ColiQuant MF

Code 3-0035

Quantity	Contents
2	Coliscan® MF, 20 mL bottle
20	Droppers, 3 mL, sterile
20	Dishes, with pads and lids, sterile
20	Pads, white
20	Membrane filter pads, with grid, sterile
1	Filter Apparatus with syringe, sterile
1	ColiQuant MF Manual
1	ColiQuant MF Colony Color Guide

Needed but not included:

- Sterile collection container
- Sterile dilution water (if sample size is less than 10 mL)
- Sterile forceps
- Gloves

Storage Conditions

Coliscan MF should be placed in the freezer (2 – 6°C) as soon as it is received. Coliscan MF can be refrozen if it has been thawed and not been used. Coliscan MF should be thawed at room temperature. Bottles may also be thawed for same day use by standing the bottles in warm water until the contents become liquid. Coliscan MF will keep for 6 weeks in the refrigerator or 12 months in the freezer.

Safety

- Coliscan MF is non-toxic. Additional information for all LaMotte reagents is available in the United States, Canada, Puerto Rico, and the US Virgin Islands from Chem-Tel by calling 1-800-255-3924. For other areas, call 813-248-0585 collect to contact Chem-Tel's International access number.
- Read the entire manual before performing any tests.
- Wear safety glasses and protective gloves when appropriate.

- Wash hands thoroughly after performing tests. Avoid placing hands in contact with eyes or mouth. Do not eat, drink, or apply cosmetics during testing or clean up.
- Follow the general safety guidelines for your organization.
- Dispose of used dishes in the proper manner.
- Wipe tabletops and work areas after each use with disinfectant, such as a 1:10 dilution of household bleach.
- Testing by students: Ensure a safe field-testing experience by using the following: safety glasses for each student, clean pail or bucket for washing hands, jug of clean water for washing hands, soap (biodegradable if possible), towels, protective gloves, eye wash bottle, first aid kit.
- Keep used Petri dishes taped shut and out of the reach of small children and animals.
- Be sure that students understand the danger of treating reagents casually or endangering others through "horseplay".

ColiQuant MF Method

Coliscan MF (membrane filter) medium is a nutrient liquid formulation which uses two color producing chemicals, one for the detection of the enzyme glucuronidase (produced by *E. coli* strains but not by general coliforms) and one for the detection of galactosidase (produced by all coliforms, including *E. coli*). A liquid sample containing *E. coli* and other coliforms is passed through a membrane filter to leave individual bacteria cells on the membrane filter. The filter is then placed on a pad soaked with the Coliscan MF medium. When incubated at a suitable temperature, the cells will grow into colony forming units (CFU) on the surface of the filter. If coliform (including *E. coli*) colonies are present, they will produce the enzyme galactosidase, which will react with its specific color producing substrate in the medium and a water insoluble pink pigment will color the colony. If *E. coli* colonies are present, they will also produce the enzyme glucuronidase, which will react with its specific color producing substrate in the medium and a water insoluble teal-green pigment will color the colony. However, since *E. coli* produces both galactosidase and glucuronidase, those colonies will be a combination of teal-green and pink pigments and will appear as some shade of blue-purple.[3]

Collecting a Water Sample

1. Remove the cap of the collection bottle. Do not touch the neck of the bottle or the inside of the cap.
2. If you are testing stream water, face upstream while holding the bottle 5 to 6 inches below the surface.
3. When the bottle has filled, replace the cap.
4. The test can be done at the site or water samples may be collected in sterile collection containers and transported back to the testing site. Water samples kept for more than one hour before testing should be kept on ice or refrigerated.
5. Do not reuse the sample collection bottle without sterilizing.

Procedure

1. Thaw a bottle of Coliscan MF overnight at room temperature. (2 mL of Coliscan MF is required for each sample to be tested). Bottles may also be thawed for same day use by standing the bottles in warm water until the contents become liquid.

