m-FC Broth with Rosolic Acid, 2 mL (6530)

Intended Use

Ampouled m-FC Broth with Rosolic Acid, 2 mL is used for enumerating fecal coliforms in water by the membrane filtration method.

Product Summary and Explanation

Ampouled m-FC Broth with Rosolic Acid, 2 mL is a prepared, ready to use medium for membrane filtration testing. Geldreich et al. formulated a medium to enumerate fecal coliforms (FC) using the membrane filter (m) technique without prior enrichment.[1] Fecal coliforms, i.e., those found in feces of warm-blooded animals, are differentiated from environmental coliforms by their ability to grow at 44.5 ± 0.5°C.[2]

Many standard method membrane filtration procedures recommend m-FC media for testing water. The American Public Health Association (APHA) specified m-FC media and incubation at 44.5 ± 0.5°C in several procedures.[2,3] The US Environmental Protection Agency specified using m-FC media in fecal coliform methods for testing water by the direct MF method or the delayed-incubation MF methods.[4,5]

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen, carbon, and minerals in m-FC Broth with Rosolic Acid. Yeast Extract is a source of vitamins and trace elements. Sodium Chloride maintains the osmotic balance. Lactose serves as a carbohydrate source. Bile Salts inhibit growth of Gram-positive bacteria. The differential indicator system combines Aniline Blue and Rosolic Acid, which is included in the ampouled broth formula.

Medium Composition: Per Liter

Enzymatic Digest of Casein	10.0 g
Enzymatic Digest of Animal Tissue	5.0 g
Yeast Extract	3.0 g
Sodium Chloride	5.0 g
Lactose	12.5 g
Bile Salts	1.5 g
Aniline Blue	0.1 g
Rosolic Acid	0.1 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Physical Characteristics

Appearance of medium:	Clear to slightly hazy, purple to dark purple
pH at 25ºC:	7.4 ± 0.2

Test Procedure

Preparation

1. Assemble the manifold or filtration flask that will supply the vacuum source, complete with rubber stopper.
2. Using a gentle twisting motion, secure the funnel adapter into the rubber stopper.
3. Using the same gentle twisting motion, secure the Neogen Filter onto the funnel adapter.

Filtration Procedure

1. Remove filtration cover and carefully pour the sample onto the filter.
2. Apply vacuum just long enough to pull the sample through the filter (if using a manifold, open only one valve at a time.)

3. Rinse the inside walls of the filter funnel with approximately 20 mL of sterile buffered solution. Apply vacuum just long enough to pull the solution through the filter, and turn off vacuum. Note: This step is optional if only water is being tested.
4. Briefly remove the filter and its funnel adapter from the rubber stopper to release any remaining vacuum pressure, and then re-secure into the stopper.
5. Add the m-FC Broth with Rosolic Acid onto the top of the filter. When doing so, be careful not to touch the filter with the tip of the ampoule.
6. Very briefly apply vacuum so that the media does not pool on top of the filter, and is visible underneath the filter. (Note: The media has been soaked correctly into the filter if there is a small pocket of air around the bottom port. The filter should be moist, but not oversaturated or dry.)
7. Remove and appropriately discard the plastic funnel. Place the filtration system cover over the filter/base assembly converting the unit to a Petri dish for sample incubation.
8. Remove the filter from the funnel adapter, and place a plug on the open bottom port.
9. Place the filtration plate into the incubator inverted so that the cover is on the bottom, and incubate at 44.5± 0.5 ºC. Read and record results after 22 – 24 hours.
10. Dispose of test materials in accordance with all applicable local, state, and federal regulations.

Expected Cultural Response:
Sterile water was added to sterile filtration units and inoculated with the cultures listed below. The inoculum was filtered followed by the ampoulized m-FC Broth with Rosolic Acid and the filtration housing removed. Plates were incubated aerobically at 44.5ºC and examined for growth at 22 – 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results
Uninoculated Media	NA	No Growth
Escherichia coli ATCC 25922	10 - 300	≥ 85% recovery, dark blue colonies
Escherichia coli ATCC 8739	10 - 300	≥ 85% recovery, dark blue colonies
Escherichia coli ATCC 11775	10 - 300	≥ 85% recovery, dark blue colonies
Pseudomonas aeruginosa ATCC 27853	300 - 10000	Suppressed to Inhibited
Proteus vulgarus ATCC 13315	300 - 10000	Suppressed to Inhibited

Results[4,5]
Examine filters for the presence of dark blue colonies. All blue colonies are counted as presumptive fecal coliforms. Report the fecal coliform density in terms of total coliforms/100 mL. Other organisms and/or non-fecal coliforms may form reddish-gray, gray or cream-colored colonies.

Storage
Store Ampouled m-FC Broth with Rosolic Acid, 2 mL at 2 - 8 ºC.

Expiration
Refer to expiration date printed on the front of the box container.

Limitations of the Procedure
1. Analyze sample as soon as possible after collection.
2. Samples containing colloidal or suspended particulate material can clog the membrane filter, thereby prevent filtration, or cause spreading of bacterial colonies which could interfere with colony identification.
3. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

m-FC Broth with Rosolic Acid, 2 mL	**Code No. 6530**	**Box of 50**
Neogen Filter "White"	**Code No. 6550**	**Box of 50**
Neogen Filter "Black"	**Code No. 6555**	**Box of 50**

References

1. Geldreich, E. E., H. F. Clark, C. B. Huff, and L. C. Best. 1965. Fecal-coliform-organism medium for the membrane filter technique. J. Am. Water Works Assoc. 57:208-214.
2. Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.). 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
3. Cowman, S., and R. Kelsey. 1992. Bottled water, p. 1031-1036. *In* C. Vanderzant, and D. F. Splittstoesser (eds.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. Bordner, R., and J. Winter (eds.). 1978. Microbiological methods for monitoring the environment. EPA-600/8-78-017. Environmental Monitoring and Support Laboratory, Office of Research and Development, U. S. Environmental Protection Agency, Cincinnati, OH.
5. Environmental Protection Agency. 1992. Manual for the certification of laboratories analyzing drinking water. EPA-814B-92-002. Office of Ground Water and Technical Support Division, U. S. Environmental Protection Agency, Cincinnati, OH

Technical Information

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