coli* Test in Recreational Waters

A revised single-step method uses modified m-TEC Agar to detect *E. coli* in recreational fresh water samples. The Modified m-TEC Agar contains an enzymatic substrate which is cleaved to produce colonies. Red or magenta colored colonies specifically represent the presence of *E. coli*. Confirmation steps are not required with this method.



1. Set up the Membrane Filter Assembly; see *Preparing Autoclavable Filter Assembly* on page 7. Using sterilized forceps, place a membrane filter, grid side up, into the assembly.

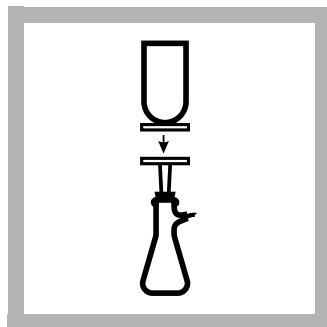
Note: To sterilize forceps, dip forceps into alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.



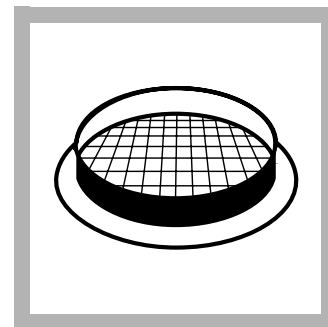
2. Prepare the necessary dilutions to obtain 20–80 *E. coli* colonies on the membranes.
Shake the sample vigorously to mix.

Pour the sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls 3 times with 20 to 30 mL of sterile buffered dilution water.

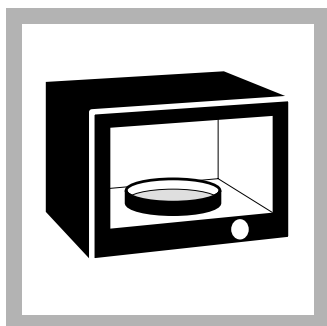
Note: See *Sample Size* on page 9 and *Diluting Samples* on page 10.



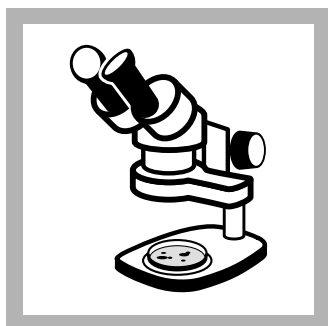
3. Turn off the vacuum and lift off the funnel top. Using sterilized forceps, transfer the membrane filter to the modified m-TEC prepared agar plate.



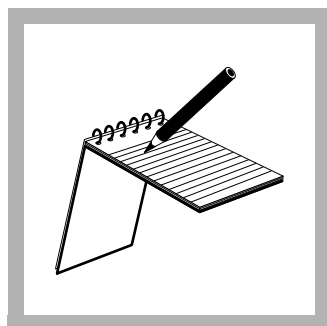
4. With a slight rolling motion, place the filter, grid side up, on the agar plate. Check for air trapped under the filter and make sure the entire filter touches the agar. Replace the petri dish lid.



5. Invert the petri dish. Incubate at 35 ± 0.5 °C for 2 hours, then at 44.5 ± 0.2 °C for 22 hours.



6. Count the number of red or magenta colonies by using an illuminated magnifier or a 10 to 15X microscope.



7. The presence of these colonies confirms *E. coli*. Record the results of the test. See *Interpreting and Reporting Results* on page 11.

***Escherichia coli*, continued**

Other Methods for Detecting *E. Coli*

m-ColiBlue24® Media

Hach's patented m-ColiBlue24 media are nutritive membrane-filtration media that simultaneously detect total coliforms and *Escherichia coli* (*E. coli*) within 24 hours. Red and blue colonies are total coliforms while blue colonies are specific to *E. coli*.

