



Bile Esculin Agar with Kanamycin

M1035

Bile Esculin Agar with Kanamycin is recommended for the selective isolation and presumptive identification of *Bacteroides fragilis* group of bacteria from mixed flora.

Composition**

Ingredients	Gms / Litre
Pancreatic digest of gelatin	5.000
Beef extract	3.000
Oxgall	20.000
Ferric citrate	0.500
Esculin	1.000
Kanamycin	0.100
Hemin	0.010
Vitamin K1	0.010
Agar	15.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 44.6 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. Cool to 45 - 50°C and pour into sterile Petri plates. DO NOT OVERHEAT.

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 Agar with Kanamycin is recommended for the selective isolation and presumptive identification of *Bacteroides fragilis* group of bacteria from mixed flora. This medium is a modification of the original formulation of Swan (6). In this medium kanamycin is added to an enriched Bile Esculin Agar, enriched with hemin and vitamin K1. Hemin and vitamin K1 enriches and enhances the growth of *Bacteroides* species. Kanamycin selectively promotes the growth of *Bacteroides fragilis* while inhibiting the growth of facultative anaerobic and aerobic gram-negative bacilli. Anaerobes that are incapable of hydrolyzing esculin do not form brown or black pigmented colonies on this medium. The plates should be reduced by keeping in anaerobic jar for 18-24 hours, just before incubation (10).

Pancreatic digest of gelatin and beef extract serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Oxgall and kanamycin 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. Viridans Streptococci sometimes exhibit a weak positive reaction. Also, *Leuconostoc*, *Pediococcus*, *Lactococcus* species causing human infections give a positive bile esculin test (11). To enhance the growth of Enterococci, Bile Esculin Agar can be supplemented with 50ml/l horse serum (3).

The test specimens can be directly streaked on the surface of the plate. The inoculated plates should be immediately incubated under anaerobic conditions at 35-37°C. Incubation should be carried out for upto 7 days.

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

Reaction

Reaction of 4.46% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

M1035: Cultural characteristics observed when incubated anaerobically, after an incubation at 35-37°C for 24-48 hours (in case of no growth incubation continued upto 7 days).

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
Cultural Response				
<i>Bacteroides fragilis</i> ATCC 25285	50-100	luxuriant	≥50%	positive reaction, blackening of medium around the colony
<i>Escherichia coli</i> ATCC 25922	50-100	none-poor	≤10%	negative reaction
<i>Fusobacterium necrophorum</i> ATCC 25286	50-100	none-poor	≤10%	negative reaction

Storage and Shelf Life

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

Reference

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