Bile Esculin Azide Agar

M493I

Bile Esculin Azide Agar is a selective medium used for isolation and presumptive identification of fecal Streptococci. The composition and performance criteria of this medium are as per the specifications laid down in ISO 7899-1:1984.

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
Esculin	1.000
Ferric ammonium citrate	0.500
Sodium azide	0.150
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 56.65 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.

Caution: 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. 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).

Bile Esculin Azide Agar is a modification of Bile Esculin Agar (6, 8) as per Isenberg (10). In this medium the bile concentration is reduced and additional sodium azide is incorporated. Bile Esculin Azide Agar, recommended by the ISO Committee (11) is a modification of Bile Esculin Azide Agar (M493), in the type of carbon sources used.

Casein enzymic hydrolysate, peptic digest of animal tissue and yeast extract serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Oxgall and sodium azide 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 (12). To enhance the growth of Enterococci, Bile Esculin Agar can be supplemented with 50ml/l horse serum (3).

Suspected water samples are filtered using membrane filters. These membrane filters are aseptically placed on Slanetz and Bartely Medium (M612I). 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 (11). Alternatively Bile Esculin Azide Agar can also be used for direct isolation of Enterococci (without membrane filter), by incubation at 35-37°C for 18-24 hours.

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 solution with a bluish tinge forms in Petri plates.

Reaction
Reaction of 5.67% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed 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	50-100	luxuriant	>=50%	positive reaction,blackening of medium around the colony
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	
Staphylococcus aureus ATCC 25923	50-100	good	40-50%	negative reaction
Proteus mirabilis ATCC 25933	50-100	good	40-50%	negative reaction
Streptococcus pyogenes ATCC 19615	50-100	none-poor	<=10%	negative reaction

Storage and Shelf Life

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

Reference

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