The m-ColiBlue24 media can be used to analyze drinking water; bottled water; beverages; surface, well, and groundwater; waste water; recreational waters; and process water for ultrapure, chemical processing and pharmaceutical applications

See *Using m-ColiBlue24® Broth PourRite™ Ampules* on page 17 for full procedure.

Other Methods

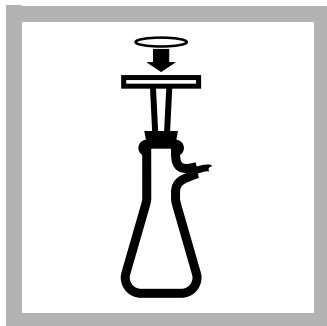
See *Using EC Medium with MUG (EC/MUG) Broth or Nutrient Agar with MUG for Confirmation of E. coli* on page 26 and *Nutrient Agar with MUG (NA/MUG) Method* on page 27 for additional tests for *E. coli*.

Enterococci

Using m-EI Prepared Agar Plates for Enterococci Test in Recreational Waters

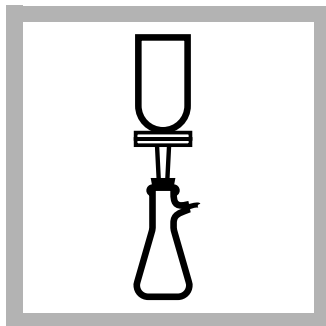
Enterococci are used as an indicator organism for recreational water quality evaluation. The m-EI Agar specifically detects enterococci in 24 hours after enzymatic cleavage of a substrate (indoxyl- β -D-glucoside) which results in formation of colonies surrounded by a blue halo.

Proposed Method 1600



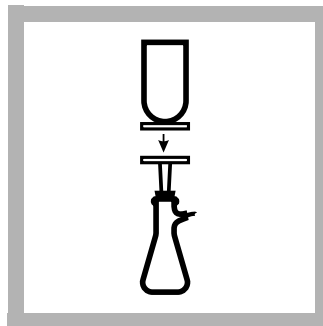
1. Set up the Membrane Filter Assembly; see *Preparing Autoclavable Filter Assembly* on page 7. Using sterilized forceps, place a membrane filter, grid side up, into the assembly.

Note: To sterilize forceps, dip forceps into alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.

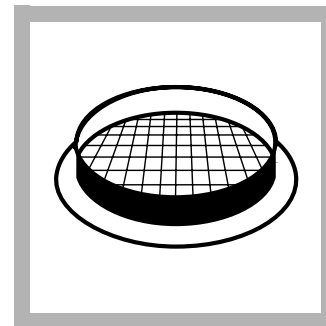


2. Prepare the necessary dilutions to obtain the proper sample size. Shake the sample vigorously to mix. Pour sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls 3 times with 20 to 30 mL of sterile buffered dilution water.

Note: See *Sample Size* on page 9 and *Diluting Samples* on page 10.

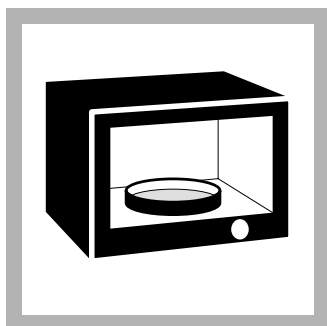


3. Turn off the vacuum and lift off the funnel top. Using sterilized forceps, transfer the membrane filter to the previously prepared agar plate.

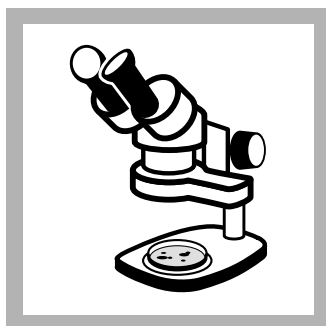


4. With a slight rolling motion, place the filter, grid side up, on the prepared m-EI agar plate. Check for air trapped under the filter and make sure the entire filter touches the agar.

