Modified Bile Esculin Azide Agar

M1150

Intended Use:

Recommended for selective isolation and enumeration of group D Streptococci.

Composition**

Ingredients	g / L
Tryptone	17.000
Peptone	3.000
Yeast extract	5.000
Bile#	10.000
Sodium chloride	5.000
Sodium citrate	1.000
Esculin	1.000
Ferric ammonium citrate	0.500
Sodium azide	0.250
Agar	13.500
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Oxgall

Directions

Suspend 56.25 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Group D Streptococci possess the group D lipoteichoic acid antigen in their cell walls. Group D species, 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 hydrolyze 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). However, other tests such as salt tolerance should be performed for identifying Enterococci (5). Modified Bile Esculin Azide Agar was formulated according to Isenberg et al (6), Swan (7), Facklam and Moody (5) and Meyer and Schonfeld (8). They reported that esculin hydrolysis and bile tolerance permit the isolation and identification of group D streptococci in 24 hours.

Tryptone, peptone, yeast extract provide all essential growth nutrients. Streptococci hydrolyze esculin to esculetin which reacts with ferric ions to form a dark brown to black coloured complex (3). Bile inhibits most of the gram-positive bacteria other than Enterococci. Sodium azide inhibits gram-negative organism except some *Proteus* species.

Type of specimen

Clinical samples - Faeces; Food and dairy samples; Water samples

Specimen Collection and Handling:

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (8).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (9,10,11).

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (12,13).

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

Warning and Precautions :

In Vitro diagnostic use. For professional use only. 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. 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.Further biochemical and serological tests must be carried out for further identification.

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 brownish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.35% Agar gel

Colour and Clarity of prepared medium

Medium amber coloured clear to slightly opalescent solution with a bluish tinge forms in Petri plates.

Reaction

Reaction of 5.63% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=50%	positive reaction, blackening of medium around the colony
Proteus mirabilis ATCC 25933	50-100	fair-good	30-40%	negative reaction
Streptococcus pyogenes ATCC 19615	50-100	none-poor	<=10%	negative reaction
Streptococcus bovis ATCC 27960	50-100	luxuriant	>=50%	positive reaction, blackening of medium around the colony
Staphylococcus aureus subsp. *aureus* ATCC 25923(00034*)	50-100	good	40-50%	negative reaction

Key : *Correspondng 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 in order 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 (12,13).

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Plot No.C-40, Road No.21Y,
MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India

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