m-Enterococcus Agar (NCM0163)

Intended Use
m-Enterococcus Agar is used for the selective isolation and enumeration of enterococci by membrane filtration in a laboratory setting. m-Enterococcus Agar is not intended for use in the diagnosis of disease or other conditions in humans.

Description
m-Enterococcus Agar was first described by Slanetz et al. for the enumeration of enterococci by the membrane filtration technique. In 1957, Slanetz and Bartley modified this medium by adding triphenyl tetrazolium chloride (TTC). Increased recovery and larger colonies were obtained by incubating the inoculated membranes on the agar surface instead of on pads saturated with liquid medium. The membrane filtration method is simple to perform, does not require confirmation, and permits a direct count of enterococci in 48 hours. m-Enterococcus Agar is also referred to as m-Azide Agar.

The presence of enterococci is a valuable bacterial indicator for determining the extend of fecal contamination of recreational surface waters. m-Enterococcus Agar is recommended for the detection of fecal streptococci using the membrane filtration technique for water testing. The food industry also has applications for testing enterococci using m-Enterococcus Agar.

Typical Formulation

Enzymatic Digest of Casein	15.0 g/L
Enzymatic Digest of Soybean Meal	5.0 g/L
Yeast Extract	5.0 g/L
Dextrose	2.0 g/L
Dipotassium Phosphate	4.0 g/L
Sodium Azide	0.4 g/L
2,3,5-Triphenyl Tetrazolium Chloride	0.1 g/L
Agar	10.0 g/L

Final pH: 7.2 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution
Refer to SDS

Preparation
1. Suspend 42 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. DO NOT AUTOCLAVE.
4. Cool to 45 - 50°C.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous, free-flowing, and light beige.

Prepared Appearance: Prepared medium is light to medium pink-beige, and trace to slightly hazy.

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Expected Cultural Response: Cultural response on m-Enterococcus Agar incubated aerobically at 35 ± 0.5°C and examined for growth at 24 - 48 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reaction
Enterococcus faecalis ATCC® 19433	10 - 100	Growth	Dark red to maroon colonies
Enterococcus faecalis ATCC® 29212	10 - 100	Growth	Dark red to maroon colonies
Enterococcus faecalis ATCC® 33186	10 - 100	Growth	Dark red to maroon colonies
Escherichia coli ATCC® 25922	10^3	Completely Inhibited	---
Staphylococcus aureus ATCC® 25923	10^3	Completely Inhibited	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedures

Membrane filtration procedure

1. Follow the membrane filtration procedure as described in appropriate references or by laboratory policy.
2. Choose a sample size resulting in the isolation of 20 - 60 colonies.
3. Transfer the filter to agar medium in a petri dish, avoiding air bubbles beneath the membrane.
4. Let plates stand for 30 minutes. Invert plates and incubate at 35 ± 0.5°C for 48 hours.

Direct plating procedure

1. If required, samples should be homogenized and diluted with saline to result in the isolation of 15 - 150 colonies.
2. Inoculate medium by spreading the sample evenly over the agar surface.
3. Incubate plates at 35 ± 2°C for 24 - 48 hours.

Results

Count all light and dark red colonies as enterococci. Count colonies using a fluorescent lamp and a magnifying lens.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container.

Limitation of the Procedure

Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow.

Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. Slanetz, Bent, and Bartley. 1955. Public Health Rep. 70:67.
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5. ISO Standard for Water Quality. Detection and enumeration of intestinal enterococci. Part 2. membrane filtration method.
6. Burkwell, M.K. and P. A. Hartman. 1964. Appl. Microbiol. 12:18-23.
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