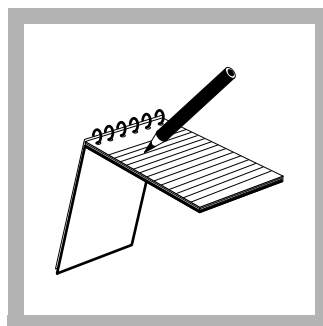
Replace the petri dish lid.



5. Invert the petri dish. Incubate at 41 ± 0.5 °C for 24 hours.



6. Count any colonies (regardless of color) with a blue halo. Use an illuminated magnifier or a 10 to 15X microscope.

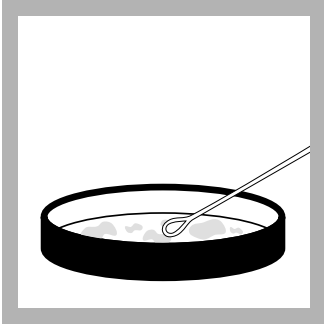


7. Record these colonies as enterococci. See *Interpreting and Reporting Results* on page 11.

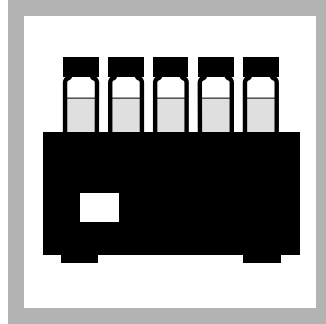
Enterococci, continued

Optional Verification of Enterococci

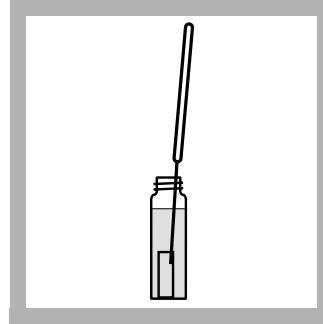
Several media can be used to verify the presence of enterococci grown on m-EI Agar. Gram-positive cocci, such as enterococci, will grow and hydrolyze esculin on Bile Esculin Agar, which results in the formation of a black or brown precipitate. Enterococci can also be confirmed by their ability to grow in Brain Heart Infusion Broth at an elevated incubation temperature of 45 °C and in Brain Heart Infusion Broth containing 6.5% NaCl.



1. Use a sterile inoculating needle to transfer cells from the centers of at least 10 well-isolated typical colonies into a Brain Heart Infusion Broth (BHIB) tube and onto a Brain Heart Infusion Agar (BHIA) slant.



2. Incubate broth tubes for 24 hours and agar slants for 48 hours at 35 ± 0.5 °C.

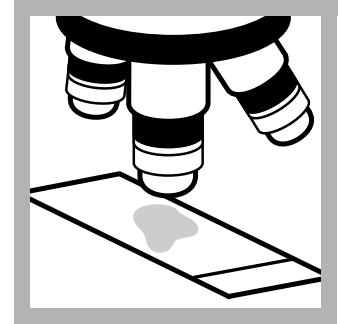


3. After 24 hours, transfer a loopful of broth from the BHIB tube to each of the following media and incubate for 48 hours:

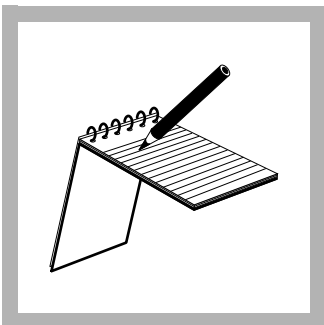
Bile Esculin Agar (BEA) and incubate at 35 ± 0.5 °C.

BHIB and incubate at 45 ± 0.5 °C.

BHIB with 6.5% NaCl and incubate at 35 ± 0.5 °C.



4. After 48 hours observe all media for growth, and apply a Gram stain to the growth from each BHIA slant.



5. Gram-positive cocci that grow and hydrolyze esculin on BEA (i.e. produce a black or brown precipitate) and grow in BHIB and BHIB with salt are verified as Enterococci.

