25696-22

MICROBIOLOGY ENVIRONMENTAL LABORATORIES—MEL

Instrument and Procedures Manual

for
MEL P/A Safe Drinking Water Laboratory

4-1-93-1ED

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SAFETY PRECAUTIONS

Important: Please read the entire manual before attempting to unpack, set up or operate this instrument. Pay close attention to all warnings, cautions and notes. Failure to do so may result in injury to the operator or damage to the equipment.

Use of Warnings, Cautions and Notes

Warnings, cautions and notes in this manual have the following significance:

WARNING
Failure to observe this information can result in personal injury or loss of life.

CAUTION
Failure to observe this information can result in damage to equipment.

NOTE
Information that requires special emphasis.

Precautionary Labels

Please pay particular attention to labels and tags attached to the instrument. Personal injury or damage to the instrument could occur if not observed.

Certification

Hach Company certifies this instrument was tested thoroughly, inspected and found to meet its published specifications when it was shipped from the factory.

SECTION 1 PORTABLE INCUBATOR

1.1 Specifications

Specifications subject to change without notice.

Ambient Operating Temperature: 0 to 40 °C

Storage Temperature: –40 to 60 °C (instrument only)

Temperature Stability: ± 0.5 °C

Temperature Range: Five degrees above ambient to 50 °C

Warm-up Time: 2 ± 1 hour

Capacity:

42—50-mm petri dishes
or 40—MPN tubes (19 mm OD)
or 6—P/A Disposable Bottles

Power Requirements: 12 Vdc or optional battery eliminator

External Dimensions: 30.5 x 30.5 x 25 cm (12 x 12 x 10")

Internal Dimensions: 20 x 20 x 15 cm (8 x 8 x 6")

Instrument Weight: 1.8 kg (4 lb)

1.2 General Description

The Hach Portable Incubator is a bacterial incubator designed primarily for field use in Hach's Microbiological Environmental Laboratories (MELs). Available MELs include the MEL P/A Safe Drinking Water Laboratory, MEL/MPN Lab for Total Coliform and *E. coli*, the MEL/MF Lab for Total Coliform, and the MEL/700 Potable Water Laboratory.

The Portable Incubator maintains temperature within ± 0.5 °C and the incubation temperature is adjustable between 30 and 50 °C. Ideally suited for total coliform, fecal coliform and *E. coli* testing, the incubator may be used for Presence/Absence (P/A), Membrane Filtration (MF) and Most Probable Number (MPN) procedures.

The instrument power cord easily plugs into an automobile cigarette lighter. For remote field use, a 12 Vdc portable battery is available. The portable battery is rechargeable and includes recharger and nylon carrying case. Battery eliminators are also available for 115 or 230 Vac.

Optional accessories include racks for P/A bottles, MPN tubes and MF petri dishes; portable battery, battery eliminators (115 or 230 Vac) and all testing media and apparatus.

1.3 Preparing for Use

Unpacking

Remove the instrument and accessories from the shipping boxes and inspect them for damage that may have occurred due to rough handling or extreme weather conditions.

Packaging Guide

Use the MEL P/A legend and Figure 1 on page 4 to verify that all components are present and to easily assemble the portable lab. The legend accompanying the packaging guide is also a materials list for the contents of the lab. Use the packaging guide to verify that your shipment is complete on receipt from the factory and also to reorder supplies when they are needed. If the shipment is not complete or any items are damaged, please contact Hach Customer Service at 800-227-4224 (U.S.A. only). For customers outside the United States, please contact the Hach office or authorized distributor serving you.

MEL P/A LEGEND

Item	Description	Cat. No.
1.	Chlorine Color Disc	21988-00
2.	Plastic Tubes (4)	46600-00
3.	UV Lamp, Portable	24152-00
4.	pH Pocket Pal	44350-00
5.	TDS Pocket Pal	44400-00
6.	Pocket Thermometer	1877-01
7.	Laboratory Pen	20920-00
8.	Color Comparator Box	1732-00
9.	Nitrate Color Disc	14038-00
10.	Portable Incubator	25699-00
11.	P/A Bottle Rack	25805-00
12.	Whirlpak Bags, with dechlor (50)	20753-33
13.	Germicidal Cloth (5)	24632-00
14.	NitraVer 5 Pdr Plws, 5 mL, 50/pk (2)	14035-99
15.	DPD Total Chlorine PermaChem, 5 mL, 100/pk	14076-99
16.	DPD Free Chlorine PermaChem, 5 mL, 100/pk	14077-99
17.	Buffer, pH 4.01 and 7.00, 20/pk (10 each)	22992-64
18.	Clippers	936-00
19.	Glass Dropper	14197-00
20.	Beaker, 100 mL	1080-42
21.	Procedure Manual	25696-88
22.	Disposable P/A with MUG Tests, 50/pk	24016-50

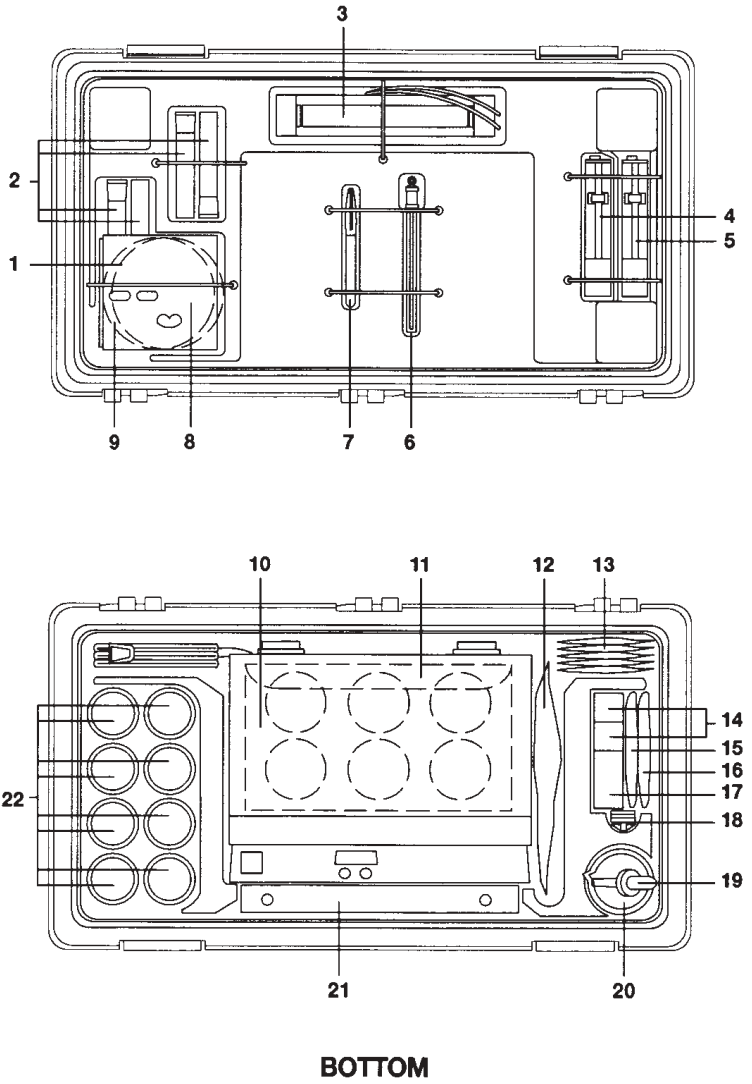


Figure 1. Packaging Guide

Missing or Damaged Products

If any of the items are missing or damaged, please contact the Customer Service Department, Hach Company, Loveland, Colorado. The toll-free number in the United States is (800) 227-4224. International customers should contact the Hach office or authorized distributor serving your area.

Hach Company
World Headquarters
P.O. Box 389
Loveland, Colorado 80539 U.S.A.
Telephone: (303) 669-3050
FAX: (303) 669-2932

Please do not return the instrument without prior authorization from Customer Service.

1.4 Operation

Power Selection

USE IN A MOTORIZED VEHICLE

Plug the power cord of the instrument into the cigarette lighter outlet. The incubator will then be powered by the 12 Vdc vehicle battery.

Note: *When using the incubator for extended periods, the automobile engine should be run periodically to ensure that the automobile battery is recharged.*

PORTABLE BATTERY USE

Plug the power cord of the instrument into the battery. The battery will operate the instrument for at least 12 hours, depending on the ambient temperature. To recharge the battery, plug the male plug of the recharger into the battery. Plug the pronged recharger plug into a 115 Vac outlet or use the appropriate adapter for other voltage requirements. The battery will completely recharge in 24 hours.

BATTERY ELIMINATOR USE

Plug the power cord of the instrument into the battery eliminator. Plug the battery eliminator into the ac outlet.

CAUTION

Before connecting the instrument power cord to any power source, ensure that the appropriate power supply is either 12 Vdc or converts power to 12 Vdc. Before connecting the battery eliminator to a power source, ensure that the appropriate line voltage is being used.

Operating Controls and Indicators

Figure 2 shows the Portable Incubator controls and indicators.

Key	Description
I O	Power switch to turn instrument on and off. The switch must be on before any systems are operational, including the control circuitry.
^	The up arrow key. Press and hold the button down until the display begins to blink, displaying the set point. Press the key to increase the incubation temperature set point.
v	The down arrow key. Press and hold the button down until the display begins to blink, displaying the temperature set point. Press the key to decrease the incubation temperature set point.

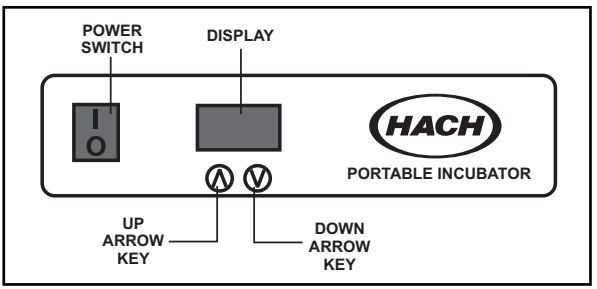


Figure 2. Portable Incubator controls and indicators.

Display

The display shows the current temperature inside the incubator in degrees Celsius (°C). When either the up or down arrow keys are pressed and held for several seconds, the display will begin to blink, displaying the temperature set point. If the arrow keys are not pressed for five seconds, the display will stop blinking and will display the temperature of the unit.

To re-calibrate the digital display to your reference thermometer, follow the calibration instructions. The incubator was calibrated at the factory at 37 °C.

Operation

1. Plug the incubator power cord into the power source.

2. Turn power switch to | (on).

3. Hold down either the up or down arrow key until the display begins to blink. Using the up or down arrow keys, adjust the display to the desired set point temperature. If the buttons are not pressed for five seconds, the display will stop blinking and display the incubator temperature.

4. Allow the incubator to warm up to the set point temperature.

NOTE: Unit is stabilized when it has been running at a constant temperature for 60 minutes.

5. Open the incubator lid. Load the unit with the desired sample rack and close the lid.

Calibration

1. Plug the incubator power cord into the power source.

2. Place a reference thermometer in the center of the incubator and allow the incubator to stabilize.

3. Turn power switch to | (on).

4. Hold down either the up or down arrow key until the display begins to blink. Using the up or down arrow keys, adjust the display to the desired set point temperature. If the buttons are not pressed for five seconds, the display will stop blinking and display the incubator temperature.

5. Allow the incubator to stabilize at the set point temperature.

NOTE: Unit is stabilized when it has been running at a constant temperature for 60 minutes.

6. Compare the reading on the reference thermometer with the digital display. If there is a difference, put the display into the calibrate mode by pressing on both the up and down arrow keys simultaneously and holding them down for about five seconds or until the two outside decimal points start to flash.

7. When the decimal points are flashing, use the up or down arrow keys until the display reads the correct value.

8. Allow the unit to stabilize again.

1.5 Maintenance

WARNING Prior to any maintenance or service on this unit, disconnect the power cord from the power supply.

Cleaning

Keep the incubator and accessories as clean as possible and store the instrument in the carrying case. Wipe spills up promptly. Wipe the outside of the incubator with a soft damp cloth. Clean the inside chamber of the incubator with mild soap and water solution. Rinse with clean water and wipe dry with a soft cloth. Foreign materials inside the unit may rust or leave rust spots. If corrosion is seen, scrub out the stains with a mild abrasive. Do NOT use steel wool. Failure to remove corrosion may permanently damage the liner.

Replacing the Fuse

To replace the fuse, unscrew the plug end of the power cord. Remove the old fuse and replace it with a new 10 amp fuse. Screw the plug end back onto the power cord.

1.6 Replacement Parts and Accessories

REPLACEMENT PARTS

Cat. No.	Description
25847-00	Control panel assembly
25850-00	Fuse, 10 amp
25848-00	Hinge
25699-00	Incubator, Portable for MELs
25849-00	Lid Assembly
25696-88	Manual, MEL P/A Safe Drinking Water Lab

PORTABLE INCUBATOR ACCESSORIES

25804-00	Battery Eliminator, 115 Vac
25804-01	Battery Eliminator, 230 Vac
25803-00	Battery, Portable, 12 Vdc, rechargeable, with carrying case and recharging adapter
25805-02	Rack, MF/General Purpose, holds 42 50-mm petri dishes (internal dimensions: 7.5 x 18.25 x 11.75 cm)
25805-01	Rack, MPN tube, holds 40 tubes, 19-mm OD
25805-00	Rack, P/A bottle, holds 6 bottles, 5-cm OD
25687-00	Sample Transport Kit Includes cooler, plastic-coated rack, 100 sampling bags with dechlorinating agent, and a refrigerant pack.

MEDIA SETS (Includes media and consumables required for testing)

24388-00	Chlorine Reagent Set, 100 tests
25801-00	**MEL/MF Media Set** for total coliform Includes 200 m-Endo Broth PourRite Ampules, 200 sterile 50-mm petri dishes with pads, 216 sterile 0.45 µm membrane filters, 216 sterile push-fit funnels, 200 Whirl-Pak bags with dechlorinating agent (media and consumables for 200 tests)
25802-00	**MEL/MPN Media Set** for total coliform and *E. coli* Includes 135 LT/MUG Broth tubes, 30 BGB Broth Tubes (for total coliform confirmation), 25 sterile 11-mL pipets, 30 sterile inoculating loops, 25 Whirl-Pak bags with dechlorinating agent (media and consumables for 25 5-tube tests)
25800-00	**MEL P/A Media Set** for total coliform and *E. coli* Includes 50 P/A with MUG Bottles, 50 Whirl-Pak bags with dechlorinating agent (media and consumables for 50 tests)
14035-99	Nitrate Reagent Set, 100 tests
22992-64	pH Buffer Pillows, 4.01 and 7.00, 10 each

MEL APPARATUS (Apparatus included in MEL)

MEL P/A Safe Drinking Water Laboratory

1080-42 Beaker, 100-mL
24632-00 Germicidal Cloths
25699-00 Incubator, Portable for MELs
25696-88 Manual, MEL P/A Safe Drinking Water Lab
44350-00 pH Pocket Pal Tester
25805-00 Rack, P/A bottle, holds 6 bottles, 5-cm OD
44400-00 TDS Pocket Pal Tester
1877-01 Thermometer, alcohol, –20 to 105 °C
46600-04 Tubes, plastic viewing

MEL/MPN for Total Coliform and *E. coli*

24632-00 Germicidal Cloths
25699-00 Incubator, Portable for MELs
25699-88 Manual, MEL/MPN
14651-00 Pipet Bulb
25805-01 Rack, MPN tube, holds 40 tubes, 19-mm OD
1877-01 Thermometer, alcohol, –20 to 105 °C

MEL/MF for Total Coliform

24846-00 Breaker for PourRite Ampules
20877-60 Burner, Alcohol, 60 mL
21411-00 Forceps, stainless steel
24632-00 Germicidal Cloths
25699-00 Incubator, Portable for MELs
25854-00 Magnifier, Illuminated, 2.5X and 5X, hand-held
25699-88 Manual, MEL/MF
25805-02 Rack, MF/General Purpose, holds 42 50-mm petri dishes
25861-00 Syringe, 140 mL (for field filtration)
1877-01 Thermometer, alcohol, –20 to 105 °C
25862-00 Vacuum Support, Field, stainless steel

For Technical Assistance, Price and Ordering

In the U.S.A.—Call toll-free (800) 227-4224 for more information.
Outside the U.S.A.—Contact the Hach office or distributor serving you.

