Modified Bile Esculin Azide Agar

M1150

Modified Bile Esculin Azide Agar is recommended for selective isolation and enumeration of group D Streptococci.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	17.000
Peptic digest of animal tissue	3.000
Yeast extract	5.000
Oxgall	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

Directions

Suspend 56.25 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Warning : Sodium Azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

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 (2). They reported that esculin hydrolysis and bile tolerance permit the isolation and identification of group D streptococci in 24 hours.

Casein enzymic hydrolysate, peptic digest of animal tissue, 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). Oxgall inhibits most of the gram-positive bacteria other than Enterococci. Sodium azide inhibits gram-negative organism except some *Proteus* species.

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

M1150: 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	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 ATCC 25923	50-100	good	40-50%	negative reaction

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expirydate on the label.

Reference

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5. Facklam R. and Moody M., 1970, Appl. Microbiol. 20 (2): 245
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