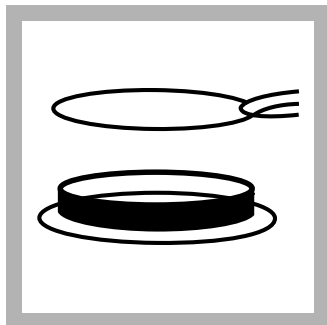
Record the results of the test. See *Interpreting and Reporting Results* on page 11.

Pseudomonas

Using Pseudomonas Broth for Detection of Pseudomonas Species

Some species of pseudomonads are opportunistic pathogens that can inhabit recreational waters such as swimming pools and hot tubs. Pseudomonas Broth is formulated to isolate species of pseudomonas. Pseudomonas Broth contains components that promote the growth of the pigment pyocyanin, which differentiates *Pseudomonas aeruginosa* from other species by forming a blue or blue-green color. Other species of pseudomonas grow on this medium without the colony color formation.

Method 8074

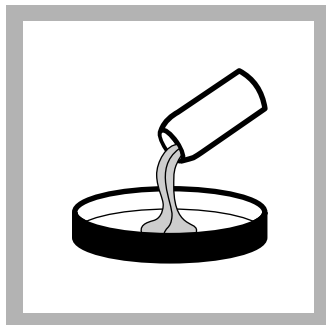


1. Place a sterile absorbent pad in a sterile petri dish using sterilized forceps. Replace the petri dish lid.

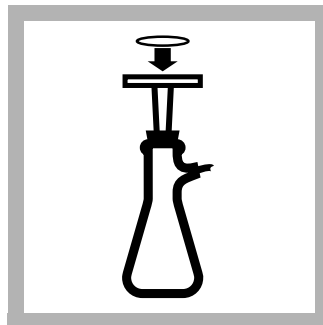
Note: Do not touch the pad or the inside of the petri dish.

Note: To sterilize forceps, dip forceps in alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.

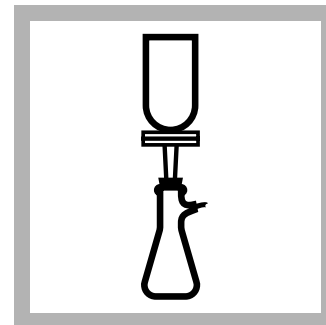
Note: Petri dishes with pads are available from Hach.



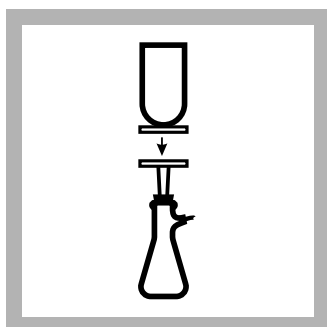
2. Invert an ampule containing Pseudomonas Broth 2 to 3 times to mix. Twist the cap to break it open. Carefully pour the contents evenly onto the absorbent pad. Replace the petri dish lid. Repeat steps 1 and 2 for each petri dish.



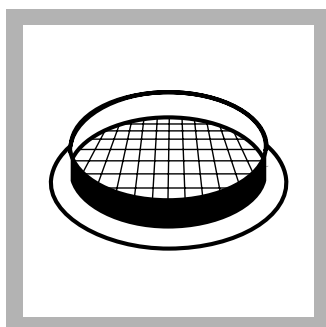
3. Set up the Membrane Filter Assembly; see *Preparing Autoclavable Filter Assembly* on page 7. Use sterilized forceps to place a membrane filter, grid side up, into the assembly.



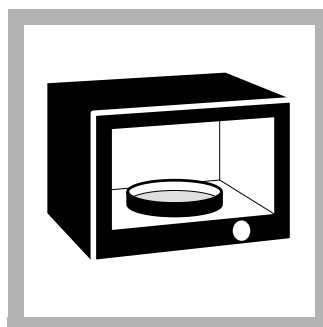
4. Shake the sample vigorously to mix. Pour 100 mL of sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls three times with 20 to 30 mL of sterile buffered dilution water.