1.7 Repair Service

For instrument service, please contact the Hach Factory Service Center serving your location.

In the United States:

Hach Company
100 Dayton Avenue
P.O. Box 907
Ames, Iowa 50010
Telephone: (800) 227-4224 (U.S.A. only)
FAX: (515) 232-1276

In Canada:

Hach Sales & Service Canada Ltd.
1313 Border Street, Unit 34
Winnipeg, Manitoba
Canada R3H 0X4
Telephone: (800) 665-7635 (Canada only)
(204) 632-5598
FAX: (204) 694-5134

All other locations:

Hach Company, World Headquarters
P.O. Box 389
Loveland, Colorado 80539 U.S.A.
Telephone: (303) 669-3050
FAX: (303) 669-2932

1.8 Warranty

Seller warrants equipment of its manufacture against defective materials or workmanship for a period of one year from the date of shipment.

The liability of Seller under this warranty is limited, at Seller's option, solely to (1) repair, (2) replacement with equivalent Hach equipment, or (3) appropriate credit adjustment not to exceed the original sales price of equipment returned to the Seller provided that:

a. Buyer promptly notifies Seller in writing on discovery of the defects, stating where applicable, the product type and serial numbers and fully describing the circumstances giving rise to the claim. Seller must receive such notification within the applicable warranty period in order for this warranty to apply.

b. On receipt of written instructions from Seller, Buyer returns the equipment as instructed with transportation charges prepaid by the Buyer, and

c. Seller's examination of such equipment discloses to its satisfaction that the defects have not resulted from any negligence, misuse, improper installation, accident or unauthorized repair or alterations by the Buyer. Seller's determination of the cause and nature of the failure of the equipment
shall be final.

This warranty is applicable to the original Buyer only and shall be in lieu of and exclude all other warranties, expressed or implied, including, but not limited to, any implied warranty of merchantability or fitness. The foregoing shall constitute the sole and exclusive remedy of Buyer and the sole and exclusive liability of Seller, whether Buyer's claims shall be breach of warranty or negligence. Seller neither assumes nor authorizes any person to assume for it any other obligation or liability in connection with the sale of the equipment. In no event shall Seller be liable for special, incidental or consequential damage.

If Seller finds that Buyer has returned the equipment without cause, Seller shall notify Buyer and return the equipment at Buyer's expense; in addition, Seller may at its sole discretion, impose a charge for testing and examination of any equipment so returned.

SECTION 2 MICROBIOLOGICAL PROCEDURES

2.1 Overview of Coliform Bacteria

Many of the microorganisms that cause serious disease, such as typhoid fever, cholera and dysentery, can be traced directly to polluted water. These disease-producing organisms, or **pathogens,** are discharged along with fecal wastes and are difficult to detect in water supplies. People may contact these pathogens in drinking water, on bathing beaches, in rivers and streams, and in swimming pools.

Testing for bacterial pathogens in water is impractical for a number of reasons, such as lengthy and involved test procedures. Most microbiological testing of water measures indicator organisms, not pathogens. **Indicator organisms** include bacteria that may not be pathogenic but usually are present when pathogens are present, and absent when pathogens are absent. No organism or group of organisms satisfies all of the criteria for an indicator; however, coliforms satisfy most of the requirements.

Total coliform tests are used for potable water supplies. Total coliform contamination indicates inadequate disinfection of drinking water. For these reasons, the microbiological quality standards for drinking water in the United States and in most developed countries are based on measuring the total coliform population.

Fecal coliform tests usually are performed on untreated water, wastewater, bathing water and swimming water. For natural water sources, the quality standards are based on fecal coliform counts. The best coliform indicator of fecal contamination from human and animal waste is *Escherichia coli (E. coli).*

2.2 Testing Techniques

Good laboratory technique is essential when accuracy is important, particularly in microbiological laboratory procedures. Careful sample collection and preservation, a clean laboratory, proper sterilization and inoculation practices, and close temperature control help assure reliable results.

2.3 Preparing Sample Containers

Take care to prevent contamination when conducting bacterial tests. All materials used for containing or transferring samples must be sterile! To collect samples use presterilized plastic bags, presterilized disposable bottles, autoclavable glass bottles, or autoclavable plastic bottles.

Presterilized Containers

Presterilized plastic bags with dechlorinating agent are included in the MELs and media sets. Plastic bags are available presterilized with or without dechlorinating agent (sodium thiosulfate). Presterilized bottles are available with a 100-mL fill-to line.

NOTE: Use dechlorinating agent with potable water or chlorinated water samples. It is not necessary for unchlorinated water samples. However, dechlorinating agent will not interfere with unchlorinated samples. For simplicity, plastic bags containing dechlorinating agent may be used for all samples.

Autoclavable Containers

Glass or plastic bottles (at least 125 mL) may be used instead of plastic bags or bottles.

PREPARING AUTOCLAVABLE CONTAINERS

1. Wash in hot water and detergent.

2. Thoroughly rinse with hot tap water, followed by a distilled water rinse to make sure that all detergent is removed.

3. If dechlorinating agent is needed (for chlorinated, potable water) add the contents of 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 may be sterilized by hot air at 170 °C for one hour.

5. Store sterile containers tightly capped in a clean environment until needed.

2.4 Collecting and Preserving Samples

Proper sampling technique ensures that results are representative of the sample source. Avoid sample contamination during collection.

Sample Size

Collect a sufficient volume of water for analysis, at least 100 mL of sample. World Health Organization guidelines suggest 200 mL per sample, while *Standard Methods for the Examination of Water and Wastewater* guidelines suggest 100 mL per sample.

Collect at least 100 mL of sample in presterilized plastic bags or bottles or in sterile glass or plastic sample bottles. Sample containers should not be filled completely. Maintain at least 2.5 cm (approximately 1") of air space to allow adequate space for mixing the sample prior to analysis.

POTABLE WATER

Potable water should contain no coliforms per 100 mL, so testing should be done on undiluted samples. Use the Membrane Filtration test, 5-tube or 10-tube Most Probable Number test, or Presence/Absence test for potable water. All these procedures except the 5-tube MPN are USEPA-approved for reporting drinking water results.

NONPOTABLE WATER

Nonpotable water testing generally requires dilution of the original sample, based on probable coliform concentration. Sample dilutions for the MF method are discussed on pages 23-24. For MPN testing, three different dilutions should be tested, using 5 tubes for each dilution, for a total of 15 tubes. Sample dilutions for the MPN method are discussed on pages 48-50.

Collecting Samples

SAMPLES FROM FAUCETS, SPIGOTS, HYDRANTS OR PUMPS

To collect representative samples, allow water to run from a faucet, spigot, hydrant or pump at a moderate rate (without splashing) for two to three minutes before sampling. Do not adjust the flow rate while collecting the sample. Valves, spigots and faucets that swivel or leak should be avoided. Attachments, such as aerators and screens, should be removed prior to sample collection.

Carefully open sample containers just prior to collection and close immediately following collection. Do not lay the lid or cap down. Avoid touching the mouths and insides of the containers. Do not rinse the containers. Properly label each sample container and analyze samples as soon as possible after collection.

SAMPLES FROM RIVERS, LAKES AND RESERVOIRS

When sampling a river, lake or reservoir, fill the sample container below the water surface. 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. (This technique excludes any surface scum.) Fill the container by positioning the mouth into the current or, in nonflowing water, by tilting the bottle slightly and allowing it to fill slowly. Do not rinse. Label each sample container and analyze samples as soon as possible after collection.

Preserving Samples

No dechlorination is necessary if the sample is added directly to the medium on site. Otherwise, samples should be dechlorinated and immediately transported for analysis.

MEL Portable Incubator Laboratories allow you to conduct all of your analysis in the field. But, if you are just transporting samples to a lab, your samples should arrive at the lab within 24 hours after collection. In warm climates, the samples must be packed in a freezing mixture to maintain the sample temperature between 4 and 10 °C. Failure to properly collect and transport samples will cause inaccurate results.

2.5 MUG Reagent for *E. coli* Screening

For detecting *E. coli*, several media contain MUG reagent (4-methylumbelliferyl-ß-D-glucuronide). MUG produces a fluorogenic product when hydrolyzed by glucuronidase, an enzyme specific to *E. coli*. Within 24 hours, MUG reagent medium detects *E. coli*.

After incubation, examine the sample under a long-wave, ultraviolet (UV) light. If the sample fluoresces, *E. coli* are present. MUG detects non-gas producing (anaerogenic) strains of *E. coli*, which go undetected with conventional methods. MUG also works well when competitive organisms are present.

NOTE: Determine fluorescence in a box or other darkened area under diffuse lighting (too much visible light masks the presence of fluorescence). Hold the tubes or bottles under a long-wave UV light, or hold the UV light so that it shines on the tubes or bottles. Germicidal lamps (short-wave UV lights) are not suitable for this purpose.

To gain confidence in differentiating between fluorescent and nonfluorescent samples, include a tube containing a known *E. coli* culture with each set of tests.

2.6 Presence/Absence (P/A) Procedures

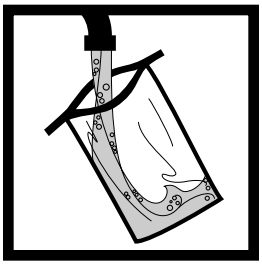
The Bromcresol Purple Acidity Method for P/A testing of total coliforms is USEPA*-approved for reporting drinking water results. This method uses lactose and lauryl tryptose broths with bromcresol purple added to detect acidity formed during lactose fermentation by the bacteria. A yellow color indicates total coliforms. P/A Medium containing MUG reagent will fluoresce under long-wave UV light if *E. coli* are present.

Potable Water Procedures

Total Coliform and *E. coli* Procedures
Method 8319—P/A Bromcresol Purple Broth
Method 8364—P/A Bromcresol Purple Broth with MUG

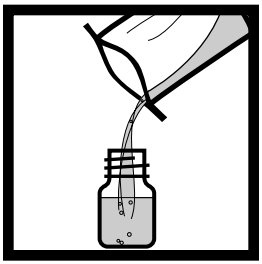
- USEPA-approved for drinking water when using P/A Broth without MUG
- Presumptive total coliform test
- Screening test for *E. coli* when using P/A Broth with MUG

Using P/A Broth Disposable Bottles with or without MUG

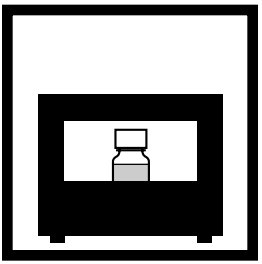


1. Collect 100 mL of sample in a sterile container. Do not contaminate the sample or sample container.

Note: *Remove screens and other aeration devices from faucets and let water run for 2 to 3 minutes before collecting the sample.*



2. Add sample to the 100-mL fill-to line on the P/A Broth Disposable Bottle. Sample may be added from a sterile container, or directly from a faucet or spigot.



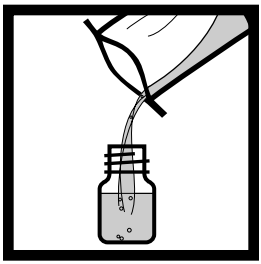
3. Incubate at 35 ± 0.5 °C for 24 to 48 hours.

4. Note the reaction after 24 hours of incubation. If sample is negative, continue incubating for another 24 hours. See Table 1 on page 19.

5. Confirm positive samples by inoculating the appropriate media from positive P/A Broth samples. See Table 2 on page 19.

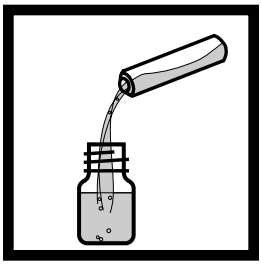
6. Dispose of completed tests appropriately. See Disposing of Completed Tests on page 20.

Using P/A Broth Ampules with or without MUG

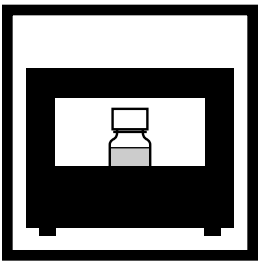


1. Collect 100 mL of sample in a sterile container. Do not contaminate the sample or sample container.

Note: *Remove screens and other aeration devices from faucets and let water run for 2 to 3 minutes before collecting the sample.*



2. Add the contents of one P/A Broth Ampule to the 100 mL of sample.



3. Incubate at 35 ± 0.5 °C for 24 to 48 hours.

4. Note the reaction after 24 hours of incubation. If sample is negative, continue incubating for another 24 hours. See Table 1 on page 19.

Confirm Positive Samples

5. Confirm positive samples by inoculating the appropriate media from positive P/A Broth samples. See Table 2 on page 19.

Dispose of all completed tests

6. Dispose of completed tests appropriately. See Disposing of Completed Tests on page 20.

Interpreting P/A Results

Table 1. Reactions using P/A Broth Ampules or Disposable Bottles with or without MUG.

Reaction	Comments	Report as:
Color change from reddish purple to yellow or yellow brown		Positive for total coliform
No color change after 24 hours	Incubate for an additional 24 hours and recheck the sample for color change.	
No color change after 48 ± 3 hours		Negative for total coliform
Fluorescence under long-wave UV light (if using P/A Broth with MUG)		Positive for *E. coli*

Confirming Positive Samples

Inoculum from incubated samples can be used to confirm the presence of bacteria. See Table 2. The media listed for fecal coliform, total coliform and *E. coli* are USEPA-approved for reporting purposes.