Sample Collection and Dilution

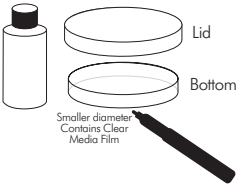
2. Collect the water sample according to the chart below.

Environmental	river, lake, pond, stream, ditch	1.0 to 5.0 mL
Drinking water	well, municipal, bottled	100 mL

Generally, use 100 mL for samples suspected of having very low coliform levels such as drinking water. Use 1.0 to 5.0 mL for samples suspected of having moderate amounts of coliform, such as environmental samples (rivers, ponds, streams, lakes or ditches). Samples that are less than 10 mL will need to be diluted with 10 – 20 mL of a sterile diluent before filtering.

For optimum results, the sample should be collected within two hours before filtering. If this is not possible, the sample may be refrigerated or kept on ice for up to 24 hours.

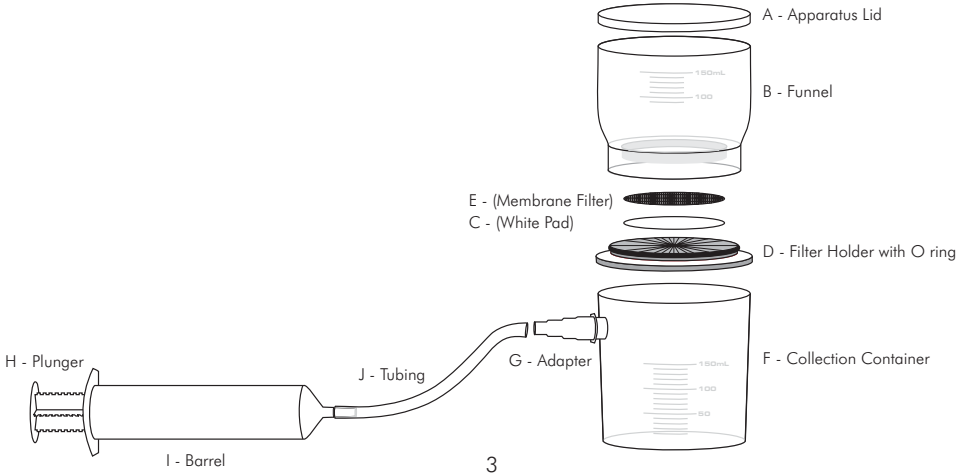
3. Use a permanent marker or wax pencil to label a dish with pad with the sample information.



4. Use the sterile 3 mL dropper to add 2mL of Coliscan MF to the pad in the dish. Cover the dish with a lid.

Set up filter apparatus

5. Remove the sterile filter apparatus from the packaging.



6. Remove the funnel and lid (A & B) from the apparatus. Use a clean forceps to place a white, sterile pad (C) on the filter holder section (D) within the circle guides.

7. Open a sterile membrane filter envelope. Use clean forceps to remove the membrane filter (E) from the pack. Carefully separate the membrane filter from the top and bottom protective backings. Do not tear or damage filter.

8. Place the membrane filter, grid side up, on top of the white pad.

9. Replace the funnel and lid on the filter holder section. Push the funnel down onto the filter holder section until it is fully and evenly seated. This will seal the connection and hold the membrane filter and white pad firmly in place.

10. Push the plunger (H) all the way into the barrel (I) of the syringe. Then attach the tubing (J) from the syringe to the adapter (G) on the side of the collection container (F).

Filter the sample

11. Swirl the water sample container to mix the contents.Remove the apparatus lid from the funnel. Pour the water sample into the funnel.

If the sample size is less than 10 mL, add 10-20 mL of sterile diluent to the funnel before attaching the syringe. Then add the sample and swirl the apparatus gently to mix the sample and the diluent. Attach the syringe.

NOTE: The diluent is used to distribute the sample more evenly over the surface of the membrane filter during filtration. The amount of diluent should not be included in the final calculation. Use only the amount of the original sample size in the calculation.

12. Pull the syringe plunger out, nearly to the end of the barrel, to create a vacuum. The water sample will be pulled through the membrane filter. Any microorganisms that are present will be deposited on the surface of the membrane filter.

