Bile Esculin Agar

M972

Intended use

Bile Esculin Agar is a differential medium 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

Directions

Suspend 64.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Mix and dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubed medium to solidify in slanted position.

Principle And 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 (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).

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 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. Also, *Leuconostoc, Pediococcus, Lactococcus* species causing human infections give a positive bile esculin test (10). To enhance the growth of Enterococci, Bile Esculin Agar can be supplemented with 50ml/l horse serum (3). 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, 5).

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 (12,13,14).

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 :

This medium is general purpose medium and may not support the growth of fastidious organisms.

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

Light yellow to brownish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Amber coloured, clear to slightly opalescent gel with a bluish tinge forms in Petri plates or in tubes as slants.

Reaction

Reaction of 6.5% w/v aqueous solution at 25°C. pH : 6.6±0.2

pH

6.40-6.80

Cultural Response

Cultural characteristics observed in an increased atmosphere of Carbon dioxide after an incubation at 35-37°C for 18-24 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
Cultural Response				
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=50%	positive reaction,blackening of medium around the colony
Proteus mirabilis ATCC 25933	50-100	luxuriant	>=50%	negative reaction
Streptococcus pyogenes ATCC 19615	50-100	none-poor	<=10%	negative reaction

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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. 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (10,11).

Reference

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