Table 2. Confirmation media

Bacteria	Confirmation Media	Incubation	Positive Result
Total Coliform (USEPA)	Brilliant Green Bile Broth **322-15** 15/pk	24-48 hours 35 ± 0.5 °C	Gas/Turbidity
Fecal Coliform (USEPA)	EC Medium Tubes **14104-15** 15/pk	24 hours 44.5 ± 0.2 °C	Gas/Turbidity
E. coli (USEPA)	EC Medium with MUG Tubes **24715-15** 15/pk	24 hours 44.5 ± 0.2 °C	Fluorescence
Presumptive Fecal Streptococci	Azide Dextrose Broth **24068-15** 15/pk	24 hours 35 ± 0.5 °C	Turbidity
Confirmed Fecal Streptococci	Bile Esculin Azide Agar **24069-15** 15/pk	24 hours 35 ± 0.5 °C	Brownish-Black with Brown Halo

Disposing of Completed Tests

Active bacterial cultures grown during incubation must be disposed of safely. This may be accomplished in one of two ways.

• **Bleach.** Used test containers may be sterilized by using a 10% bleach solution. Pour the test container contents and the test containers into the bleach solution. Allow 10 to 15 minutes contact time with the bleach. Pour the liquid down the drain. Dispose of the test containers in the normal garbage.

• **Autoclave.** Place used test containers in a contaminated items bag or a biohazard bag and seal tightly. Autoclave used test containers at 121 °C for 15 minutes at 15 pound pressure. Test containers must be placed in a bag before autoclaving to prevent the solution from leaking into the autoclave. Once sterile, dispose of the test containers in the normal garbage.

Media and Apparatus for P/A Testing

Required Media and Apparatus

Cat. No.	Description	Quantity
20753-33	Bags, Whirl-Pak with dechlorinating agent, 170-mL	100
25699-00	Incubator, Portable for MEL	1
25800-00	Presence/Absence Media Set Includes 50 P/A Broth with MUG Disposable Bottles (24016-50) and 50 Whirl-Pak Bags (170-mL) with dechlorinating agent (20753-33)	1
25805-00	Rack, P/A Bottle (for use with Portable Incubator)	1
24152-00	UV Lamp, long-wave, portable, 4 watt	1

Optional Media and Apparatus

24633-00	Bags, for contaminated items	200
24950-12	Bottles, presterilized, 100-mL fill-to line	12
24950-50	Bottles, presterilized, 100-mL fill-to line	50
25640-00	Breaker, P/A Ampule	1
24632-00	Germicidal Clothes	50
22454-10	Inoculating Loops, sterile and disposable (for confirmation)	10
24949-25	P/A Broth Ampules	25
24955-25	P/A Broth Ampules with MUG	25
23232-12	P/A Broth Disposable Bottles	12
23232-50	P/A Broth Disposable Bottles	50
24016-12	P/A Broth Disposable Bottles with MUG	12
24016-50	P/A Broth Disposable Bottles with MUG	50
20920-00	Pen, Laboratory	1
21843-00	UV Lamp, long-wave, 115V	1
21843-02	UV Lamp, long-wave, 230V	1

2.7 Membrane Filtration (MF) Procedures

The MF Method is a fast, simple way of estimating bacterial populations in water. In the initial step, an appropriate sample volume is passed through a membrane filter with a pore size small enough (0.45 microns) 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 coliforms. The petri dish containing the filter and pad is incubated, upside down, for 24 hours at the appropriate temperature. After incubation, the colonies that have grown are identified and counted by using illuminated magnifier or a 10-15X microscope.

The MF Method is especially useful for testing drinking water because large numbers of samples can be analyzed in a short time.

Preparing Materials

Start the incubator while preparing other materials. Adjust the incubator temperature setting: 35 °C for total coliforms or 44.5 °C for fecal coliforms.

USING PRESTERILIZED EQUIPMENT AND MEDIA

You will need sterile materials, a disinfected work area and proper handling techniques, or contamination may give false results. To simplify technique and minimize the possibility of contamination, use presterilized equipment and media. Hach offers presterilized and disposable membrane filters, pipets, petri dishes, absorbent pads, inoculating loops, filter pads, buffered dilution water in 99-mL bottles, sampling bags, and prepared growth media. MELs include presterilized consumables and field filtration assembly.

If you are using a conventional filter funnel assembly, it will require sterilization. Sanitize the funnel by immersing it in boiling water for 5 minutes prior to use. You will also need to sterilize the forceps included with any portable lab. Just dip the forceps in alcohol and flame.

USING FIELD FILTRATION APPARATUS

1. Flame sterilize the top surface of the stainless steel Field Vacuum Support.

2. Attach the luer tip of the syringe to the tubing attached to the vacuum support.

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 upper rim of the funnel to snap it onto the vacuum support.

6. Pour the sample into the funnel.

NOTE: See specific procedures for the sample volume required.

7. Pull on the syringe plunger to draw the sample through the filter apparatus.

8. Remove the funnel.

9. Press the lever on the vacuum support stem to lift the membrane filter from the surface of the vacuum support.

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 luer tip of the syringe from the tubing attached to the vacuum support. Dispose of the liquid in the syringe.

13. Follow steps 1-12 to filter remaining samples.

USING AUTOCLAVABLE EQUIPMENT

When numerous samples must be run on a routine basis, you may prefer to use an autoclave for nondisposable materials.

1. Wash all 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 demineralized water and 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 base of the filter funnel into an autoclavable rubber stopper that will fit 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 may be sterilized with dry heat at 170 °C for a minimum of 1 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 contaminate the funnel by touching the inner surfaces that will be exposed to the sample.

3. Insert the funnel with rubber stopper into the filtering flask or filter funnel manifold and connect to the water trap and aspirator with rubber tubing.

4. Using sterile forceps, 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 assure a good seal on the filter and connections before running the sample through.

Sample Size

Sample size is governed by bacterial density as well as turbidity.

• 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 volume for fecal coliform testing yields approximately 20 to 60 coliform colonies and not more than 200 colonies for all types per filter.

To accomplish these ideal situations, three different volumes should be filtered for samples where the coliform number is uncertain. Tables 3 and 4 list recommended volumes for various types of samples.

When the sample is less than 20 mL (diluted or undiluted), 10 mL of sterile dilution water should be added to the filter funnel before vacuum is applied. This aids in the uniform distribution of the bacteria over the entire membrane filter.

Table 3.
Suggested Sample Volumes for MF Total Coliform Test*

	Volume to be Filtered (mL)							
Water Source	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*, 18th ed., page 9-56

Table 4.
Suggested Sample Volumes for MF Fecal Coliform Test*

	Volume to be Filtered (mL)						
Water Source	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*, 18th ed., page 9-60

Diluting Samples

As indicated by Tables 3 and 4, very small sample volumes may be required for testing water samples high in turbidity or coliform number. Because it is almost impossible to measure these small volumes accurately, a series of dilutions should be made. The following procedure describes one method of preparing a series of dilutions.

DILUTION TECHNIQUE

1. Wash 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 Steps 3-5 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 A into 99 mL of sterile 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 B into 99 mL of sterile 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 C into 99 mL of sterile dilution water. Filter 100 mL of this dilution to obtain the 0.01-mL sample.

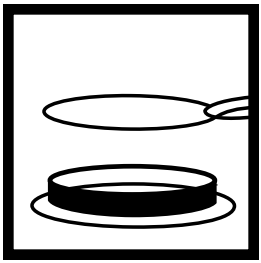
E. If 0.001-mL sample is required:
Transfer 11 mL of the 0.01-mL dilution from D into 99 mL of sterile dilution water. Filter 100 mL of this dilution to obtain the 0.001-mL sample.

F. If 0.0001-mL sample is required:
Transfer 11 mL of the 0.001-mL dilution from E into 99 mL of sterile dilution water. Filter 100 mL of this dilution to obtain the 0.0001-mL sample.

Potable Water Procedures

Total Coliform Procedure
Method 8074

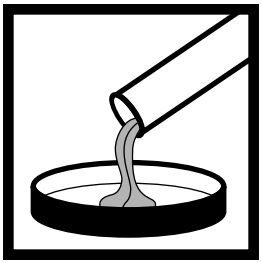
- USEPA-approved for drinking water
- Presumptive total coliform test using m-Endo Broth
- The procedure can also be used for nonpotable water, if the sample has been appropriately diluted (see Table 3 on page 23).



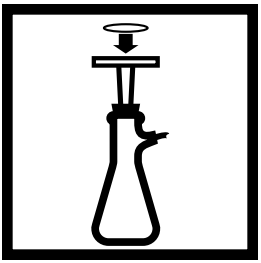
1. Place a sterile absorbent pad in a sterile petri dish (use sterilized forceps). Replace petri dish lid.

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

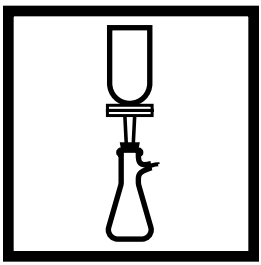
Note: *To sterilize forceps, dip forceps in alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.*



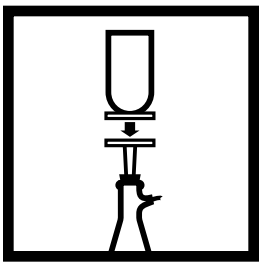
2. Open an ampule of m-Endo Broth. Pour the contents evenly over the absorbent pad. Replace petri dish lid.



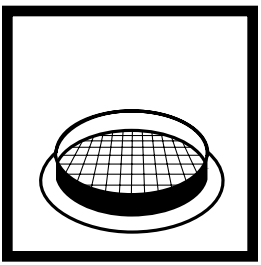
3. Set up the Membrane Filter Apparatus; see Preparing Materials on pages 21-22. With sterile forceps, place a membrane filter, grid side up, into the assembly.



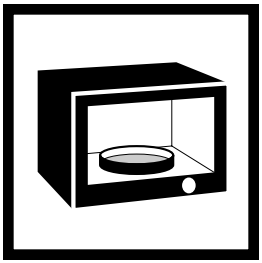
4. Shake the sample vigorously to mix. Pour 100 mL of sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls 3 times with 20 to 30 mL of sterile buffered dilution water.



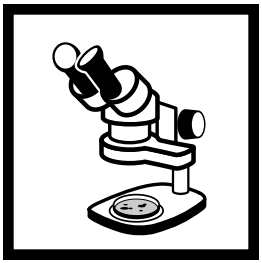
5. Turn off the vacuum and lift off the funnel top. Using sterile forceps, transfer the filter to the previously prepared petri dish.



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 petri dish lid.



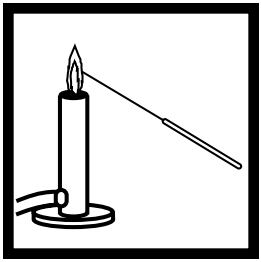
7. Invert the petri dish and incubate at 35 ± 0.5 °C for 24 hours.



8. After incubation count the greenish-gold metallic sheen colonies by using illuminated magnifier or a 10-15X microscope. Use the Confirmation Procedures on pages 27-32 to confirm results.

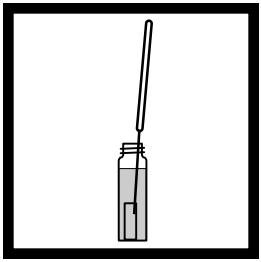
Total Coliform Confirmation Procedure
Method 8074

- USEPA-approved for drinking water samples
- All sheen colonies, or a minimum of 5 such colonies, must be confirmed to ensure they are total coliforms
- Confirming total coliform test using LT Broth and BGB Broth

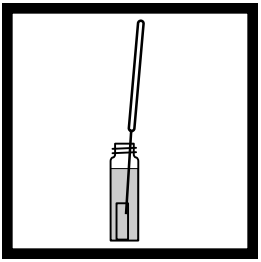


1. Sterilize an inoculating needle. Or use a sterile, disposable inoculating needle.

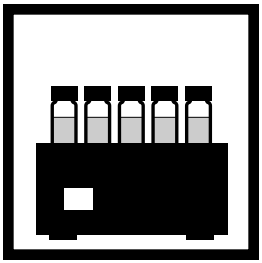
Note: *To sterilize an inoculating needle, heat to red hot in an alcohol or Bunsen burner. Let the needle cool before use.*



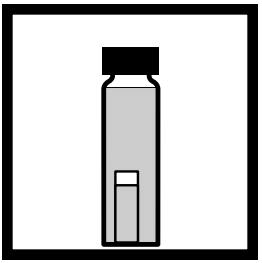
2. Touch the needle to a total coliform (sheen) colony. Transfer it to a single-strength LT Broth Tube.



3. Again touch the same coliform colony with the needle. Transfer it to a 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 for 1 hour. After 1 hour, invert the tubes to remove trapped air in the inner vial; then continue incubation.

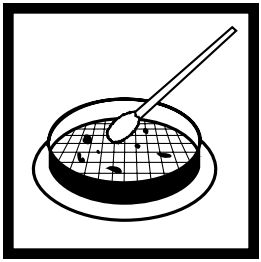


5. After 24 ± 2 hours, check the inner vials for gas bubbles. Gas bubbles in both the LT and BGB Broth Tubes verify that the colonies are total coliforms. If no gas is present in one or both tubes, continue incubating both tubes for another 24 hours.

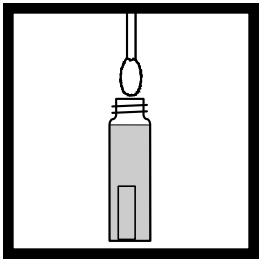
- If no gas is present in the LT Broth Tube, the colony is not a coliform and additional testing is unnecessary.
- If gas is present in the LT Broth Tube but not in the BGB Broth Tube, inoculate another BGB Broth Tube from the gas-positive LT Broth Tube. Incubate this BGB Broth Tube and check for gas after 24 hours and/or after 48 hours. If gas is produced within 48 ± 3 hours, the colony is confirmed as total coliform.

Fecal Coliform Confirmation Procedure
Method 8074

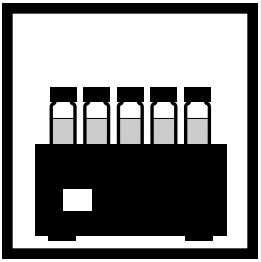
- USEPA-approved for drinking water
- Total coliform positive drinking water samples must be analyzed for the presence of fecal coliform or *E. coli*
- Confirming fecal coliform test using EC Medium



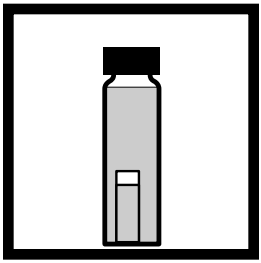
1. Using a sterile cotton swab, swab the entire surface of the total coliform positive membrane filter.



2. To transfer the colonies on the swab, swirl the swab in an EC Medium Tube. Remove the swab from the medium. Use the same swab to transfer colonies to other broth media if desired.



3. Invert the tube to eliminate any air bubbles trapped in the inner vial. Incubate the tube at 44.5 ± 0.2 °C for 1 hour. After 1 hour, invert the tube to remove trapped air in the inner vial; then continue incubation.

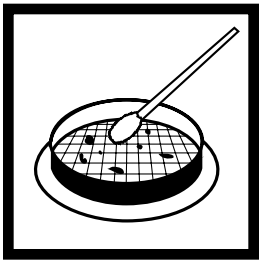


4. After 24 ± 2 hours, check the inner vial for gas bubbles. Gas bubbles in the EC Medium Tube confirms the presence of fecal coliforms.