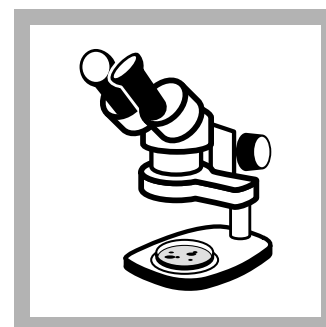
5. Turn off the vacuum and lift off the funnel top. Using sterilized forceps, transfer the filter immediately to the previously prepared petri dish.



6. With a slight rolling motion, center the filter, grid side up, on the absorbent pad. Check for air trapped under the filter and make sure the filter touches the entire pad. Replace the petri dish lid.

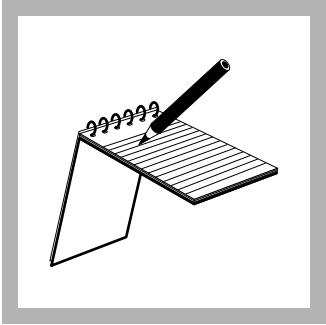


7. Invert the petri dish and incubate at 35 ± 0.5 °C for 22–24 hours.



8. After incubating, count the colonies using an illuminated magnifier or a 10 to 15X stereoscopic microscope.

Pseudomonas, continued



9. Colonies present on the filter after incubation are pseudomonas species. Colonies with a blue-green, green, or yellow-green color are *Pseudomonas aeruginosa*.

Record the results of the test.
See *Interpreting and Reporting Results* on page 11.

Media, Reagents, and Apparatus

REQUIRED MEDIA AND REAGENTS

Description	Quantity	Cat. No.
Brilliant Green Bile Broth Tubes (for total coliform confirmation).....	15/pkg.....	322-15
Dilution Water, buffered, 99-mL bottles	25/pkg.....	14305-98
EC Medium Broth Tubes (for fecal coliform confirmation).....	15/pkg.....	14104-15
EC Medium with MUG Broth Tubes (for <i>E. coli</i> confirmation).....	15/pkg.....	24715-15
KF Streptococcus Broth Ampules, plastic	50/pkg.....	28127-50
Lauryl Tryptose Broth Ampules, sterile (for enrichment technique).....	20/pkg.....	14725-20
Lauryl Tryptose Broth Tubes, single-strength (for total coliform confirmation).....	15/pkg.....	21623-15
m-ColiBlue24® Broth Ampules, glass	20/pkg.....	26084-20
m-ColiBlue24® Broth, 100 mL glass bottle	1 each.....	26084-42
m-ColiBlue24® Broth Ampules, plastic	50/pkg.....	26084-50
m-ColiBlue24® prepared agar plates	bulk.....	28052-01
m-ColiBlue24® prepared agar plates	15/pkg.....	28052-15
m-Endo prepared agar plates	15/pkg.....	28116-15
m-Endo Broth Ampules, plastic	50/pkg.....	23735-50
m-Endo Broth, 100 mL glass bottle	1 each.....	23735-42
m-FC prepared agar plates	bulk.....	28115-01
m-FC prepared agar plates	15/pkg.....	28115-15
m-FC Broth Ampules, plastic	50/pkg.....	23732-50
m-FC w/Rosolic Acid Broth Ampules, plastic.....	50/pkg.....	24285-50
m-EI prepared agar plates, 47 mm	bulk.....	28117-01
m-EI prepared agar plates, 47 mm	15/pkg.....	28117-15
m-Endo Broth PourRite™ Ampules (for total coliform presumptive)	20/pkg.....	23735-20
m-FC Broth PourRite™ Ampules (for fecal coliform presumptive)	20/pkg.....	23732-20
m-FC with Rosolic Acid Broth PourRite™ Ampules (for fecal coliform presumptive)	20/pkg.....	24285-20
m-TEC Agar Tubes, 2 tests/tube (for <i>E. coli</i> determination)	6/pkg.....	25611-06
For m-TEC Agar Method:		
Phenol Red Sodium Salt.....	5 g.....	25639-22
Sulfuric Acid, 0.02 N.....	100 mL.....	203-42
Urea Reagent, ACS	100 g.....	11237-26
m-TEC, modified, prepared agar plates, 47 mm	bulk.....	28118-01
m-TEC, modified, prepared agar plates, 47 mm	15/pkg.....	28118-15
Nutrient Agar with MUG prepared plates	15/pkg.....	21821-15
Nutrient Agar with MUG Tubes, 2 tests/tube (for <i>E. coli</i> confirmation).....	6/pkg.....	24373-06

