Ready Prepared Media

Technical Information

Bile Esculin Agar Plate

Product Code: PM 1972

Application: Recommended for isolation and presumptive identification of group D Streptococci from food and pharmaceutical products.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
HM peptone B #	3.000
Bile □	40.000
Esculin	1.000
Ferric citrate	0.500
Agar	15.000
Final pH (at 25°C)	6.6±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef extract
□ Equivalent to Oxgall

Principle & Interpretation

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 (8). The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld (10). Enterococci and Group D Streptococci hydrolyze esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate (9). The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix (12). Bile Esculin Agar was originally formulated by Swan (4) for the isolation and identification of Group D Streptococci from food. Facklam and Moody (2,5) 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 (11) on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci (3).

The medium is highly nutritious. Peptone and HM peptone B serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Bile inhibits most of the other accompanying 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. Viridians Streptococci sometimes exhibit a weak positive reaction. Also, *Leuconostoc, Pediococcus, Lactococcus* species causing human infections give a positive bile esculin test (6). Inoculate and incubate the test sample in Todd Hewitt Broth (M313). After 24 hours incubation add two drops of the culture onto the surface of slant or plate media (3, 9).

Type of specimen

Food samples

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,13,14).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the pack. 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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. It is recommended to store the plates ta 24-30°C to avoid minimum condensation.

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.

Methodology

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

Quality Control

Appearance
Sterile Bile Esculin Agar in 90 mm disposable plates.
Colour of medium
Amber coloured medium
Quantity of medium
25 ml of medium in 90 mm disposable plates.
Reaction
6.40-6.80
Sterility Test
Passes release criteria
Cultural Response
Cultural characteristics observed in an increased atmosphere of Carbon dioxide after an incubation at 35-37°C for 18-24 hours.

Oragnism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=50%	positive reaction,blackning of medium around the colony
Proteus mirabilis ATCC 25933	50-100	luxuriant	>=50%	Negitive reaction
Streptococcus pyogenes ATCC 19615	50-100	non-poor	<=10%	Negitive reaction

Key : * - Corresponding WDCM numbers

Storage and Shelf Life

On receipt store between 20-30°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 (6,7).

Further Reading

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6. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
7. 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.
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12. Rochaix, 1924, Comt. Rend. Soc. Biol., 90:771.
13. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
14. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

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Disclaimer

- User must ensure suitability of the product(s) in their application prior to use.
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