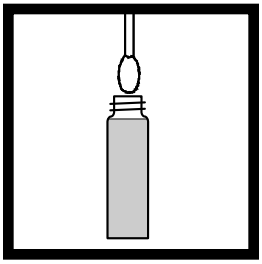
***E. coli* Procedures**
Method 8074

- Total coliform positive drinking water samples may be analyzed for the presence of *E. coli* in lieu of fecal coliform.
- The two following methods are available to confirm the presence of *E. coli* from a total coliform positive membrane filter.

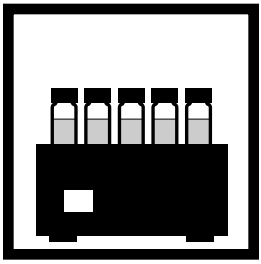
EC Medium with MUG Method



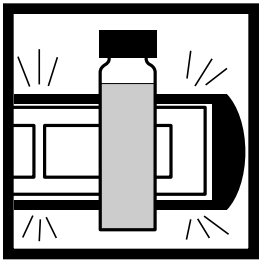
1. Using a sterile cotton swab, swab the entire surface of the total coliform positive membrane filter.



2. To transfer the colonies on the swab, swirl the swab in an EC Medium with MUG Tube. Remove the swab from the medium. Use the same swab to transfer colonies to other broth media if desired.

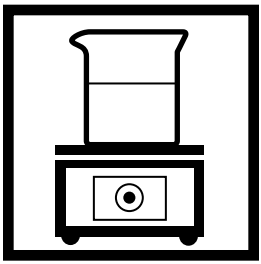


3. Invert the tube to mix and incubate at 44.5 ± 0.2 °C for 24 ± 2 hours.

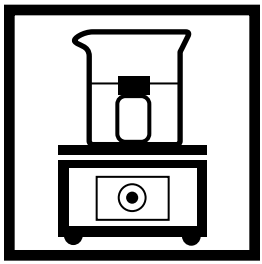


4. Check the tube for fluorescence by using a long-wave UV lamp. Fluorescence indicates the presence of *E. coli.*

Nutrient Agar with MUG Method

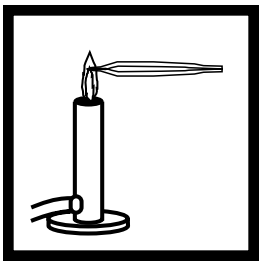


1. Heat beaker of water or water bath to boiling.



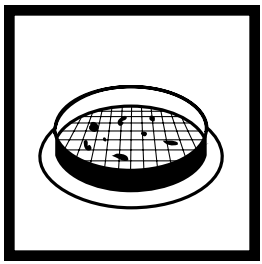
2. Place Nutrient Agar with MUG Tubes into boiling water. When agar melts, carefully remove tubes from boiling water with a test tube holder.

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.

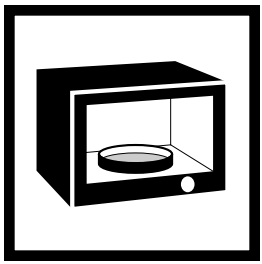


4. Using sterilized forceps, lift the membrane filter with total coliform colony(ies) off of the absorbent pad.

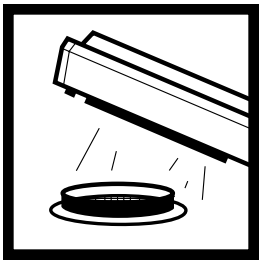
Note: *To sterilize forceps, dip forceps in alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.*



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



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



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

Nonpotable Water Procedures

Wastewater, river, bathing and other nonpotable waters usually are tested for fecal coliforms. Fecal coliform testing requires special medium and an elevated incubation temperature to inhibit growth of nonfecal coliforms. Sample sizes for these waters are selected from Table 4 on page 23.

If you need to test for total coliforms in nonpotable water, see Table 3 on page 23 and use the procedure for total coliforms on pages 25-28.

TOTAL COLIFORM TEST FOR STRESSED ORGANISMS

Organisms exposed to adverse environments, such as water treatment processes, grow slowly or not at all under bacteriological testing conditions. These organisms are called **stressed organisms**. Stressed organisms can give false negative results in membrane filter testing. Stressed organisms are found in chlorinated effluents, saline waters and natural waters polluted with substances such as heavy metal ions or toxic organic wastes. Sampling conditions, abrupt temperature changes, extremes in pH, low nutrient concentrations, and disinfectants also can produce stressed organisms.

When using the MF Method, stressed coliforms may require special techniques to get complete recovery. An enrichment technique using Lauryl Tryptose (LT) Broth Pillows is described below. Consult your local or state authorities as to 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 Pillow and pour off any excess liquid.

3. Filter sample through a membrane filter.

4. Place a membrane filter onto the saturated pad.

5. Incubate the petri dish without inverting for 1.5 to 2 hours at 35 ± 0.5 °C with 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 petri dish.

9. Carefully transfer the filter from the lid to the new pad.

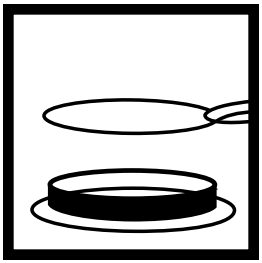
10. Discard the old pad (the enrichment pad saturated with LT). Replace petri dish lid.

11. Invert the petri dish and incubate at 35 ± 0.5 °C for 20 to 22 hours.

12. After incubation, count the greenish-gold metallic sheen colonies by using an illuminated magnifier or a 10-15X microscope. Use the Confirmation Procedure on page 36 to confirm results.

Fecal Coliform Procedure
Method 8074

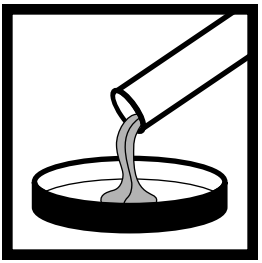
- USEPA-approved for wastewater samples
- Presumptive fecal coliform test using m-FC Broth



1. Place a sterile absorbent pad in a sterile petri dish (use sterilized forceps). Replace petri dish lid.

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

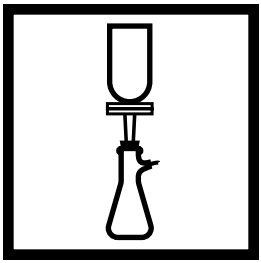
Note: *To sterilize forceps, dip forceps in alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.*



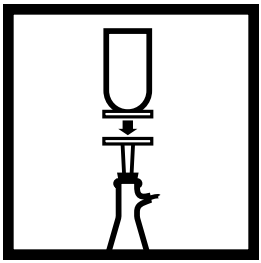
2. Open an ampule of m-FC Broth. Pour the contents evenly over the absorbent pad. Replace petri dish lid.

Note: *m-FC Broth with Rosolic Acid may be used for increased specificity when high levels of non-coliform bacteria may be present.*

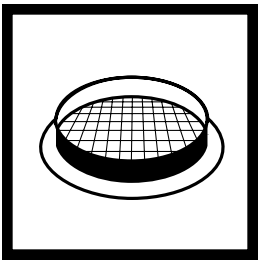
3. Set up the Membrane Filter Apparatus; see Preparing Materials on pages 21-22. With sterile forceps, place a membrane filter, grid side up, into the assembly.



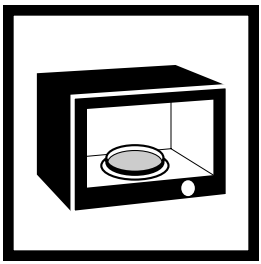
4. Prepare the necessary dilutions to obtain the proper sample size (see Diluting Samples on page 24) and shake vigorously. Pour sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls 3 times with 20 to 30 mL of sterile buffered dilution water.



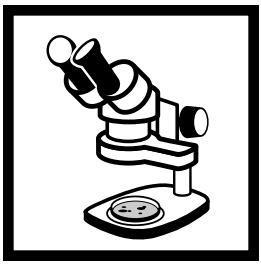
5. Turn off the vacuum and lift off the funnel top. Using sterile forceps, transfer the filter to the previously prepared petri dish.



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 petri dish lid.



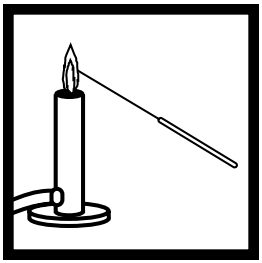
7. Invert the petri dish and incubate at 44.5 ± 0.2 °C for 24 ± 2 hours.



8. After incubation count the blue colonies by using an illuminated magnifier or a 10-15X microscope. Follow Interpreting Results on page 40 and record the data. If stressed organisms were tested, confirm not less than 10% of the blue (fecal coliform) colonies.

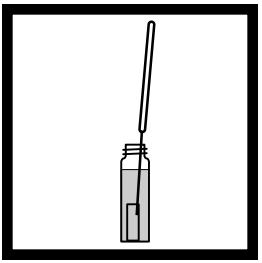
Fecal Coliform Confirmation Procedure
Method 8074

- USEPA-approved for wastewater samples
- Confirming fecal coliform test using Lauryl Tryptose (LT) Broth

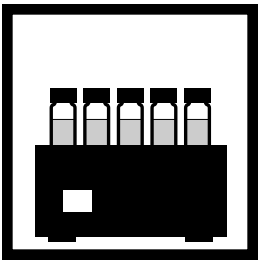


1. Sterilize an inoculating needle. Or use a sterile, disposable inoculating needle.

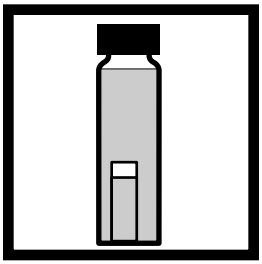
Note: *To sterilize an inoculating needle, heat to red hot in an alcohol or Bunsen burner. Let the needle cool before use.*



2. Touch the needle to a fecal coliform (blue) colony. Transfer it to a Lauryl Tryptose (LT) Broth Tube.



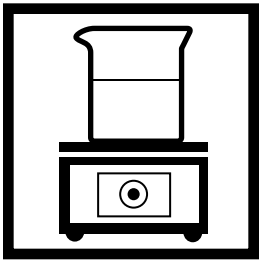
3. Invert the tube to eliminate any air bubbles trapped in the inner vials. Incubate the tubes at 35 ± 0.5 °C for 48 ± 3 hours. If gas is not produced in 48 hours, the colony is not fecal coliform. If gas is produced in 48 hours, use a sterile inoculating loop to inoculate one EC Medium Tube from each gas-positive LT Broth Tube.



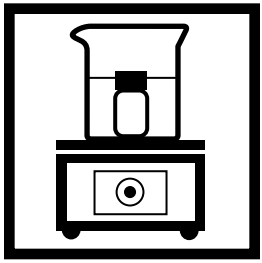
4. Incubate the EC Medium Tubes at 44.5 ± 0.2 °C for 24 ± 2 hours. Gas produced at 44.5 °C confirms the presence of fecal coliforms.

***E. coli* Procedure**
Method 8367

- This procedure is recommended in *Standard Methods for the Examination of Water and Wastewater* for examining fresh, estuarine, or marine natural recreational waters (stream, lake, ocean or hot spring) that may be contaminated with wastewater.
- This procedure is also accepted by the American Society of Testing Materials (ASTM).
- Presumptive *E. coli* test using m-TEC Agar Tubes

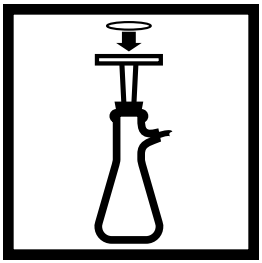


1. Heat beaker of water or water bath to boiling.



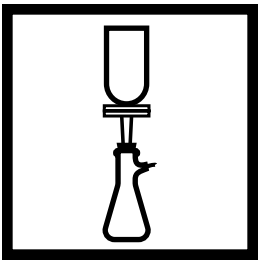
2. Place m-TEC Agar Tubes into boiling water. When agar melts, carefully remove tubes from boiling water with a test tube holder.

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.

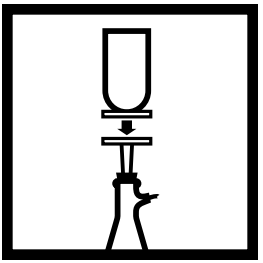


4. Set up the Membrane Filter Assembly; see Preparing Materials on pages 21-22. With sterile forceps, place a membrane filter, grid side up, into the assembly.

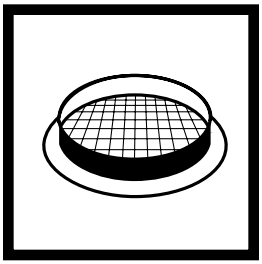
Note: *To sterilize forceps, dip forceps in alcohol and flame in an alcohol or Bunsen burner. Let forceps cool before use.*



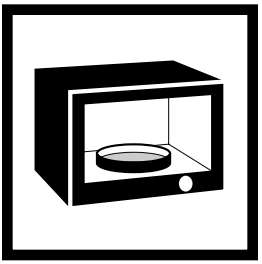
5. Prepare the necessary dilutions to obtain the proper sample size (see Diluting Samples on page 24) and shake vigorously. Pour sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls 3 times with 20 to 30 mL of sterile buffered dilution water.



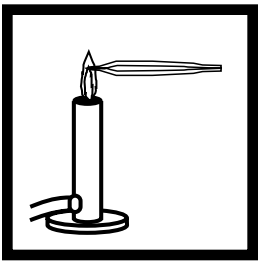
6. Turn off the vacuum and lift off the funnel top. Using sterile forceps, transfer the filter to the previously prepared petri dish.



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



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



9. Using sterile forceps, transfer the filter to a pad saturated with at least 2 mL of urea substrate.

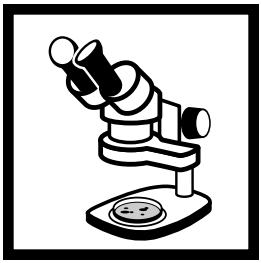
Note: Preparing urea substrate

1. Dissolve 2.0 g of urea in 100 mL of demineralized water.

2. Add 10 mg of phenol red sodium salt indicator to the urea solution.

3. Adjust the pH using a weak acid solution until the solution is yellow (pH 5.0 ± 0.2).

4. 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 10-15X microscope. Report as *E. coli* colonies per 100 mL. See Interpreting Results on page 40.

Interpreting MF Results

Coliform density is reported as 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 testing, samples should produce 20 to 60 fecal coliform colonies.

Equation A is used to calculate coliform density. Note the mL sample refers to actual sample volume, and not volume of the dilution.

Equation A—Coliform density on a single membrane filter

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

- 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 (coliforms plus non-coliforms) 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, a new sample must be run using a dilution that will give about 20 to 80 coliform colonies and not more than 200 colonies of all types.

When testing nonpotable water, if no filter meets the desired minimum colony count, the average coliform density can be calculated with Equation B and used for reporting.

