Bile Esculin Azide Medium

MF004/E*/F▽

Intended Use

Recommended for isolation and presumptive identification of faecal Streptococci.

Directions

The test sample should be filtered through a sterile membrane filter having pore size of 0.22µ / 0.45µ. Rehydrate the nutrient pad with 2.0-2.5 ml sterile distilled / purified water. After filtration, remove the membrane filter aseptically using sterile forceps. Place the membrane filter on rehydrated nutrient pad. Incubate the inoculated nutrient. Interpret the results qualitatively by observing the presence or absence of growth and quantitatively by counting the number of colonies on the surface of the membrane filter and calculating CFU/ml.

Principle And Interpretation

This is ready to use sterile culture media in the form of a 50 mm biological inert absorbent pads impregnated with Bile Esculin Azide Medium, then dried and sterilized in 55 mm petri plate. They eliminate the need of laborious media preparation and autoclaving procedures. The nutrient pads are to be just rewetted with sterile distilled water and are ready to use. Use of nutrient pads allows larger sample volumes to be tested at a time. Interpretation of results is directly by counting the CFUs and also quantifies the microbial load present in the sample. Group D Streptococci possess the group D lipoteichoic acid antigen in their cell walls. Former Group D species, which are predominant normal inhabitants of the human gastrointestinal tract, are termed as faecal Streptococci or Enterococci (1). The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld (2). Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate (3). The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix (4). Bile Esculin Agar was originally formulated by Swan (6) for the isolation and identification of Group D Streptococci from food. Facklam and Moody (7, 8) further reported that using Bile Esculin Agar, Group D Streptococci could be differentiated from non Group D Streptococci. Bile Esculin Agar was also shown to aid differentiation of Enterobacteriaceae, *Klebsiella, Enterobacter, Serratia* from other *Enterobacteriaceae* genera (9) on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci (5). Bile Esculin Azide Agar is a modification of Bile Esculin Agar (6, 8) as per Isenberg (10). In this medium the bile concentration is reduced and additional sodium azide is incorporated.

Peptones serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Oxgall and sodium azide inhibits most of the other accompyning bacteria. Esculin in the medium is hydrolyzed to esculetin and dextrose. Esculetin reacts with ferric citrate to form a dark brown or black complex, visualized as a zone of black precipitate around the colonies. If the media is dispensed in tubes in the form of slants, a positive reaction is indicated by blackening of more than half of the slant within 24-48 hours. If blackening is totally absent or if less than half of the slant is blackened within 24-48 hours, the test is negative. Viridans Streptococci sometimes exhibit a weak positive reaction.

Suspected water samples are filtered using membrane filters. These membrane filters are aseptically placed on Slanetz and Bartely Medium (M612I). Red or maroon coloured colonies observed after incubation are further confirmed by aseptically transferring the membrane filter on to Bile Esculin Azide Agar plate preheated to 44°C. Incubation at 44 ± 0.5°C for 2 hours is done following the inoculation. All typical colonies exhibiting a brown black colouration in the surrounding medium are counted as intestinal Enterococci (11). Alternatively Bile Esculin Azide Agar can also be used for direct isolation of Enterococci (without membrane filter), by incubation at 35-37°C for 18-24 hours.

Type of specimen

Food and dairy samples; Pharmaceutical samples.

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,7).
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 clinical specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

1. Since it is a general purpose medium, bacterial cultures will also grow .
2. Further isolation and biochemical tests should be carried out for confirmation.

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
Dry filter membrane pad of 50mm diameter

Colour
Pale coloured nutrient pad

Sterility test
Passes release criteria

Cultural response
Cultural characteristics observed after incubation at 35-37ºC for 18-48 hrs

Organism	Growth	Colour of colony
Enterococcus faecalis ATCC 29212 (00087*)	luxuriant	Positive (Blackening of nutrient membrane)
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	good	negative (No change)
Proteus mirabilis ATCC 25933	good	negative (No change)
Streptococcus pyogenes ATCC 19615	none-poor	negative (No change)

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

On receipt store between 10-30°C. Use before expiry date on the label.
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 (3,4).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.

2. Carlier G. I. M., 1984, Brit. J. Derm. Syph., 60:61

3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

4. 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.

5. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.

6. Sabouraud R., 1892, Ann. Dermatol. Syphil. 3 : 1061.

7. Sabouraud R., Les Teignes, Paris: Masson et Cie, 1910, p 553

8. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

Revision : 01 / 2019

Note:
MF000E* - Sterile pad packed individually in sterile disposable plastic bag without Membrane Filter
MF000F▽ - Sterile pad packed individually in sterile Petri plate with sterile Membrane Filter (0.45 mm).