Ready Prepared Media

Technical Information

Bile Esculin Azide Agar Plate

Product Code: PM 1493

Application : Bile Esculin Azide Agar Plate is a selective medium used for isolation and presumptive identification of faecal Streptococci.

Composition**

Ingredients	Gms / Litre
Tryptone	17.000
HM peptone B #	5.000
Proteose peptone	3.000
Bile$	10.000
Esculin	1.000
Ferric ammonium citrate	0.500
Sodium chloride	5.000
Sodium azide	0.150
Agar	15.000
Final pH (at 25°C)	7.1±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(10). The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld (12). Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate (11). The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix (14).

Bile Esculin Agar was originally formulated by Swan (16) for the isolation and identification of Group D Streptococci from food. Facklam and Moody (4,6) 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 (3) on the basis ofesculin 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 (6,16) as per Isenberg (7). In this medium the bile concentration is reduced and additional sodium azide is incorporated.

Tryptone, proteose peptone and HM peptone B serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Bile and sodium azide 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.Also, *Leuconostoc, Pediococcus, Lactococcus* species causing human infections give a positive bile esculin test (13). Suspected water samples are filtered using membrane filters. These membrane filters are aseptically placed on Slanetz and Bartely Medium (DM1612I). 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 (13).Alternatively Bile Esculin Azide Agar can also be used for direct isolation of Enterococci (without membranefilter), by incubation at 35-37°C for 18-24 hours.

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Type of specimen

Food and dairy samples; Water samples

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,15,17). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(2) 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. Viridians Streptococci sometimes exhibit a weak positive reaction.Proper anaerobic conditions must be maintained for optimal recovery of organisms.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium .
3. 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.
4. Further biochemical tests must be carried out for confirmation.
5. 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 Azide Agar in 90 mm disposable plates.
Colour of medium
Amber coloured coloured medium
Quantity of medium
25 ml of medium in 90 mm disposable plates.
pH
6.90-7.30
Sterility Test
Passes release criteria
Cultural Response
Cultural characteristics observed 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
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	

Staphylococcus aureus ATCC 25923 (00034*)	50-100	good	40-50%	negative reaction
Proteus mirabilis ATCC 25933	50-100	good	40-50%	negative reaction
Streptococcus pyogenes ATCC 19615	50-100	non-poor	<=10%	negative 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 (8,9).

Further Reading

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14. Rochaix, 1924, Comt. Rend. Soc. Biol., 90:771.
15. 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.
16. Swan, 1954, J. Clin. Pathol., 7:160.
17. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Disclaimer

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