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

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

Media, Reagents and Apparatus for MF Testing

Required Media and Apparatus

Cat. No.	Description	Quantity
20753-33	Bags, Whirl-Pak with dechlorinating agent, 170-mL	100
24846-00	Breaker, PourRite Ampule	1
20877-60	Burner, Alcohol, 60-mL	1
21411-00	Forceps	1
25863-00	Funnels, Push-Fit with 0.45 µm gridded membrane, sterile	72
25699-00	Incubator, Portable for MEL	1
25854-00	Magnifier, Illuminated, 2.5 and 5X	1
13530-01	Membrane Filters, 0.45 µm pore size, gridded, sterile	200
25801-00	Membrane Filtration Media Set Includes 216 membrane filters, 200 petri dishes with pads, 216 Push-Fit Funnels, 200 m-Endo Broth PourRite Ampules, and 200 Whirl-Pak Bags for sampling.	1
14717-99	Petri Dish, polystyrene, sterile, disposable, with pads	100
25805-02	Rack, MF Petri Dish (for use with Portable Incubator)	1
25861-00	Syringe, 140-mL, polypropylene, autoclavable	1
25862-00	Vacuum Support, Field, Stainless Steel	1

Optional Media, Reagents and Apparatus

24630-00	Autoclave, Automatic, 120 V	1
24630-02	Autoclave, Automatic, 240 V	1
24633-00	Bags, for contaminated items	200
322-15	Brilliant Green Bile Broth Tubes (for total coliform confirmation)	15
22453-00	Bottles, polysulfone, autoclavable (use for buffered dilution water)	12
24950-12	Bottles, sterile, 100-mL fill-to line, disposable	12
24950-50	Bottles, sterile, 100-mL fill-to line, disposable	50
14305-98	Buffered Dilution Water, 99-mL, sterile	25
14363-69	Dechlorinating Reagent Powder Pillows	100
14104-15	EC Medium Tubes (for fecal coliform confirmation)	15
24715-15	EC Medium with MUG Tubes (for *E. coli* determination)	15
23735-20	m-Endo Broth PourRite™ Ampules, 2-mL each (for total coliform determination)	20
23732-20	m-FC Broth PourRite Ampules, 2-mL each (for fecal coliform determination)	20
24285-20	m-FC Broth with Rosolic Acid PourRite Ampules, 2-mL each (for fecal coliform determination)	20
13529-00	Filter Holder, magnetic coupling (use with 24861-00)	1
24861-00	Filter Funnel Manifold, Aluminum, 3-place (use with 13529-00)	1
546-49	Filtering Flask, 500-mL	1
24632-00	Germicidal Cloths	50
45900-00	Incubator, 25-Well Dri-Bath, 115/230 V, 50/60 Hz	1
45900-02	Incubator, 25-Well Dri-Bath, 115/230 V, 50/60 Hz with continental-European power cord and fuses	1
22454-10	Inoculating Loops, sterile and disposable (for confirmation)	10
21121-00	Inoculating Loop, nichrome wire	1

21779-00	Inoculating Needle, wire	1
14725-64	Lauryl Tryptose Broth Pillows, sterile, 2-mL (for enrichment technique)	24
21623-15	Lauryl Tryptose Broth Tubes, single-strength (for total coliform confirmation)	15
25853-00	Magnifier, Illuminated, 10X, portable	1
23174-00	Microscope, 10X	1
24373-06	Nutrient Agar with MUG Tubes, 2 tests/tube (for *E. coli* determination)	6
20920-00	Pen, Laboratory	1
21429-64	Peptone Powder Pillows, 1-g	30
25639-22	Phenol Red Sodium Salt	5 g
2097-98	Pipet, Serological, 11-mL sterile, disposable	25
21431-66	Potassium Dihydrogen Phosphate & Magnesium Chloride Pillows	25 of each
2119-07	Stopper, Rubber, one-hole, No. 7	6
25543-00	Swabs, cotton, sterile (for confirmation)	100
25611-06	m-TEC Agar Tubes, 2 tests/tube (for *E. coli* determination)	6
559-19	Tubing, Rubber, 5/16" (0.8 cm) ID	3.7 m
11237-26	Urea Reagent	100 g
21843-00	UV Lamp, long-wave, 115 V	1
21843-02	UV Lamp, long-wave, 230 V	1
24152-00	UV Lamp, long-wave, portable, 4 watt	1
14697-00	Vacuum/Pressure Pump, portable, 115 V	1
14283-00	Vacuum Pump, Hand-operated	1
24638-00	Water Bath Incubator, 120 V, with gable cover	1
24638-02	Water Bath Incubator, 240 V, with gable cover	1
20978-10	Wicks, replacement, used with Alcohol Burner (20877-60)	10

2.8 Most Probable Number (MPN) Procedures

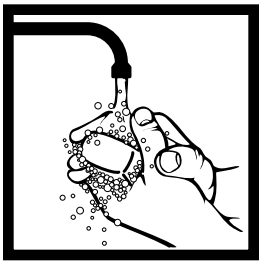
The following procedures are USEPA-approved. The Most Probable Number (MPN) method uses a specified number of test tubes to statistically predict the number of organisms present in a sample. The MPN method is ideal for wastewater and sludge applications, because analysts can use highly turbid samples by diluting the sample prior to analysis. Since the sample is diluted before analysis, no filtering is necessary.

The MPN procedure is performed by using tubes containing presterilized broth media. Several MPN media tubes also contain an inverted inner vial (durham tube) for gas collection. The MPN method involves adding sample to a tube of medium and incubating. If coliforms are present, gas is produced and trapped in the inner vial.

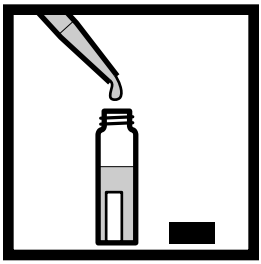
Potable Water Procedures

Total Coliform Procedure
Method 8001

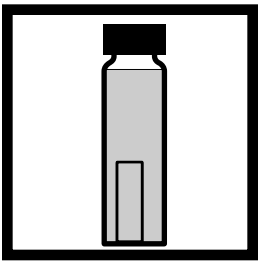
- USEPA-approved method for drinking water when using Lauryl Tryptose (LT) Broth
- Presumptive total coliform test when using either LT Broth or LT/MUG Broth
- Quick screening test for *E. coli* when using LT/MUG Broth



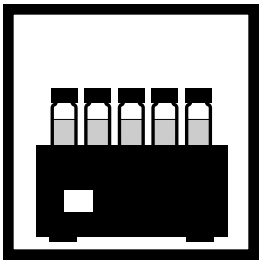
1. Wash hands thoroughly. Open the sealed package and remove 5 or 10 LT Broth Tubes or LT/MUG Broth Tubes. (10 LT Broth Tubes are required for USEPA reporting.)



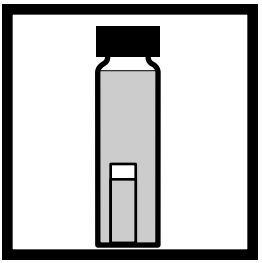
2. Remove the cap and pipet 10 mL of sample into each tube with a sterile pipet. Do not touch the open end of the tube or the inside of the cap. See Note A on page 47.



3. Replace the cap after sample addition. Invert the tube 3 to 5 times to mix and to eliminate any air bubbles trapped in the inner vial.



4. Incubate at 35 ± 0.5 °C for 1 hour. After 1 hour, invert the tube to remove trapped air in the inner vial; then continue to incubate. See Note B on page 47.



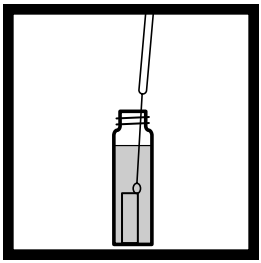
5. After 24 ± 2 hours check each tube.

- If the broth is cloudy and the inner vial contains gas bubbles, coliform bacteria are present.
- If no gas is present reincubate the tubes and check after 48 ± 3 hours. Tubes containing gas are positive. Tubes with no gas are negative.
- Fluorescence under long-wave UV light indicates *E. coli* are present. Fluorescence without gas production is an indication of an anaerogenic (non-gas-producing) strain(s) of *E. coli.*

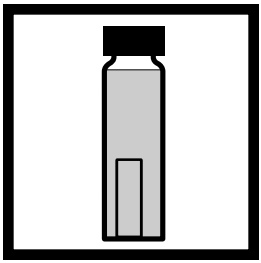
6. All tubes containing gas should be confirmed. Confirmation tests are used to eliminate false positive results.

Total Coliform Confirmation Procedure
Method 8001

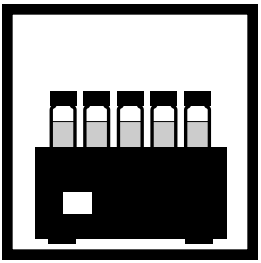
- USEPA-approved for drinking water when confirming from LT Broth Tubes
- Confirming total coliform test using Brilliant Green Bile (BGB) Broth



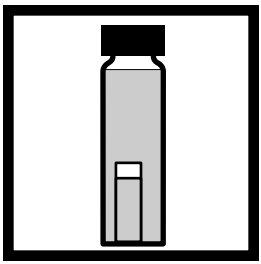
1. Using a sterile loop, transfer one loop from each positive LT Broth Tube or LT/MUG Broth Tube to a BGB Broth Tube. Do not touch the rim of either tube. See Note C on page 47.



2. Replace the cap after sample addition. Invert the tube 3 to 5 times to mix and to eliminate any air bubbles trapped in the inner vial.

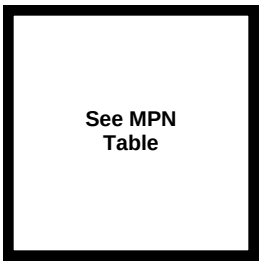


3. Incubate at 35 ± 0.5 °C for 1 hour. After 1 hour, invert the tube to remove trapped air in the inner vial; then continue to incubate. See Note B on page 47.



4. After 24 ± 2 hours check each tube.

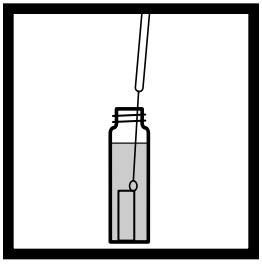
- If the inner vial contains gas bubbles, total coliform bacteria are confirmed.
- If no gas is present reincubate the tubes and check after 48 ± 3 hours. Tubes containing gas are confirmed positive. Tubes with no gas are confirmed negative.



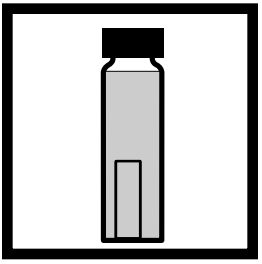
5. Express results of the test as Most Probable Number (MPN). See Table 6 on page 58 for the 5-tube MPN value, or Table 7 on page 58 for the 10-tube MPN value.

Fecal Coliform and *E. coli* Confirmation Procedure
Method 8001

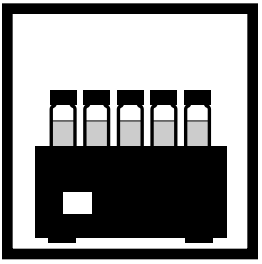
- USEPA-approved for drinking water
- Confirming fecal coliform test using EC Medium
- Confirming *E. coli* test using EC Medium with MUG
- USEPA requires that samples positive for total coliform should be tested for fecal coliform or *E. coli*



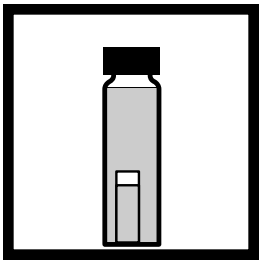
1. Using a sterile loop, transfer one loop from each positive LT Broth Tube to an EC Medium Tube or an EC Medium with MUG Tube. Do not touch the rim of either tube. See Note C on page 47.

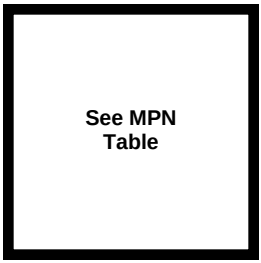


2. Replace the cap after sample addition. Invert the tube 3 to 5 times to mix and to eliminate any air bubbles trapped in the inner vial.



3. Incubate at 44.5 °C for 1 hour. After 1 hour, invert the tube to remove trapped air in the inner vial; then continue to incubate. See Note B on page 47.





4. After 24 ± 2 hours check each EC Medium Tube for gas bubbles. Check each EC Medium with MUG Tube for fluorescence. EC Medium Tubes that contain gas in the inner vial are confirmed positive for fecal coliform. EC Medium with MUG Tubes that fluoresce under long-wave UV light are confirmed positive for *E. coli.* Tubes with no gas are confirmed negative for fecal coliform. Tubes that do not fluoresce are confirmed negative for *E. coli.*

5. Express results of the test as Most Probable Number (MPN). See Table 6 on page 58 for the 5-tube MPN value, or Table 7 on page 58 for the 10-tube MPN value.

NOTES FOR TESTING POTABLE WATER

A. Instead of pipetting, add sample until the broth tube is level full. Then invert the tube to fill the inner vial. This technique may save time when USEPA reporting is not required. If USEPA reporting is required, measure the sample by pipetting.

B. Remember to check tubes for air in the inner vials after 1 hour of incubation. Bubbles formed during the first hour result from dissolved gases released by heating and are not due to bacteria. Trapped air should be removed by inverting and carefully returning the tubes to their upright position. Loosen the caps slightly before returning the tubes to the incubator.

C. The cap transfer method of inoculation may be used for non-reporting purposes. Shake or invert a positive presumptive tube to wet the liner of the cap. Exchange this cap with the cap on the confirmation tube. Invert the confirmation tube to mix the transferred bacteria with the medium.

Nonpotable Water and Wastewater Procedures

MPN methods are also useful for detecting coliforms in recreational waters, swimming pools, lakes, shellfish-growing waters, heavily polluted waters and wastewater. In these test situations, the multiple tube decimal dilution procedure should be used for determining the MPN indices for both total and fecal coliforms.

SAMPLE SIZE AND DILUTION

Testing nonpotable water samples requires inoculating a series of tubes with appropriate decimal dilutions of the original sample. The decimal dilutions are based on probable coliform density. Three different dilutions should be inoculated into MPN tubes. For example, when examining swimming pool water, add undiluted sample to 5 tubes, sample with a 1:10 dilution to 5 tubes, and sample with a 1:100 dilution to 5 tubes. Table 5 on pages 49-50 specifies typical dilutions used for various sample types.

PREPARING DILUTION WATER

Sterile buffered dilution water is available in 99-mL bottles or it can be prepared with one of the following methods.

A. Add the contents of 1 Peptone Powder Pillow to 1 liter of distilled or demineralized water. Dispense in amounts to yield 99 ± 2 mL after autoclaving. Sterilize in an autoclave for 15 minutes.

B. Add the contents of 1 pH adjusted Potassium Dihydrogen Phosphate Pillow and 1 Magnesium Chloride Pillow to 1 liter of distilled or demineralized water. Dispense in amounts to yield 99 ± 2 mL after autoclaving. Sterilize in an autoclave for 15 minutes.