REQUIRED APPARATUS

Ampule Breaker, PourRite™	each.....	24846-00
Counter, hand tally	1.....	14696-00
Dish, Petri, with pad, 47 mm, sterile, disposable, Gelman	100/pkg.....	14717-99
Dish, Petri, with pad, 47 mm, sterile, disposable, Millipore	100/pkg.....	28177-00
Filter Holder, magnetic coupling (use with 24861-00)	1.....	13529-00
Filter Funnel Manifold, aluminum, 3-place (use with 13529-00).....	1.....	24861-00
Filters, Membrane, 47 mm, 0.45 µm, gridded, sterile, Gelman	200/pkg.....	13530-01
Filters, Membrane, 47 mm, 0.45µm, gridded, sterile, Millipore	200/pkg.....	28176-00
Filtering Flask, 1000-mL.....	1.....	546-53
Forceps, stainless steel	1.....	21411-00
Incubator, Culture, 120 V ac, 50/60 Hz	each.....	26192-00
Inoculating Needle, disposable	25/pkg.....	27489-25
Loop, inoculating, disposable.....	25/pkg.....	27491-25
Microscope Illuminator (use with 23174-00).....	1.....	23175-00
Microscope, Stereo Binocular, 10X (15X available)	1.....	23174-00

Media, Reagents, and Apparatus, continued

REQUIRED APPARATUS, continued

Description	Quantity	Cat. No.
Pipet, serological, 10–11 mL, sterile, disposable	25/pkg	2097-98
Pump, vacuum/pressure, 115 V ac, 60 Hz	1	14697-00
Stopper, rubber, one-hole, No. 7	6/pkg	2119-07
Swabs, cotton, sterile (for confirmation)	100/pkg	25543-00
Tubing, rubber, 0.8 cm (5/16 in.) ID	3.7 m (12 ft.)	559-19