NOTE: Larger water samples may not be completely filtered with a single pull of the plunger. To complete the filtration, carefully remove the tubing and syringe from the adapter on the side of the filter apparatus. Push the plunger all the way into the barrel of the syringe. Reattach the tube to the apparatus and pull out the plunger.

13. When the entire water sample has passed through the membrane filter, remove the tubing and syringe. Carefully remove the funnel from the apparatus.

14. Use clean forceps to lift the membrane filter from the filter holder section of the apparatus. Place the membrane filter, grid side up, on top of the pad in a

prepared dish. Make sure that there are no air bubbles between the filter and the pad. Cover the dish with a lid. Tape the lid to the dish bottom with clear tape.

Incubation

15. Incubate the dish in a warm place or in an incubator. If using an incubator, incubate at 35°C for 18-24 hours. If an incubator is not used, place the dish in a place that will be at constant, warm temperature. Do not place it in direct sunlight or over a direct heat source, radiator, furnace duct etc. The dish may be placed near one of these sources or in a warm spot in a kitchen. Allow 24-48 hours for growth to begin. Once growth has begun, incubate for an additional 24 hours for complete growth.

16. Once the incubation is complete, count the colonies. Colonies are normally reported as Colony Forming Units (CFU) per 100 mL of water. Refer to the ColiQuant MF Colony Color Guide.

Interpretation of Results

17. To determine the result as ***E. coli*** or **Fecal Coliform,** count the **blue** colonies. Disregard any light-blue, blue-green or white colonies. Refer to the ColiQuant MF Colony Color Guide.

To report the result in terms of *E. coli* or Fecal Coliform per mL of water: Divide the number of colonies by the original sample size

$$\text{*E. Coli* or Fecal Coliform Colonies per mL (CFU/mL)} = \frac{\text{\# Colonies}}{\text{Amount of sample used}}$$

To report the results in terms of *E. coli* or Fecal Coliform per 100 mL of water: Multiply the number of colonies by 100. Then divide by the original sample size. (Most states use this measurement to report water quality results.)

$$\text{*E. Coli* or Fecal Coliform Colonies per 100mL (CFU/100mL)} = \frac{\text{\# Colonies x 100}}{\text{Amount of sample used}}$$

18. To determine the result as **General Coliforms** count the number of **pink to red** colonies.

To report the result in terms of General Coliforms per mL of water: Divide the number of colonies by the original sample size

$$\text{General Coliform colonies per mL (CFU/mL)} = \frac{\text{\# Colonies}}{\text{Amount of sample used}}$$

To report the results in terms of General Coliforms per 100 mL of water: Multiply the number of colonies by 100. Then divide by the original sample size.

$$\text{General Coliform colonies per 100mL (CFU/100mL)} = \frac{\text{\# Colonies X 100}}{\text{Amount of sample used}}$$

19. To determine the result as **Total Coliforms** count the number of **dark purple/blue** and **pink to red** colonies.

To report the result in terms of Total Coliforms per mL of water: Divide the number of colonies by the original sample size

$$\text{Total Coliform Colonies per mL (CFU/mL)} = \frac{\text{\# Colonies}}{\text{Amount of sample used}}$$

To report the results in terms of Total Coliforms per 100 mL of water: Multiply the number of colonies by 100. Then divide by the original sample size.

$$\text{Total Coliform Colonies per 100mL (CFU/100mL)} = \frac{\text{\# Colonies x 100}}{\text{Amount of sample used}}$$

20. If there are more than 80 CFU (colonies) of one color, the result should be recorded as Too Numerous To Count (TNTC). If the *E. coli* colony count is less than 80, even though there are more than 80 total colonies of all colors and types the *E. coli* may be counted and reported. Record TNTC for coliforms or non-coliforms that are present at greater than 80 CFU.

21. Presence/Absence

The presence of at least one dark blue/purple or pink/red colony at least 0.5 mm in diameter indicates the sample is total coliform positive. The presence of at least one blue/purple colony indicates the sample is positive for *E. coli*. The presence of at least one pink/red colony indicates the sample is positive for general coliforms.