DILUTION TECHNIQUE

1. Wash hands.

2. Open a bottle of sterile Buffered Dilution Water.

3. Shake the sample collection container vigorously, approximately 25 times.

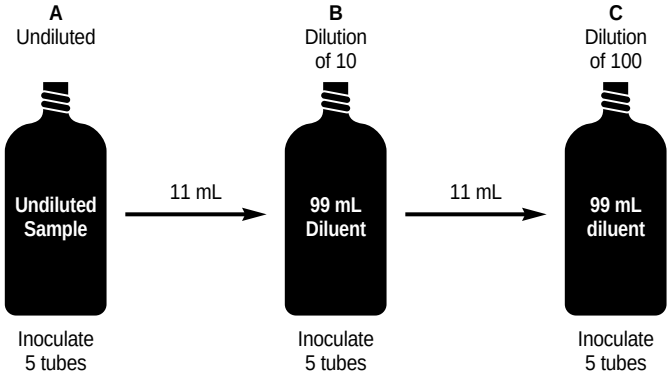
4. Use a sterile transfer pipet to pipet the required amount of sample into the sterile Buffered Dilution Water.

5. Recap the buffered dilution water bottle and shake vigorously 25 times.

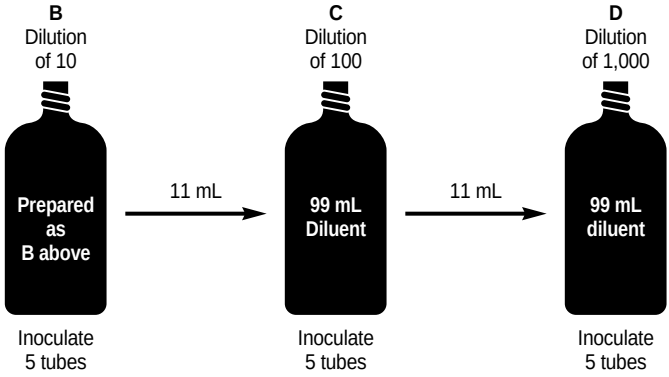
6. If more dilutions are needed, repeat Steps 3-5 using clean, sterile pipets and additional bottles of sterile Buffered Dilution Water.

Table 5. Typical Sample Dilutions for Nonpotable Water

Swimming Pools Water, Chlorinated: *Dilution Factor = 1*



Bathing Beach Water; Lake Water; Unpolluted River Water: *Dilution Factor = 10*



Final Effluent, Chlorinated: *Dilution Factor = 100*

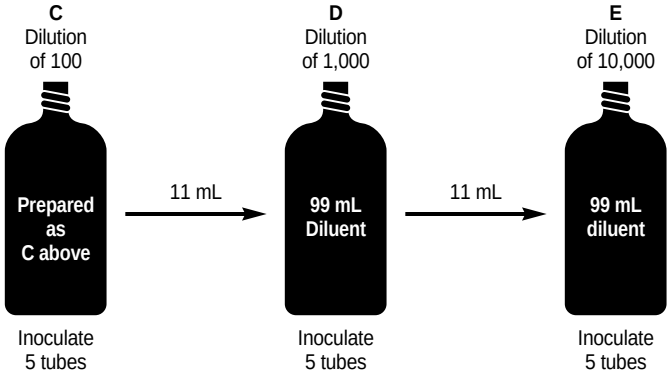
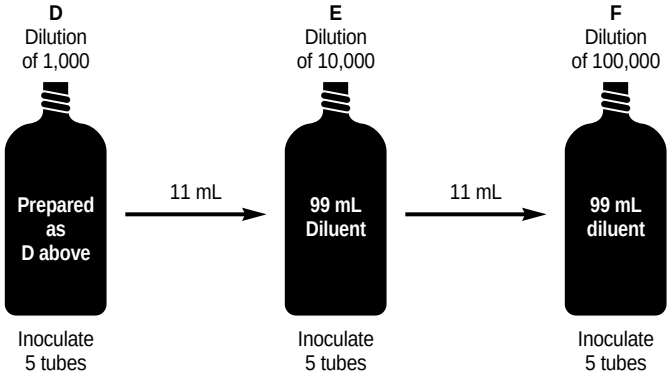
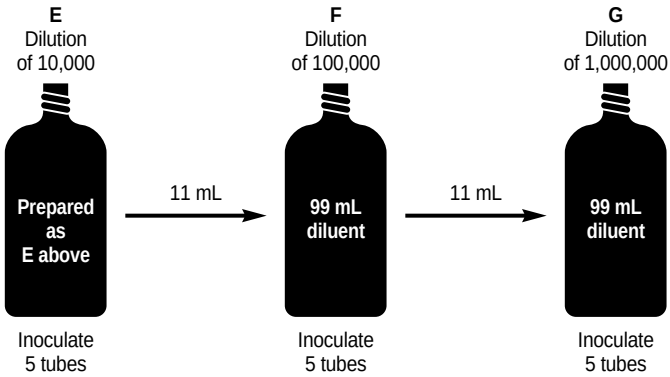


Table 5. Typical Sample Dilutions for Nonpotable Water, continued

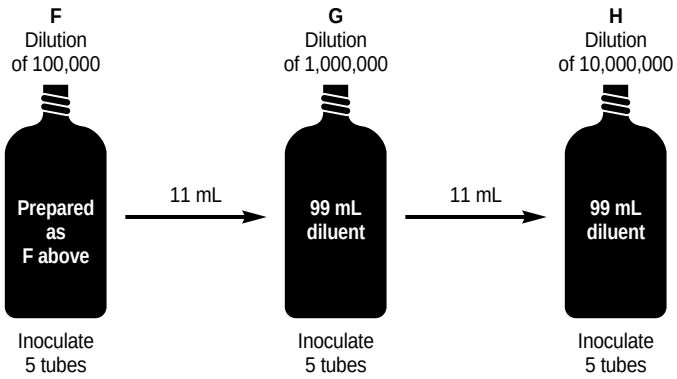
River Water, Polluted: ***Dilution Factor = 1,000***



Storm Water, Unchlorinated Final Effluent: ***Dilution Factor = 10,000***

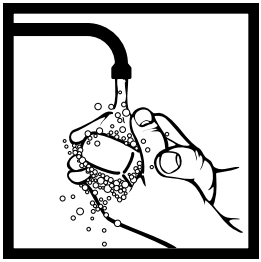


Raw Sewage: ***Dilution Factor = 100,000***

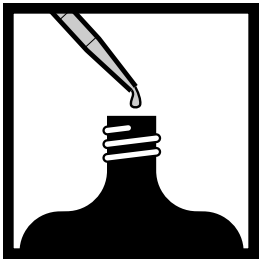


Total Coliform Procedure
Method 8001

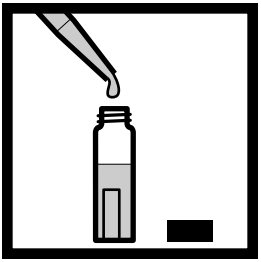
- USEPA-approved for wastewater when using Lauryl Tryptose (LT) Broth
- Presumptive total coliform test when using either Lauryl Tryptose (LT) Broth or LT/MUG Broth
- Quick screening test for *E. coli* when using LT/MUG Broth



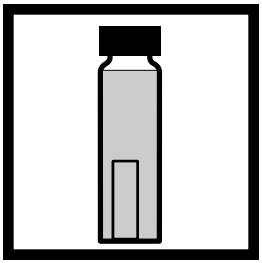
1. Wash hands thoroughly.



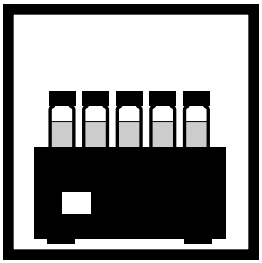
2. Using sterile Buffered Dilution Water, prepare 3 decimal dilutions of the sample (see Table 5 on pages 49-50). Follow steps 3 to 7 for each of the dilutions.



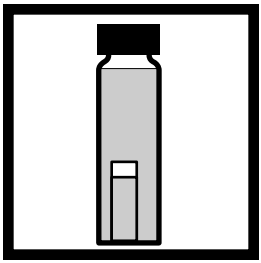
3. Open the sealed package and remove 5 LT Broth Tubes or LT/MUG Broth Tubes. Remove the cap and pipet 10 mL of sample into each tube with a sterile pipet. Do not touch the open end of the tube or the inside of the cap. See Note A on page 47.



4. Replace the cap after sample addition. Invert the tube 3 to 5 times to mix and to eliminate any air bubbles trapped in the inner vial.



5. Incubate at 35 ± 0.5 °C for 1 hour. After 1 hour, invert the tube to remove trapped air in the inner vial; then continue to incubate. See Note B on page 47.



6. After 24 ± 2 hours check each tube.

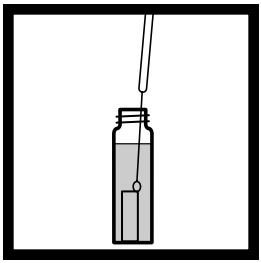
- If the broth is cloudy and the inner vial contains gas bubbles, coliform bacteria are present.
- If no gas is present reincubate the tubes and check after 48 ± 3 hours. Tubes containing gas are positive. Tubes with no gas are negative.
- Fluorescence under long-wave UV light indicates *E. coli* are present. Fluorescence without gas production is an indication of an anaerogenic (non-gas-producing) strain(s) of *E. coli.*

Confirm Positive Samples

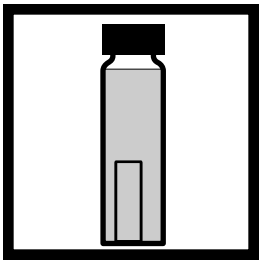
7. All tubes containing gas should be confirmed. Confirmation tests are used to eliminate false positive results.

Total Coliform Confirmation Procedure
Method 8001

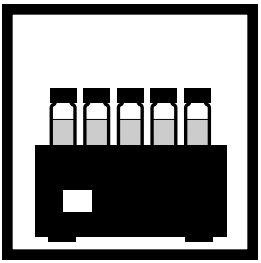
- USEPA-approved for wastewater when using Lauryl Tryptose (LT) Broth
- Confirming total coliform test using Brilliant Green Bile (BGB) Broth



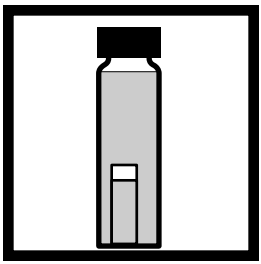
1. Using a sterile loop, transfer one loop from each positive LT Broth Tube or LT/MUG Broth Tube to a BGB Broth Tube. Do not touch the rim of either tube. See Note C on page 47.



2. Replace the cap after sample addition. Invert the tube 3 to 5 times to mix and to eliminate any air bubbles trapped in the inner vial.

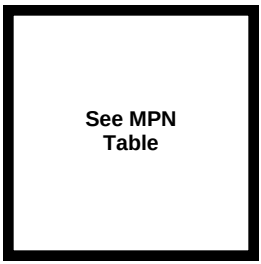


3. Incubate at 35 ± 0.5 °C for 1 hour. After 1 hour, invert the tube to remove trapped air in the inner vial; then continue to incubate. See Note B on page 47.



4. After 24 ± 2 hours check each tube.

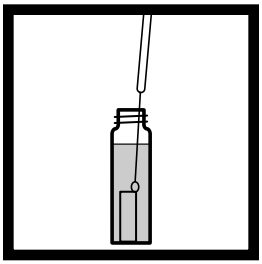
- If the inner vial contains gas bubbles, total coliform bacteria are confirmed.
- If no gas is present reincubate the tubes and check after 48 ± 3 hours. Tubes containing gas are confirmed positive. Tubes with no gas are confirmed negative.



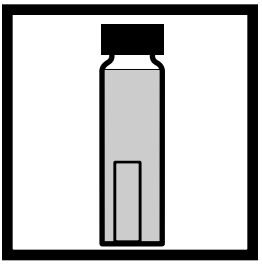
5. Express results of the test as Most Probable Number (MPN). See Table 8 on page 60 for the MPN value.

Fecal Coliform and *E. coli* Confirmation Procedure
Method 8001

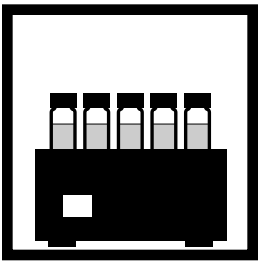
- USEPA-approved for wastewater when using EC Medium
- Confirming fecal coliform test using EC Medium
- Confirming *E. coli* test using EC Medium with MUG



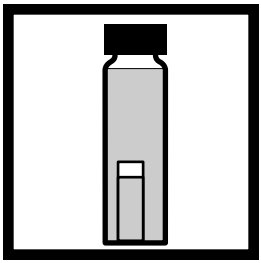
1. Using a sterile loop, transfer one loop from each positive LT Broth Tube to an EC Medium Tube or an EC Medium with MUG Tube. Do not touch the rim of either tube. See Note C on page 47.

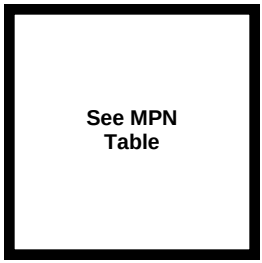


2. Replace the cap after sample addition. Invert the tube 3 to 5 times to mix and to eliminate any air bubbles trapped in the inner vial.



3. Incubate at 44.5 °C for 1 hour. After 1 hour, invert the tube to remove trapped air in the inner vial; then continue to incubate. See Note B on page 47.





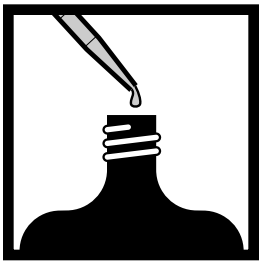
4. After 24 ± 2 hours check each EC Medium Tube for gas bubbles. Check each EC Medium with MUG Tube for fluorescence. EC Medium Tubes that contain gas in the inner vial are confirmed positive for fecal coliform. EC Medium with MUG Tubes that fluoresce under long-wave UV light are confirmed positive for *E. coli*. Tubes with no gas are confirmed negative for fecal coliform. Tubes that do not fluoresce are confirmed negative for *E. coli*.

5. Express results of the test as Most Probable Number (MPN). See Table 8 on page 60 for the MPN value.

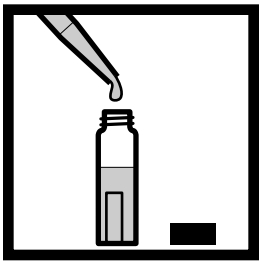
Fecal Coliform Procedure
Method 8368

- Fecal coliform test using A-1 Medium Broth
- No confirmation is required
- This procedure is recommended in *Standard Methods for the Examination of Water and Wastewater* for examining source water, seawater, recreational water, and treated wastewater.

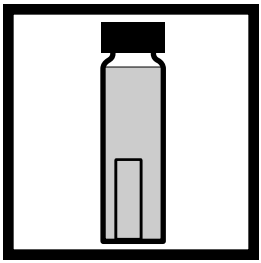
1. Wash hands thoroughly.