OPTIONAL MEDIA AND REAGENTS

Alcohol Burner	1	20877-42
Aspirator, water	1	2131-02
Autoclave, Automatic, 120 V ac, 50/60 Hz	1	24630-00
Autoclave, Automatic, 240 V ac, 50/60 Hz	1	24630-02
Autoclave tape, 55 m (60 yd.) 2.54 cm wide (1 in.)	1 roll	24631-00
Bags, Whirl-Pak [®] , with dechlorinating agent, 180-mL	100/pkg	20753-33
Bags, Whirl-Pak [®] , without dechlorinating agent, sterile, 205 mL	100/pkg	22331-99
Bags, Whirl-Pak [®] , without dechlorinating agent, sterile, 710 mL	10/pkg	14372-97
Agar, Bacteriological	500 g	23250-34
<i>Aspergillus niger</i> Aqua QC-Stiks [™]	3/pkg	27069-03
Beaker, 600 mL	1	500-52
Bile Esculin Agar	500 g	28156-34
Bottles, sample, glass, with cap, 118 mL	3/pkg	21631-03
Bottles, sample, sterilized, 100-mL fill-to line, disposable	12/pkg	24950-12
Bottles, sample, sterilized, 100-mL fill-to line, disposable	50/pkg	24950-50
Bottles, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	12/pkg	25991-12
Bottles, sample, sterilized, 100-mL fill-to line, disposable with dechlorinating agent	50/pkg	25991-50
Brain Heart Infusion Agar	500 g	24056-34
Brain Heart Infusion Broth	500 g	28155-34
Bunsen Burner with tubing	1	21627-00
<i>Citrobacter freundii</i> Aqua QC-Stiks [™]	3/pkg	27067-03
Dechlorinating Reagent Powder Pillows	100/pkg	14363-69
Dish, Petri, 47 mm, sterile, disposable	500/pkg	14852-00
Dish, Petri, 47 mm, sterile, disposable	100/pkg	14852-99
EC Medium, MPN tubes	15/pkg	14104-15
<i>Enterobacter aerogenes</i> Aqua QC-Stiks [™]	3/pkg	27070-03
<i>Enterococcus faecalis</i> Aqua QC-Stiks [™]	3/pkg	27064-03
<i>Escherichia coli</i> Aqua QC-Stiks [™]	3/pkg	27063-03
Filtering Flask, 500-mL	1	546-49
Filter Funnel Manifold, plastic, 3-place (use with 13529-00)	1	24861-50
Filter Holder, reusable (use with 26566-00)	1	26567-00
Filter Unit, sterile, disposable with gridded membrane (use with 26567-00)	12/pkg	26566-00
Filtration Support (for field use), stainless steel	1	25862-00
Funnels, Push-Fit and membrane filters (use with 25862-00)	72 of each/pkg	25863-00
Germicidal Cloths	50/pkg	24632-00
Graduated Cylinder, 100-mL	1	508-42
Incubator, portable, 12 V dc	1	25699-00
Incubator, Culture, 220 V ac, 50/60 Hz	each	26192-02
Incubator, 25-Well Dri-Bath, 115/230 V ac, 50/60 Hz with North American style plug	each	45900-00
Incubator, 25-Well Dri-Bath, 115/230 V ac, 50/60 Hz with European style plug	each	45900-02
Incubator, Water Bath, 120 V ac, 50/60 Hz	each	26163-00
Incubator, Water Bath, 240 V ac, 50/60 Hz	each	26163-02
Inoculating Loop, nichrome wire, with handle	1	21121-00