Clean up

Empty the filtered water from the collection container (F) into a sink. Clean the funnel before the next use by rinsing with alcohol. The white filter pads (C) and the funnel (B) can be sterilized by microwaving for 1-2 minutes. Dry the funnel with a clean paper towel before microwaving. Place the white filter pads on a clean paper towel to microwave. The white filter pads should be saved and reused.

Disposal

Any materials containing living or viable microbes should be disinfected or sterilized before being discarded. Before disposal in normal trash, treat the test kit components in one of the following ways:

- Pour one tablespoon of undiluted household bleach in the dish. Wait 10 minutes. Place the dish in a waterproof bag. Discard in trash.
- Place the dish in a large pan. Cover with water. Boil for 45 minutes. Discard in trash. (Warning: May cause unpleasant odors.)
- Place the dish in a sealed ovenproof bag and heat in a 300°F oven for 45 minutes. Discard in trash. (Warning: May cause unpleasant odors.)
- Place dish in a pressure cooker and cook at 15 pounds for 15 minutes. Discard in trash. (Warning: May cause unpleasant odors.)

Hints

Apparatus

The equipment supplied with this kit – bottles, dishes, droppers, pads and apparatus - is sterile. Take care that it remains sterile. Do not touch the inside of dishes, bottle necks or inside of caps, or tips of droppers with fingers.

Coliscan MF

- Coliscan MF is non-toxic before it is inoculated. It can be stored safely in a freezer with food.
- Coliscan MF can be refrozen if it has been thawed and not used.
- Coliscan MF can be stored in the freezer for up to 12 months.
- Coliscan MF will keep for 6 weeks in the refrigerator or two weeks at room temperature.
- Coliscan MF should be thawed at room temperature overnight. Bottles may also be thawed for same day use by standing the bottles in warm water until the contents become liquid.
- Coliscan MF contains dissolved solids that may settle out. Shake the bottle vigorously to suspend the solids and allow it to stand for several minutes to let air bubbles dissipate before using it.
- If Coliscan MF is contaminated and growth is evident in the bottle, do not use it. If contamination is present, growth will usually be apparent throughout the contents of the bottle, not just on the bottom, and shaking will not make solids disappear.

Interpretation of Results

- Non-fecal coliforms are widely distributed in nature, being found both as naturally occurring soil organisms, and in the intestines of warm-blooded animals and humans. Fecal coliforms, such as *E. coli*, are coliforms found naturally only in the intestines of warm-blooded animals and humans. The presence of fecal coliforms is therefore the result of some form of fecal contamination from either animal or human.
- Be aware of animals, like ducks or geese, which may be upstream from where the sample was taken because their feces will increase the *E. coli* and coliform count temporarily, therefore, the results will not reflect the true nature of the water quality.
- Proper application of the Coliscan MF filtration method will result in accurate results. Therefore, if the results indicate dangerously contaminated water, contact your local health department for help in performing an official assessment of the water quality.
- Water containing *E. coli* should not be used for drinking water. Contact your local health department for guidelines regarding *E. coli* and coliforms in recreational water.
- Most standard counts are reported as CFU/100mL of water
- Check your state water quality guidelines (see references).

Sample Dilution

- Samples with large coliform concentrations may have to be diluted to bring the number of colonies into a practical range. The target range should be between 20 and 80 colonies. Below 20 colonies the results will not be significant. Colonies greater than 80 will be too numerous to count (TNTC).
- Samples that are less than 10 mL will need to be diluted with 10-20 mL of a sterile diluent before filtering. Samples can be diluted with 1% peptone water or Butterfield Phosphate Buffer if they are available. Sterile water from a medical supply company or pharmacy or boiled, cooled tap water is also acceptable.