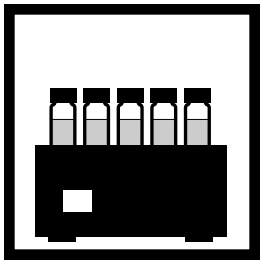
2. Using sterile Buffered Dilution Water, prepare 3 decimal dilutions of the sample (see Table 5 on pages 49-50). Follow steps 3 to 7 for each of the dilutions.



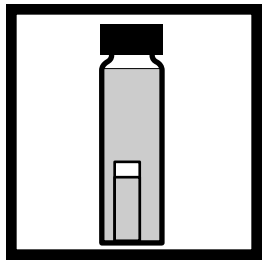
3. Open the sealed package and remove 5 A-1 Medium Broth Tubes. Remove the cap and pipet 10 mL of sample into each tube with a sterile pipet. Do not touch the open end of the tube or the inside of the cap. See Note A on page 47.



4. Replace the cap after sample addition. Invert the tube 3 to 5 times to mix and to eliminate any air bubbles trapped in the inner vial.



5. Incubate at 35 ± 0.5 °C for 3 hours. After 3 hours, invert the tube to remove trapped air in the inner vial; then continue to incubate at 44.5 ± 0.2 °C for an additional 21 hours. See Note B on page 47.



6. After 24 ± 2 hours check each tube.

- If the inner vial contains gas bubbles, fecal coliform bacteria are present.
- If no gas is present, fecal coliform are not present.

See MPN Table

7. Express the results of the fecal coliform test as MPN/100 mL. See Table 8 on page 60 for the MPN value.

Interpreting MPN Results

When testing potable water, the USEPA has established minimum requirements* for sampling. Certain areas may have more stringent requirements. If all 5 tubes (for a 5-tube MPN test) or all 10 tubes (for a 10-tube MPN test) of the confirmed coliform test are negative, the sample is accepted as meeting bacterial standards. To assure that you interpret results in accordance with appropriate standards and regulations, contact the appropriate agency for reporting information.

Using statistical methods you can estimate the number of coliform organisms from any combination of positive and negative test results. Use Table 6 for the 5-tube test, Table 7 for the 10-tube test, and Table 8 for the 15-tube MPN test. Report results as Most Probable Number (MPN)/100 mL. See Table 9 for an example of an MPN data sheet.

FIVE-TUBE MPN TEST

Use Table 6 to determine MPN/100 mL for 5-tube MPN tests. For example, if 4 of the 5 tubes showed a positive response, the MPN/100 mL would be 16.0.

Table 6.
5-tube MPN undiluted sample, 10 mL per tube. 95% confidence limits.

Positive Tubes	MPN/100 mL
0	< 2.2
1	2.2
2	5.1
3	9.2
4	16.0
5	> 16.0

TEN-TUBE MPN TEST

Use Table 7 to determine MPN/100 mL for 10-tube MPN tests. Example, if 6 of the 10 tubes showed a positive response, the MPN/100 mL would be 9.2.

Table 7.
10-tube MPN undiluted sample, 10 mL per tube. 95% confidence limits.

Positive Tubes	MPN/100 mL
0	< 1.1
1	1.1
2	2.2
3	3.6
4	5.1
5	6.9
6	9.2
7	12.0
8	16.1
9	23.0
10	> 23.0

*National Primary Drinking Water Regulations published in *Federal Register*, June 29, 1989.

FIFTEEN-TUBE MPN TEST

Use Table 8 to determine MPN/100 mL for tests using 5 tubes each of 3 different sample dilutions.

Example: 5 undiluted samples, 5 dilutions of 10, and 5 dilutions of 100 were inoculated initially. Results observed were: 5 positive tubes from the tubes containing undiluted sample; 4 positive tubes from the tubes containing dilutions of 10; and 2 positive tubes from the tubes containing dilutions of 100. These results are expressed as 5, 4, 2. This sequence can be located in the horizontal rows of Table 8. The MPN/100 mL for this sample is 220.

The values in Table 8 are based on tests using the following dilutions: undiluted, dilution of 10, and dilution of 100. If other dilutions are used, the value in Table 8 should be adjusted. To obtain the correct MPN value, simply multiply the MPN index from Table 8 by the smallest dilution factor used.

Example:

If your decimal dilutions were:	**And the number of positive tubes were:**
10	5
100	1
1000	1

The MPN index obtained from Table 8 is: 5, 1, 1 = 50
Smallest Dilution Factor Used = 10
50 x 10 = 500 coliforms/100 mL

NOTE: Any series of decimal dilutions should contain both positive and negative tubes to be of most value. If all tubes of the 3 dilutions are positive, a greater degree of dilution is needed. Similarly, if all tubes of the 3 dilutions are negative, the degree of dilution is too great.

Table 8. MPN Index* (Index based on 95 percent confidence limits)

Number of tubes giving positive reaction out of				Number of tubes giving positive reaction out of			
5 undiluted samples (dilution factor-1)	5 dilutions of 10 (dilution factor-10)	5 dilutions of 100 (dilution factor-100)	MPN Index per 100 mL	5 undiluted samples (dilution factor-1)	5 dilutions of 10 (dilution factor-10)	5 dilutions of 100 (dilution factor-100)	MPN Index per 100 mL
0	0	0	< 2	4	2	1	26
0	0	1	2	4	3	0	27
0	1	0	2	4	3	1	33
0	2	0	4	4	4	0	34
1	0	0	2	5	0	0	23
1	0	1	4	5	0	1	30
1	1	0	4	5	0	2	40
1	1	1	6	5	1	0	30
1	2	0	6	5	1	1	50
2	0	0	4	5	1	2	60
2	0	1	7	5	2	0	50
2	1	0	7	5	2	1	70
2	2	0	9	5	2	2	90
2	2	0	9	5	3	0	80
2	3	0	12	5	3	1	110
3	0	0	8	5	3	2	140
3	0	1	11	5	3	3	170
3	1	0	11	5	4	0	130
3	1	1	14	5	4	1	170
3	2	0	14	5	4	2	220
3	2	1	17	5	4	3	280
4	0	0	13	5	4	4	350
4	0	1	17	5	5	0	240
4	1	0	17	5	5	1	300
				5	5	2	500
4	1	1	21	5	5	3	900
4	1	2	26	5	5	4	1600
4	2	0	22	5	5	5	1600

Multiply the MPN index by the smallest dilution factor from the series used when dilutions other than 1, 10 and 100 are used.
**Standard Methods*, 18th ed.

Table 9. Data Sheet

Row	Tube Number	Dilution Factor	Presumptive 24 hr	Presumptive 48 hr	Confirmed 24 hr	Confirmed 48 hr	Positive Tubes No. Code	Fecal 24 hr	Positive Tubes No. Code
A	1	1	+		+		5	0	0
	2		+		+				
	3		+		+				
	4		+		+				
	5		+		+				
B	1	10	+		–	+	5	0	0
	2		+		+				
	3		–	+	–	+			
	4		+		–	+			
	5		+		+				
C	1	100	–	–			3	0	0
	2		–	+	+				
	3		+		+				
	4		–	+	–	+			
	5		–	–					

Confirm Code: 5,5,3 MPN Index from Table 8: 900

Coliforms Count:: 900/100 mL

Fecal Count: <2/100 mL

Collection Data
Sample Description: final effluent
Sample Number: 241
Date Collected: 1 Nov. 92
Time Collected: 8:20 am
Collected by: K.R.

Laboratory Data
Date:1 Nov. 92
Time: 9:00 am
By: A.L.

Media, Reagents and Apparatus for MPN Testing

Required Media and Apparatus

Cat. No.	Description	Quantity
20753-33	Bags, Whirl-Pak with dechlorinating agent, 170-mL	100
25699-00	Incubator, Portable for MEL	1
22454-10	Inoculating Loop, sterile and disposable	10
25802-00	Most Probable Number Media Set Includes 135 LT/MUG Broth Tubes, 30 Brilliant Green Bile Broth Tubes, 30 presterilized inoculating loops, 25 presterilized 11-mL pipets, and 25 Whirl-Pak Bags for sampling.	1
14651-00	Pipet Bulb	1
25805-01	Rack, MPN Tube (for use with Portable Incubator)	1
24152-00	UV Lamp, long-wave, portable, 4 watt	1

Optional Media, Reagents and Apparatus

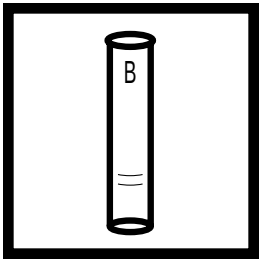
25609-15	A-1 Medium Broth Tubes, concentrated	15
24630-00	Autoclave, Automatic, 120 V	1
24630-02	Autoclave, Automatic, 240 V	1
24633-00	Bags, for contaminated items	200
22331-00	Bags, Whirl-Pak without dechlorinating agent, 207-mL	500
322-15	Brilliant Green Bile Broth Tubes (total coliform confirmation)	15
22453-00	Bottles, polysulfone, autoclavable (use for buffered dilution water)	12
24950-12	Bottles, presterilized, 100-mL fill-to line	12
24950-50	Bottles, presterilized, 100-mL fill-to line	50
14724-41	Bottles, wide-mouth, 250-mL, polypropylene, autoclavable	3
14305-98	Buffered Dilution Water, sterile, 99-mL	25
20877-81	Burner, Alcohol, 120-mL	1
21627-00	Burner, Bunsen with tubing	1
20658-00	Clippers, large	1
14363-69	Dechlorinating Reagent Powder Pillows	100
14104-15	EC Medium Tubes (fecal coliform confirmation)	15
24715-15	EC Medium with MUG Tubes (fecal coliform and *E. coli* confirmation)	15
23611-00	Fluorescence Standard, for *E. coli*	1
24632-00	Germicidal Cloth	50
45900-00	Incubator, 25-Well Dri-Bath, 115/230 V, 50/60 Hz	1
45900-02	Incubator, 25-Well Dri-Bath, 115/230 V, 50/60 Hz with continental-European power cord and fuses	1
21121-00	Inoculating Loop, nichrome wire	1
21013-15	Lactose MPN Tubes, concentrated (for non-reporting purposes)	15
21014-15	Lauryl Tryptose Broth Tubes (total coliform presumptive)	15
14404-15	Lauryl Tryptose MPN Tubes, dehydrated medium (for non-reporting purposes)	15
21821-15	Lauryl Tryptose with MUG Tubes	15
21431-66	Magnesium Chloride & Potassium Dihydrogen Phosphate Powder Pillows	25 of each
21429-64	Peptone Powder Pillows, 1-g	30
2097-98	Pipet, sterile, disposable, 11-mL	25
20926-48	Pipet, sterile, disposable, individually wrapped, 10-mL	12
25517-01	Pipet Aid with 110 V Recharger (UL, CSA approved) and 4 replacement filters	1
25517-02	Pipet Aid with 220 V Recharger (UL, CSA approved) and 4 replacement filters	1
2215-00	Rack, Coliform Tube	1
21843-00	UV Lamp, long-wave, 115 V	1
21843-02	UV Lamp, long-wave, 230 V	1

SECTION 3 FREE AND TOTAL CHLORINE MEASUREMENT

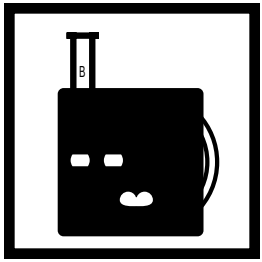
Measuring Hints and Test Information

- Rinse all viewing tubes thoroughly before testing.
- The powdered reagent does not have to dissolve entirely to obtain correct results.
- If a high level of monochloramine (combined chlorine) is present, it can interfere with the Free Chlorine test after 1 minute of developing time. Read the result of the Free Chlorine test within 1 minute as stated in Step 4 of the procedure.
- In the Total Chlorine test, do not let color development proceed for more than 6 minutes.
- Use clippers to open plastic powder pillows. Foil PermaChem pillows may be torn open.

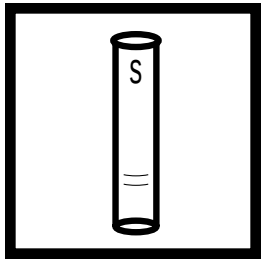
Free Chlorine Procedure



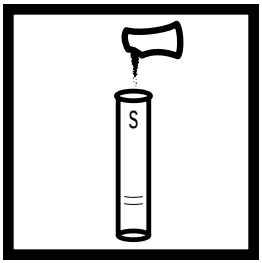
1. Fill viewing tube to the 5-mL mark with sample and label “B” for blank.



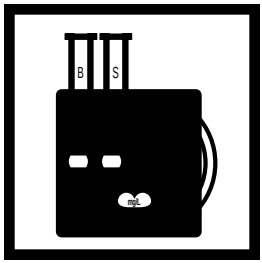
2. Place this tube in the left top opening of the color comparator.



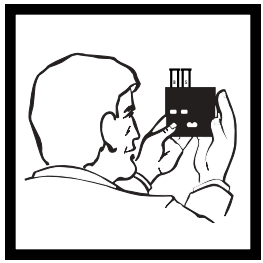
3. Fill the other viewing tube to the 5-mL mark with sample and label “S” for sample.



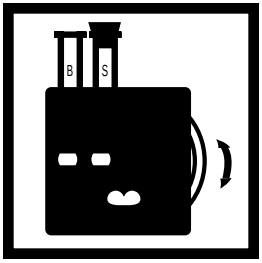
4. Add the contents of a DPD Free Chlorine Reagent Powder Pillow. Swirl to mix.



5. Place this tube in the right top opening of the color comparator.



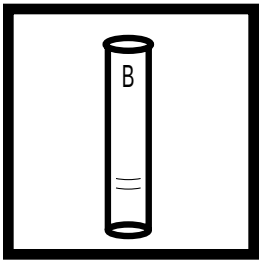
6. Hold comparator up to a light source (sky, light or window). Look through the openings in front.



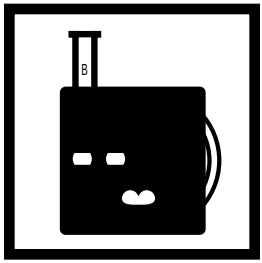
7. Rotate the color disc until the color matches in the two openings.

8. Within 1 minute, read the mg/L free chlorine through the scale window.

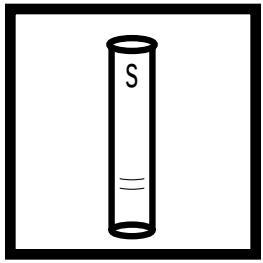
Total Chlorine Procedure



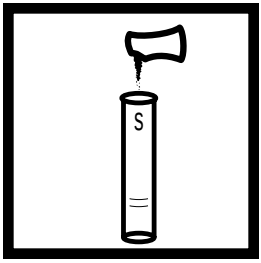
1. Fill viewing tube to the 5-mL mark with sample and label “B” for blank.



