

# PRODUCT INFORMATION AND QUALITY CONTROL SHEET

## m-ENTEROCOCCUS AGAR

### I. INTENDED USE

**m-Enterococcus Agar** is used for the selective isolation and enumeration of enterococci by membrane filtration.

### II. SUMMARY AND EXPLANATION

m-Enterococcus Agar was first described by Slanetz et al. for the enumeration of enterococci by the membrane filtration technique.<sup>1</sup> In 1957, Slanetz and Bartley modified this medium by adding triphenyltetrazolium chloride (TTC).<sup>2</sup> 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 enterococcus group are fecal streptococci and include *E. faecalis*, *E. faecium*, *E. gallinarum*, and *E. avium*.<sup>3</sup> Enterococci are differentiated from other streptococci by their ability to grow in 6.5% Sodium Chloride, at pH 9.6, and at 10°C and 45°C.<sup>3</sup> The presence of enterococci is a valuable bacterial indicator for determining the extent of fecal contamination of recreational surface waters.<sup>3</sup> m-Enterococcus Agar is used in standard methods for the detection of fecal streptococci using the membrane filtration technique.<sup>3</sup>

### III. PRINCIPLES OF THE PROCEDURE

Enzymatic Digest of Casein and Enzymatic Digest of Soybean Meal provide the nitrogen, minerals, and amino acids in m-Enterococcus Agar. Yeast Extract is the vitamin source and Dextrose supplies carbon. Dipotassium Phosphate acts as a buffer. Sodium Azide is the selective agent used to suppress the growth of gram-negative organisms. Agar is the solidifying agent. Triphenyl Tetrazolium Chloride (TTC) is the dye used as an indicator of bacterial growth. TTC is reduced to insoluble formazan inside the bacterial cell, resulting in the production of red colonies.

### IV. TYPICAL FORMULA AND APPEARANCE

Appearance = light to medium pink-beige, and clear to slightly hazy.

(Approximate formula\* per liter of processed water)

Enzymatic Digest of Casein .....	15 g
Enzymatic Digest of Soybean Meal .....	5 g
Yeast Extract.....	5 g
Dextrose.....	2 g
Dipotassium Phosphate.....	4 g
Sodium Azide.....	0.4 g
2,3,5-Triphenyl Tetrazolium Chloride.....	0.1 g
Agar .....	10 g

Final pH: 7.2 ± 0.2 at 25°C

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

### V. PRECAUTIONS

This product is for IN VITRO diagnostic use only. Culture specimens may contain microorganisms which can be potentially infectious to the user. Strict adherence to aseptic techniques and established precautions against microbiological hazards should be followed throughout the procedure. Carefully dispose of all items which contact patient specimens or isolated bacteria.

### VI. STORAGE/SHELF LIFE

Plated media should be stored at 2-8°C (36-46°F), media side up, in the unopened or resealed package protected from light. DO NOT FREEZE OR EXPOSE TO HIGH TEMPERATURES. Allow unopened plates to warm to room temperature prior to inoculation. Prior to and during inoculation procedures, plates should be handled in a manner

that minimizes product exposure to the environment. Product which has exceeded the assigned expiration date noted on the label should not be used. Do not use plates that exhibit evidence of drying, cracking, discoloration, microbial contamination or any other signs of deterioration. The presence of excessive condensate may indicate plates which have been damaged by exposure to temperature extremes.

### VII. SPECIMEN COLLECTION

The quality of culture results depends primarily on the adequacy and condition of the specimen submitted for examination. Proper specimen collection techniques must be followed to ensure the most accurate culture results.

### VIII. MATERIALS PROVIDED

Cat No. 1079 - m-Enterococcus Agar Plates (10/pkg)  
Cat No. 1079DD – m-Enterococcus Agar Plates (10/pkg)  
Cat No. 1105 – m-Enterococcus Agar 60mm Plates (10/pkg)

### IX. MATERIALS REQUIRED BUT NOT PROVIDED

Incubator maintaining 33-37°C.  
Ancillary culture media, reagents and laboratory equipment as required.

### X. PROCEDURE

#### Membrane filtration procedure

1. Follow the membrane filtration procedure as described in standard methods or by laboratory policy.<sup>3</sup>
2. Choose a sample size resulting in 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.
5. Invert plates and incubate at 35 ± 0.5°C for 48 hours.

#### Direct plating procedure

1. Inoculate medium with a specimen using the steak plate method.
2. Incubate plates at 35 ± 2°C for 24 - 48 hours.

### XI. EXPECTED RESULTS

Cultural response on m-Enterococcus Agar at 35°C after 18 – 48 hours incubation.

Microorganism	Response	Reactions
Enterococcus faecalis ATCC 19433	growth	dark red colonies
Enterococcus faecalis ATCC 29212	growth	dark red colonies
Escherichia coli ATCC 25922	Inhibited	---
Staphylococcus aureus ATCC 25923	Inhibited	---

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

### XII. LABORATORY RESULTS

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

### XIII. LIMITATIONS

The ability to detect microorganisms by culture techniques can be affected by the following factors: improper specimen collection, storage and inoculation, improper culture incubation temperatures and atmospheres, improper length of culture incubation, and improper storage and handling of culture media.

### XIV. REFERENCES

1. **Slanetz, Bent, and Bartley.** 1955. Public Health Rep. **70**:67.
2. **Slanetz, and Bartley.** 1957. J. Bacteriol. **74**:591.
3. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19<sup>th</sup> ed. American Public Health Association, Washington, D.C.

## **USER QUALITY ASSURANCE/ QUALITY CONTROL PROCEDURES AND INFORMATION**

HealthLink recommends that the following quality assurance and quality control procedures be performed on each batch of product.

### **I. QUALITY ASSURANCE**

The following quality assurance procedures must be performed to assure the product will perform according to its intended use within the assigned expiry date:

1. Daily, document that product storage refrigerator maintains temperature within 2-8<sup>o</sup>C.
2. Daily, document that laboratory incubator maintains temperature within the recommended range: 35 ± 2<sup>o</sup>C.

### **II. QUALITY CONTROL**

The following incoming inspection procedures must be performed for each batch (batch = same lot, same shipment) of culture media received in the laboratory:

Inspect plates according to instructions contained in the Section VI "STORAGE/SHELF LIFE"

Note: Notify Technical Service immediately if media does not meet the inspection criteria.

### **TECHNICAL SERVICE**

HealthLink provides a toll free technical service line (1-800-638-2625) to assist with product usage. To have technical questions answered; please call between the hours of 9:00 am to 5:00 pm EST.

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**1-800-638-2625**

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