Background of Water Testing

In 1972, the U.S. public was concerned about the pollution in our nation's water. Congress responded by passing the Federal Water Pollution Control Act Amendments, also known as, the Clean Water Act of 1972. The goal of the Act was to protect human health by preventing water pollution. Water pollution comes in many forms and from many sources. The U.S. Environmental Protection Agency (EPA or USEPA) manages the water quality standards program, recommends water quality criteria and works with states to develop standards for different water use, such as beach swimming areas, rarely used swimming areas, offseason swimming areas, or fish and wildlife habitat.[2]

Water can be contaminated or polluted with disease causing organisms (pathogens). When people swim in these waters, they can get sick with ear or skin infections, respiratory infections, or intestinal infections. The pathogens responsible for these diseases can be bacteria (Cholera), viruses (Hepatitis A), or parasites (Giardia and

Cryptosporidium) that live in the gastrointestinal tract and are shed into the water through the waste (feces) of warm-blooded animals. However, analyzing for all possible pathogens is impractical. It is difficult, time consuming, and expensive. The EPA recommends using indicator organisms, such as fecal bacteria, as indirect evidence of the possible presence of pathogens in water.[2] The two most important fecal bacteria indicators are *E. coli* and Enterococcus. We will discuss the use of *E. coli* as it relates to the USEPA standards for water pollution.[2]

Escherichia coli (*E. coli*) is the United States Environmental Protection Agency's (USEPA) most commonly recommended indicator of fecal contamination in fresh waters. It is present in high numbers in the gastrointestinal tracts of warm-blooded animals (humans, dogs, bears, cows, ducks, geese) and therefore provides a sensitive measure of fecal pollution. Researchers have found that its presence in water is often associated with water borne illness outbreaks actually caused by the other pathogens mentioned above. *E. coli* do not live long in water so that their presence indicates a fairly recent contamination event (up to 48 hours).

The current EPA water quality standard for *E. coli* relates to approximately 8 gastrointestinal illnesses per 1,000 swimmers. This is considered an acceptable level of disease for people who come in contact with the tested water. If the indicator counts are measured above the standard acceptable level, then the risk of becoming infected from that water source is unacceptably high. In the past fecal coliforms were used as the indicator for water standards with maximum allowable counts of 200 FC(fecal coliforms) per 100 mL.[2]

Coliform bacteria are members of the family Enterobacteriaceae, which are gram negative, non-spore forming rods that ferment the sugar lactose to produce gas and acid. *E. coli* is a genus (*Escherichia*) and species (*coli*) in the total coliform group. It is also a fecal coliform (FC), a smaller subset of total coliforms. Fecal coliforms are defined as coliforms that can grow at 44.5°C, which is too hot for most of the other coliforms. Fecal coliforms live in the gastrointestinal tract of warm-blooded animals, such as dogs, cats, birds, mammals, and people. *E. coli* belongs to both groups of bacteria that are reported in water quality studies; *E. coli* is a fecal coliform (FC) and a total coliform (TC).[3]

New guidelines from the USEPA recommend *E. coli* or Fecal Coliform standards that vary with the potential human exposure to the water. For example, during summer on a lake which allows swimming and water skiing, the standards will be stricter than during the winter on that same lake or if it was a rarely used swimming area. Each state works with the EPA to determine the best standards that apply to their water bodies. Refer to the EPA document, "Bacterial Water Quality Standards for Recreational Waters (Freshwater and Marine Waters)-Status Report" at this website: **www.epa.gov/waterscience/beaches/local/statreptac.pdf** for more specific standards from your state. [2]

Normal testing for *E. coli* cannot determine the source of the contamination (e.g. animal vs. human), since it is present in all warm-blooded mammals. Drinking water is often drawn from rivers, lakes, streams, and other reservoirs open to non-point source or point source pollution. Water company scientists measure fecal coliform bacteria, in addition to dissolved oxygen, pH, temperature, and turbidity to determine water quality. High levels of fecal coliform or *E. coli* have caused closures of shellfish beds, beaches, and other recreational water sources. Point source pollution may be caused by septic systems, municipal sewage discharges, animal feed operations, farms, and boaters discharging wastes. Non-point source pollution from *E. coli* may be related to runoff of fecal waste from the land after a heavy rainfall. For example, in urban areas, *E. coli* runoff occurs from runoff of pet feces in the park or on the street into creeks, rivers, streams, lakes, or groundwater. *E. coli* levels in urban storm water

can reach as high as 100,000/100 mL. *E. coli* levels reaching over 27,000/100 mL were found in an area where gulls routinely roost in Milwaukee.