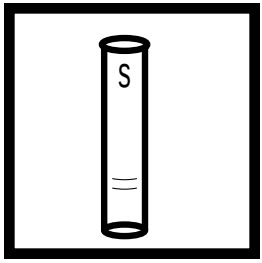
2. Place this tube in the left top opening of the color comparator.



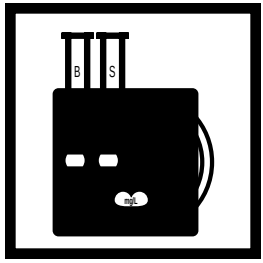
3. Fill the other viewing tube to the 5-mL mark with sample and label “S” for sample.



4. Add the contents of a DPD Total Chlorine Reagent Powder Pillow. Swirl to mix.

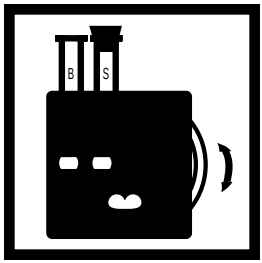


5. Let stand for 3 minutes, but not more than 6 minutes.



6. Place this tube in the right top opening of the color comparator.

7. Hold comparator up to a light source (sky, light or window). Look through the openings in front.



8. Rotate the color disc until the color matches in the two openings.

9. Read the mg/L total chlorine through the scale window.

Required Reagents and Apparatus

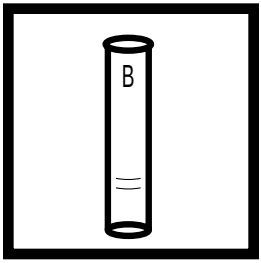
Cat. No.	Description	Quantity
14077-99	DPD Free Chlorine Reagent Powder Pillows	100
14076-99	DPD Total Chlorine Reagent Powder Pillows	100
936-00	Clippers for opening powder pillows	1
1732-00	Color Comparator	1
1730-00	Color Viewing Tube, glass	2
46600-04	Color Viewing Tube, plastic	4
21988-00	DPD Chlorine Color Disc, 0-3.5 mg/L	1

SECTION 4 NITRATE MEASUREMENT

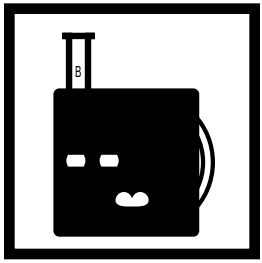
Measuring Hints and Test Information

- Rinse all viewing tubes thoroughly before testing.
- The powdered reagent does not have to dissolve entirely to obtain correct results.
- Use clippers to open plastic powder pillows. Foil PermaChem pillows may be torn open.

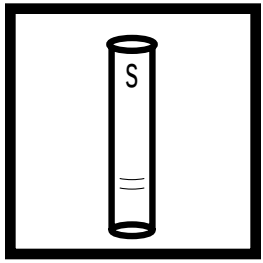
Nitrate Procedure



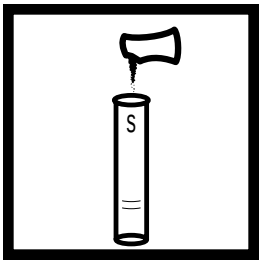
1. Fill viewing tube to the 5-mL mark with sample and label “B” for blank.



2. Place this tube in the left top opening of the color comparator.

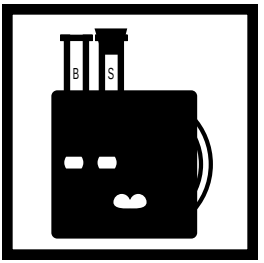


3. Fill the other viewing tube to the 5-mL mark with sample and label “S” for sample.

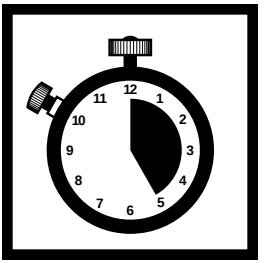


4. Add the contents of a NitraVer 5 Powder Pillow. Cap the tube and shake vigorously for 1 minute.

Note: *Use a 15 mg/L nitrate-nitrogen standard to learn proper shaking technique and use of the color comparator. Practice with the standard until repeated results are within 1 mg/L of each other. The standard should read near 15 mg/L. If the experimental concentration is less than 14 mg/L or greater than 17 mg/L, repeat the procedure until satisfactory results are obtained.*

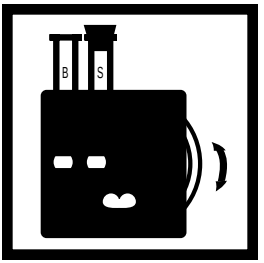


5. Place this tube in the right top opening of the color comparator.



6. Wait 5 minutes for the color to develop.

7. Hold comparator up to a light source (sky, light or window). Look through the openings in front.



8. Rotate the color disc until the color matches in the two openings.

9. Read the mg/L nitrate through the scale window. Take the average of 3 readings to determine the mg/L NO_3^-–N in the sample.

Note: *Readings before 5 minutes or after 10 minutes will result in inaccurate values.*

Required Reagents and Apparatus

Cat. No.	Description	Quantity
1732-00	Color Comparator Box	1
14038-00	Color Disc, Nitrate-Nitrogen, High Range	1
46600-04	Color Viewing Tubes with caps, plastic	4
14035-99	NitraVer 5 Nitrate Reagent Powder Pillows	100
24151-32	Nitrogen Stock Solution, 15 mg/L, 100 mL MDB	1

SECTION 5 pH MEASUREMENT

Calibrating the pH Pocket Pal Tester

1. Add a pH 7.00 Buffer Powder Pillow to 50 mL of water. Swirl the beaker to dissolve the buffer.

2. Slide the on/off switch to on.

3. Remove the protective cap from the Pocket Pal. See Figure 3.

4. Immerse the electrode tip 2.5-3.5 cm (1-1.5") into the solution and stir gently.

5. Read the pH of the solution and verify that it reads 7.0 pH units.

6. If necessary, adjust the calibration. Place a small screwdriver into the hole in the back of the Pocket Pal. See Figure 4. Then, turn the screwdriver until you obtain a pH reading of 7.0.

NOTE: Large difference in pH readings may be caused by a dry electrode or low batteries. To improve performance, dip the electrode tip into tap water at least once a week.

Replacing the Battery

1. Remove the case top from the Pocket Pal. See Figure 5.

Caution: *Do not over extend the attached wires.*

2. Replace the 4 batteries (positive terminals up) with EverReady E675E, Duracell RM675 or equivalent. See Figures 4 and 5.

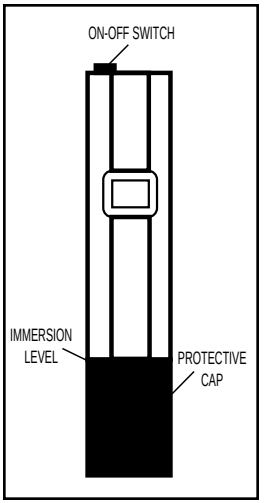


Figure 3

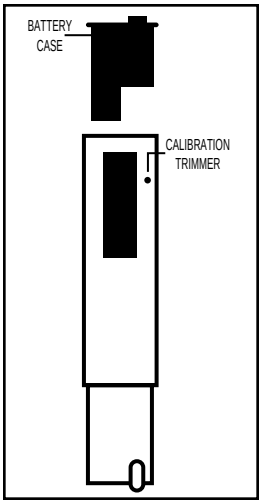


Figure 4

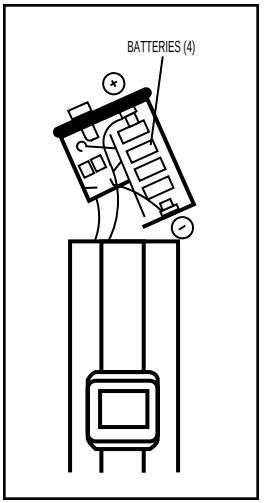
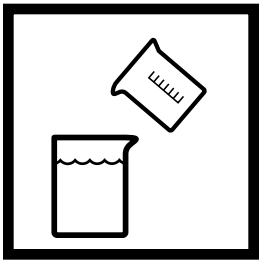
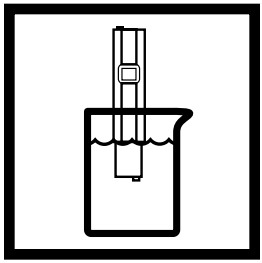


Figure 5

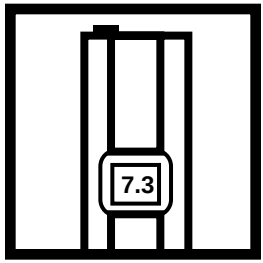
pH Procedure



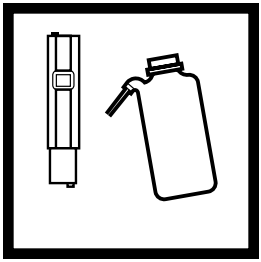
1. Add sample to a 50-mL plastic beaker.



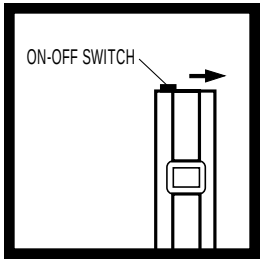
2. Immerse the electrode tip 2.5-3.5 cm (1-1.5") into the sample and stir gently.



3. Record the stable pH reading to the nearest 0.1 pH unit.



4. Rinse the electrode tip with deionized water. Wipe with a tissue before continuing to the next sample.



5. When testing is complete, rinse the electrode tip, slide the on/off switch to off, and replace the protective cap.

Note: *Place several drops of water in the protective cap to prevent the glass bulb from drying out. This will provide faster response and longer Pocket Pal life.*

Required Reagents and Apparatus

Cat. No.	Description	Quantity
23678-00	Batteries, 1.4 V, set offers 1000 hours of continuous use	4
1080-41	Beaker, polypropylene, 50 mL	1
22270-66	Buffer Powder Pillows, pH 7.00	50
44350-00	pH Pocket Pal Tester, range is 0-14 pH units	1

SECTION 6 TOTAL DISSOLVED SOLIDS (TDS) MEASUREMENT

Calibrating the TDS Pocket Pal Tester

1. Add a solution with a known TDS value to a 50-mL beaker.

NOTE: The greatest accuracy (±2%) is obtained when samples are measured at the same temperature (25 °C) as that of the standards used for calibration. When samples are measured at different temperatures, the Pocket Pal will compensate for the difference by adjusting the reading 2% °C. The accuracy of these temperature-compensated readings will be ±10% in the temperature range of 0 to 50 °C.

2. Slide the on/off switch to on.

3. Remove the protective cap from the Pocket Pal. See Figure 3.

4. Immerse the stainless steel probe 2.5-3.5 cm (1-1.5") into the solution and stir gently.

5. Read the stable display. Multiply the reading by 10 to determine the TDS value in mg/L as NaCl.

NOTE: Multiply the reading by 20 to determine the TDS value in µS/cm.

6. If necessary, adjust the calibration. Place a small screwdriver into the hole in the back of the Pocket Pal. See Figure 4. Turn the screwdriver until you obtain the known TDS value of the solution.

NOTE: Periodically clean the stainless steel probe with alcohol to maintain optimal performance.

Replacing the Battery

1. Remove the case top from the Pocket Pal. See Figure 5.

Caution: *Do not over extend the attached wires.*

2. Replace the 4 batteries (positive terminals up) with EverReady E675E, Duracell RM675 or equivalent. See Figures 4 and 5.

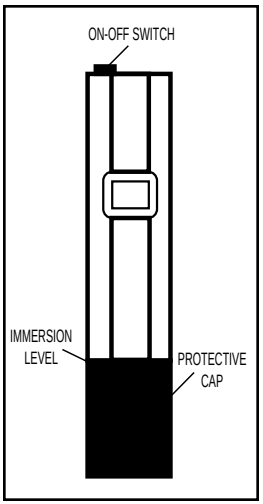


Figure 3

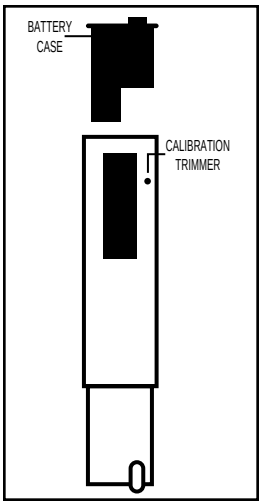


Figure 4

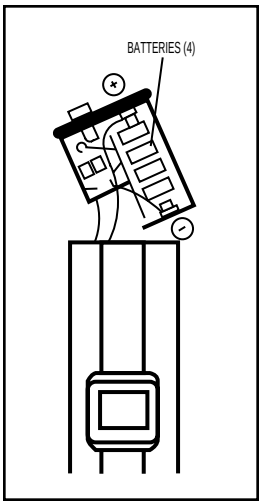
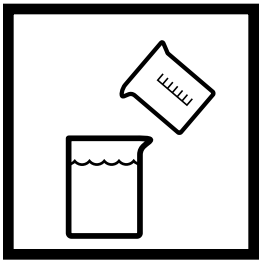
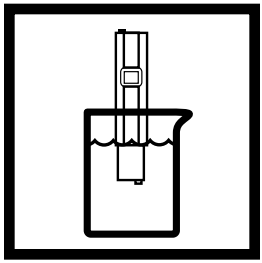


Figure 5

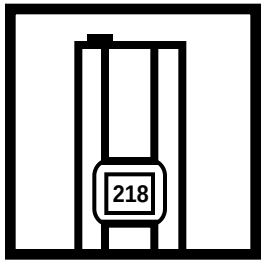
TDS Procedure



1. Add sample to a 50-mL plastic beaker.

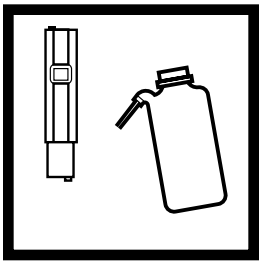


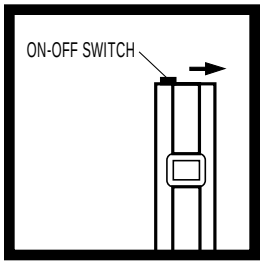
2. Immerse the stainless steel probe 2.5-3.5 cm (1-1.5") into the sample and stir gently.



3. Record the stable reading. Multiply the reading by 10 to determine the TDS value in mg/L.

Note: *Multiply the reading by 20 to determine the TDS value in µS/cm.*





4. Rinse the probe with deionized water. Wipe with a tissue before continuing to the next sample.

5. When testing is complete, rinse the probe, slide the on/off switch to off, and replace the protective cap.

Required Reagents and Apparatus

Cat. No.	Description	Quantity
23678-00	Batteries, 1.4 V, set offers 1000 hours of continuous use	4
1080-41	Beaker, polypropylene, 50 mL	1
44400-00	TDS Pocket Pal Tester, range is 10 to 1990 TDS	1
23075-42	TDS Standard, Sodium Chloride, 85.47 mg/L NaCl, 100 mL	1
14400-42	TDS Standard, Sodium Chloride, 491 mg/L NaCl, 100 mL	1