Enterococcus Agar Base

M2077

Intended use

For selective isolation and differentiation of *Enterococcus faecalis* and *Enterococcus faecium*

Composition**

Ingredients	Gms / Litre
BHI powder	8.500
Peptone	10.000
HM Peptone B#	8.500
Dipotassium hydrogen phosphate	2.500
Sodium azide	0.250
Dextrose (Glucose)	10.000
Bromo thymol blue	0.020
Agar	15.000
Final pH (at 25°C)	7.20±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 54.77 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add rehydrated contents of one vial of TTC solution 1% (FD057). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Enterococci were formerly classified as faecal streptococci. Enterococci serves as an indicator organism in monitoring food samples as it is cause of faecal contamination. Of the various species of Enterococci, *E.faecalis* and *E.faecium* are frequently found in humans. The presence of Enterococci in food samples has been studied. (2,6). A variety of selective media have been recommended for the isolation of *Enterococcus* species (3). This medium is designed for the selective isolation and differentiation between *Enterococcus faecalis* and *Enterococcus faecium.*

The differentiation is based depending upon the reduction of tetrazolium. *Enterococcus faecalis* produces colonies with a deep red center and a narrow white periphery with yellow background, whereas Enterococcus faecium produces white or pale pink coloured colonies.

Proteose peptone, BHI powder and HM Peptone B serves as a source of nitrogen and vitamins. Dextrose (Glucose) serves as a source of carbohydrate and bromothymol blue is the pH indicator. Sodium azide helps in inhibition of contaminating flora.

Type of specimen

Food samples

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (2,6).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Due to variable nutritional requirements, some strains show poor growth on this medium.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light yellow to pale green coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.45% w/v aqueous solution at 25°C. pH : 7.20±0.1

pH

7.10 - 7.30

Cultural Response

Cultural characteristics observed with added TTC Solution 1% (FD057) after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Enterococcus faecalis ATCC 29212 (00087*)	50-100	good-luxuriant	>=50%	red or maroon
Enterococcus faecium ATCC 19434 (00010*)	50-100	good-luxuriant	>=50%	Colourless to pale pink
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	

Key : (*) Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. Barnes,E.M.(1956)Methods for the isolation of faecal streptococci(Lancefield group D)from bacon factories. J. Appl. Bacteriol.19,193-203.
2. Devriese, L.A., Pot, B., Van Damme, L., Kersters, K and Haesebrouk, F. (1995) Identification of *Enterococcus* species isolated from food of animal origin. Int. J. Food Microbiol. 26, 187-197.
3.Domig, K.J., Mayer, H.K. and Kneifel, W (2003a) Methods used for isolation, enumeration, characterization and identification of *Enterococcus* species.1. Media for isolation and enumeration. Int.J.Food Microbiol.88 147-164.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. Knudtson, L.M. and Hartman, P.A. (1993) Enterococci in pork processing. J.Food Prot. 56, 6-9.