

## TM 2177 - M-AZIDE BROTH BASE

### INTENDED USE

For cultivation and enumeration of Enterococci from water samples using membrane filter technique.

### PRODUCT SUMMARY AND EXPLANATION

Enterococci may be considered an essential part of the autochthonous microflora of humans and animals. Because of its wide distribution, Enterococci can also occur in different food commodities, especially those of animal origin. The most important feature of this genus is their high level of endemic antibiotic resistance. In bodies of water, the acceptable level of contamination is very low. In 2004, *Enterococcus* species took the place of fecal coliform as the new federal standard for water quality at public beaches. It is believed to provide a higher correlation than fecal coliforms with many of the human pathogens often found in sewage.

M-Azide Broth was formulated by Slanetz, Bent and Bartely and is especially recommended for the enumeration of Enterococci from water samples and other specimens using membrane filter technique. In this technique, a measured volume of the water sample is filtered through a membrane with a pore size small enough to retain the indicator bacteria to be counted. The membrane is then aseptically placed and incubated on a selective indicator medium (or sterile absorbent cotton pads saturated with the selective medium), so that the indicator bacteria grow into colonies on its upper surface.

For membrane filter technique, 2.2 ml medium is added per absorbent pad. Using this medium, Slanetz et al observed better recovery of pure cultures of *Enterococcus faecalis* by membrane filter technique than MPN technique.

### COMPOSITION

Ingredients	Gms / Ltr
Tryptose	40.000
Yeast extract	10.000
Dextrose	2.000
Saccharose	100.000
Dipotassium phosphate	4.000
Sodium azide	0.400

### PRINCIPLE

Tryptose, yeast extract provide essential growth nutrients. Dextrose and saccharose are the fermentable carbohydrates. Sodium azide is used as selective agent, which inhibits gram-negative bacteria. Mallmann et al reported that sodium azide exerts bacteriostatic effect on gram-negative bacteria allowing unrestricted growth of gram-positive cocci, particularly Enterococci. TTC imparts pink to red colour to the colonies.

### INSTRUCTION FOR USE

- Dissolve 15.64 grams in 100 ml distilled water.
- Heat if necessary to dissolve the medium completely.
- Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
- Cool to 50°C and aseptically add 1 ml of 1% 2, 3, 5 Triphenyl Tetrazolium Chloride.
- Mix well before dispensing.

Caution: Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

### QUALITY CONTROL SPECIFICATIONS



**Appearance of Powder** : Cream to yellow homogeneous free flowing powder  
**Appearance of prepared medium** : Light yellow coloured clear solution without any precipitate  
**pH (at 25°C)** : 7.1±0.2

### INTERPRETATION

Cultural characteristics observed after an incubation with added 1% 2, 3, 5 Triphenyl Tetrazolium Chloride.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Colour of colony (on membrane filter)	Incubation Temperature	Incubation Period
<i>Escherichia coli</i>	25922	$\geq 10^3$	Inhibited	0%	-	35-37°C	48 Hours
<i>Enterococcus faecalis</i>	29212	50-100	Good-luxuriant	$\geq 50\%$	pink to red	35-37°C	48 Hours

### PACKAGING:

In pack size of 500 gm bottles.

### STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

**Product Deterioration:** Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.













### DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

### REFERENCES

1. Belzer, R., Inaug. Dissert, Univ. München, 1983.
2. Burkwall, M. K., Hartman, P. A., Appl. Microbiol., 12; 18-23 (1964).
3. Jin G., Jeng H., Bradford H., Englande A., 2004, Water Environ Res 76 (3): 245-55.
4. Slanetz L. W., Bent D. F. and Bartley C. H., 1955, Pub. Hlth. Rep.70: 67.
5. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds) Mackie and McCartney, Practical Medical Microbiology, 1996, 14th edition, Churchill Livingstone.
6. Mallmann W. L., Botwright W. E. and Churchill E. S., 1941, J. Inf. Dis., 69:215.
7. MacFaddin J. F., 1985, Media for Isolation-Identification-Cultivation-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.



 GMP Good Manufacturing Practices Certified	 IVD For In Vitro Diagnostic Use	 QTY. Quantity	 LOT/ B. NO. Lot / Batch Number	 REF Catalogue Number	 Manufacturer
 Temperature Unit	 EC REP Authorized Representative <small>MedNet GmbH Buckstrasse 10, 49163 Muenster, Germany</small>	 European Conformity	 QR Code	 Consults Instructions for Use	 Best Before

**NOTE:** Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

**\*For Lab Use Only**  
**Revision: 08 Nov., 2019**