Media, Reagents, and Apparatus, continued

OPTIONAL MEDIA AND REAGENTS, continued

Description	Quantity	Cat. No.
Inoculating Needle, nichrome, 76 mm	1	21779-00
Indoxyl beta-D-glucouronide	2 g	28139-35
<i>Klebsiella pneumonia</i> Aqua QC-Stiks™	3/pkg	27921-03
Kovac's Indole Reagent.....	30 mL.....	27161-23
Lamp, long-wave, ultraviolet, 115 V ac, 60 Hz	1	21843-00
Lamp, long-wave, ultraviolet, 230 V ac, 50/60 Hz	1	21843-02
Lamp, long-wave, ultraviolet, portable, 4 watt	1	24152-00
Magenta G1CA	2 g	28150-35
(5-bromo-6-chloro-3-indoyl-beta-D-glucuronide)		
Magnifier, illuminated, 2.5X and 5X	1	25854-00
Magnifier, illuminated, 10X, portable.....	1	25853-00
M-E Agar.....	100 g.....	22812-26
M-E Agar.....	500 g.....	22812-34
m-Green Yeast & Mold Broth Ampules, glass	20/pkg.....	24283-20
m-Green Yeast & Mold Broth Ampules, plastic	50/pkg.....	24283-50
m-HPC prepared agar plates	15/pkg.....	28114-15
m-HPC Broth Ampules, plastic	50/pkg.....	28124-50
<i>Micrococcus luteus</i> Aqua QC-Stiks™	3/pkg.....	27066-03
m-TEC Agar, dehydrated	100 g.....	22811-26
m-TGE Broth Ampules, glass	20/pkg.....	23738-20
m-TGE Broth Ampules, plastic	50/pkg.....	23738-50
m-TGE w/TTC Broth Ampules, glass	20/pkg.....	24284-20
m-TGE w/TTC Broth Ampules, plastic.....	50/pkg.....	24284-50
m-TSB/USP Broth Ampules, plastic	50/pkg.....	28126-50
Nalidixic Acid	25 g.....	24071-24
Nutrient Agar with MUG plates.....	15/pkg.....	28121-15
Orange Serum Broth Ampules, plastic	50/pkg.....	28125-50
Oxidase Reagent.....	0.5 mL.....	26225-00
Pad, absorbent, with dispenser	1000/pkg.....	14918-00
Pen, colony counter (Felt-tip pen attached to a counter, that marks, beeps, and registers accumulative count on an LCD display).....	1	26132-00
Pen, laboratory	1	20920-00
Peptone Powder Pillows, 1 g.....	30/pkg.....	21429-64
Phosphate Buffer, pillows.....	50/pkg.....	23236-68
Potassium Dihydrogen Phosphate and Magnesium Chloride Powder Pillows for buffered dilution water (25 of each)	50/pkg.....	21431-66
Proteose Peptone #3	500 g.....	28151-34
<i>Pseudomonas aeruginosa</i> Aqua QC-Stiks™	3/pkg.....	27065-03
Pseudomonas Broth Ampules, plastic	50/pkg.....	28122-50
Pump, vacuum, hand-operated	1	14283-00
Pump, vacuum/pressure, 220 V ac, 50 Hz	1	14697-02
R2A Agar Tubes, 2 tests/tube	6/pkg.....	27241-06
R2A prepared agar plates	bulk.....	28142-01
R2A prepared agar plates	15/pkg.....	28142-15
R2A Broth Ampules, plastic	50/pkg.....	28123-50
Rose Bengal prepared agar plates	15/pkg.....	28119-15
<i>Saccharomyces cerevisiae</i> Aqua QC-Stiks™	3/pkg.....	27068-03
Sodium Azide, Micro Grade	1 each.....	27345-26

Media, Reagents, and Apparatus, continued

OPTIONAL MEDIA AND REAGENTS, continued

Description	Quantity	Cat. No.
Sodium Sulfite, Micro Grade	1 each	23860-26
<i>Staphylococcus epidermidis</i> Aqua QC-Stiks™	3/pkg	27071-03
Sterilization Indicators, Autoclave.....	15/pkg	28111-15
Sterilization Indicators, Autoclave.....	100/pkg	28111-99
Syringe, 140-mL, polypropylene (use with 25862-00).....	1	25861-00
Tryptic Soy Agar.....	100 g	25659-26
Tryptic Soy Broth, ampules	50/pkg	28126-50
TTC Solution, 1%, presterilized	100 mL	24060-42
Wicks, replacement (used with Alcohol Burner 20877-42)	10/pkg	20978-10

For a complete listing of Hach's micro products, consumables, equipment, apparatus and labware, see the Hach Products for Analysis Catalog.

PourRite is a trademark of Hach Company.

Whirl-Pak is a registered trademark and patented product of Nasco. U.S. Patent 2,973,131.

Aqua QC-Stiks is a trademark of MicroBioLogics.



HACH COMPANY
WORLD HEADQUARTERS
P.O. Box 389
Loveland, Colorado 80539-0389
Telephone: (970) 669-3050
FAX: (970) 669-2932

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:

In the U.S.A. - **Call toll-free 800-227-4224**

Outside the U.S.A. - **Contact the HACH office or distributor serving you.**

On the Worldwide Web - **www.hach.com; E-mail - techhelp@hach.com**
