

ECA Check® MF Membrane Filter Instructions

Read entire instructions before beginning. If using your own filter apparatus, follow the procedure for setup included with that apparatus, otherwise you may follow all the instructions that follow.

Items needed (minimum):

- | | |
|--|-------------------------|
| 1 Filter Apparatus (with vacuum device) | 1 ECA Check MF bottle |
| 10 Membrane Filters | 10 50 mm dishes w/ pads |
| 10 3 mL Calibrated Droppers (or pipette, any size) | |

Preparation and setup

1. Thaw the desired number of bottle(s) of ECA Check MF by leaving at room temperature overnight. For rapid same-day thawing, stand in warm water until liquid. All unused bottles should be left in freezer. Collect the water to be tested in the appropriate volume and dilution (see table below). It is best to do this within a couple hours prior to filtering or, if this is not possible, water may be stored in refrigerator for no more than 24 hours.

Water amount to be collected

| Water Sources | Amount to collect |
|--|---|
| <u>Environmental:</u> River, lake, pond, stream, ditch | 1.0 to 5.0 mL added to sterile dilution water (10 to 90 mL) |
| <u>Drinking water:</u> Well, municipal, bottled | 100 mL |

2. Open a dropper or pipette and sterilely add 1.75-2 mL ECA Check MF to each pad in each dish that is to be used.
3. Filter apparatus setup. The filter apparatus comes in a sterile pack. Open the pack and remove the apparatus. The clear top of the apparatus is the funnel, which is calibrated for 100 and 150 mL samples and is covered with a lid. It fits on the bottom collection container and is sealed with an O-ring. There is a side port with a tip for the attachment of the vacuum syringe. Twist it and it can also be removed. It contains a plug in its tip which can be removed. The contents of the bottom collection container are most easily poured out when the tip is removed. It is easily replaced by twisting back on.
4. To prepare the apparatus for use, remove the funnel and using a clean forceps place a sterile pad on the top grid-work (in the blue circle) of the container.

5. Open a sterile filter envelope and with the clean forceps, carefully remove the membrane filter from the pack. **Be sure to separate the filter from the protective backing and handle the filter carefully so it is not torn or damaged.** Place the filter, grid side up, on top of the sterile pad. Push the funnel down so that it is held and sealed by the O-ring and the filter and pad are held firmly in place. The funnel must be pushed down as far as possible to obtain a good seal.
6. Attach the syringe to the filter apparatus by pushing the end of the hose on to the side port tip of the funnel contained. Be sure that the syringe plunger is not pulled out.

Filtering the water

7. Pour the water sample into the funnel, swirl to mix and create a vacuum by pulling out the plunger of the syringe. The water will be pulled through the filter, depositing any microorganisms present onto the filter surface.
8. When the water sample has been completely passed through the filter, disconnect the syringe, remove the funnel and with the clean forceps remove the filter and place grid side up directly on top of the pad of a dish prepared earlier. Make sure that there are no air spaces (bubbles) between the pad and the membrane filter. Place the lid back on the dish.
9. The filtered water in the collection container should be emptied and the filter apparatus prepared for repeat use. Before the funnel is used again it should be cleaned. This may be done by rinsing with alcohol or radiating for 1 minute with germicidal UV. The absorbent pad can generally be reused as it will only contain filtered water (sterile).

Incubation and interpretation

10. Incubate in an incubator or a warm place. If using an incubator, incubate at 35° for 18- 24 hours. If an incubator is not available, find a place that will be warm for a 24 hour period. **DO NOT** place in direct sunlight or over a direct heat source, radiator, furnace duct etc. You may place them near one of these sources or in a warm spot in the kitchen. Allow 24-48 hours for growth to begin. Once growth begins you can incubate another 24 hours for complete growth to take place.
11. Once the incubation period is complete, a count of the colonies can be done. Count all **dark blue colonies** as ***E. coli* (fecal coliform)**, **blue-gray colonies** as **general coliforms**, **pink colonies** as ***Aeromonas spp.*** and **green colonies** as ***Salmonella spp.*** The sum of the dark blue and blue-gray is the **total coliform** population.

If you have any questions, call 1.888.327.9435.

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