Reducing bacterial water pollution involves a whole community working together. State, Federal, and Local Water agencies, EPA, community leaders, teachers, students, neighbors, farmers, chemical companies- everybody must work together to plan a best management strategy for your community. Additional tools are available under Resources to make an action plan. Education programs show the impact of individual behaviors on public health and water quality and create a culture for change.

Definitions

CFU:	colony-forming units or colonies or cells, one CFU is larger than a period(.), can be seen without magnification, and may contain more than 10,000 individual bacterial cells, all clones of the original bacterial cell
E. coli (Escherichia coli):	those bacteria which grown as dark blue/purple colonies on the Coliscan® plate as a result of the production of both glucuronidase and galactosidase enzymes. These bacteria are of fecal origin.
Total Coliforms:	those bacteria which make up the sum of the *E. coli* (blue/purple colonies) + other coliforms. The other coliforms will appear as pink/magenta colonies because they produce galactosidase, but NOT glucuronidase. Species of the genera *Citrobacter*, *Enterobacter*, *Escherichia*, and *Klebsiella* are the main groups of coliform bacteria.
Non-Coliforms:	bacteria that form colonies that are not blue/purple or pink/magenta on Coliscan® medium and are not considered to be of fecal origin. They live naturally in soil and water.
Pathogen:	disease causing microbe
Nonpoint source pollution (NPS):	pollution that cannot be traced to a single point, such as an outlet or pipe, because it comes from many individual places or a widespread area (typically urban, rural, and agricultural runoff, acid mine drainage).
Point source pollution (PS):	pollution that can be traced to a single point source, such as a pipe or culvert (Example: industrial and wastewater treatment plant, and certain storm water discharges).
Pollution:	contaminants in the air, water, or soil that cause harm to human health or the environment.

References

Many EPA webpages are valuable resources

[1]www.epa.gov/waterscience/beaches/technical.html#tech
Beach and recreational water quality monitoring and reporting handbook; How to design and implement community water quality monitoring program

[2] www.epa.gov/waterscience/beaches/local/statreptac.pdf
Bacterial water quality standards adopted by states for marine and fresh water recreational and fish and wildlife use

www.epa.gov/owow/monitoring/volunteer
Volunteer monitoring programs: fact sheets, methods, newsletter, national directory of volunteer monitoring programs, and much more

www.epa.gov/maia/html/fecal.html
What can you do about fecal water contamination?

www.alabamawaterwatch.org
More than a decade of experience in monitoring Alabama water bodies for pollution

[3]www.micrologylabs.com/html/education_ideas.html
Microbiology for Everyone, Jonathan N. Roth – many science fair ideas, lab activities order booklet at

[4]USEPA 1997. Volunteer Stream Monitoring: A Methods Manual. EPA 841-B-97-003 pgs 180-184.

www.ccme.ca/sourcetotap/ecoli.html
Canadian Council of Ministers of the Environment *E. coli* fact sheet and what individuals can do to help avoid contaminating our water supply.

Additional Resources

Protecting Our Watershed (LaMotte product code 5-0093)
Community Action Package How to Manage water pollution, teacher guide, activity notebook, posters to help students pinpoint problems, handy tote bag

GREEN Standard Water Monitoring Kit (LaMotte product code 5848)
This kit will identify 9 different test factors, such as dissolved oxygen, pH, phosphate, turbidity, temperature that contribute to water pollution

Leaf Pack Experiments Stream Ecology Kit (LaMotte product code 5946)
Water quality kit for studying living aquatic macroinvertebrates

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