



Bile Esculin Agar, Modified

M972A

Bile Esculin Agar is a differential medium recommended for isolation and presumptive identification of group D Streptococci/Enterococci from food and pharmaceutical products.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Beef extract	3.000
Oxgall	20.000
Esculin	1.000
Ferric citrate	0.500
Agar	15.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 44.5 grams of 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

This medium is a modification of Bile esculin agar, M972 that differs in content of oxgall. The value of bile tolerance together with hydrolysis of esculin as a means of presumptive identification of group D streptococci/ enterococci is widely recognized (1). Bile esculin agar was formulated by Swan (2) for the isolation and identification of Group D Streptococci from food. This medium was also shows differentiation of *Enterobacteriaceae*, *Klebsiella*, *Enterobacter-Serratia* division from other *Enterobacteriaceae* genera on the basis of esculin hydrolysis (3).

The medium contains 2% oxgall that inhibits gram-positive bacteria other than group D Streptococci and Enterococci. Enterococci and Group D Streptococci hydrolyzes esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate (4). Originally Bile esculin test was used for identification of Enterococci. But it was found that this test is also shared by group D Streptococci (1) and therefore it is recommended that other tests such as salt tolerance be performed while identifying Enterococci (5).

Quality Control

Appearance

Light yellow to brownish yellow coloured homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow coloured clear to slightly opalescent gel with/without a bluish tinge forms in petri plates.

Reaction

Reaction of 4.5% w/v aqueous solution at 25°C pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

M972A: Cultural characteristics observed after an incubation at 35°C-37°C for 18-24 hours in an increased atmosphere of carbon dioxide.

Organism	Growth	Esculin hydrolysis
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Cultural Response

<i>Enterococcus faecalis</i> ATCC 29212	luxuriant	Positive
<i>Streptococcus pyogenes</i> ATCC 12344	luxuriant	Negative
<i>Enterobacter aerogenes</i> ATCC 13048	luxuriant	Positive
<i>Proteus mirabilis</i> ATCC 25933	luxuriant	Negative

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

- 1.Facklam R., 1972, Appl. Microbiol., 23:1131.
- 2.Swan A., 1954, J. Clin. Pathol., 7:160
- 3.Edberg S.C., Pittman S., and Singer J.M., 1977, J. Clin. Microbiol., 6:111.
- 4.MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification-,,Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
- 5.Facklam R., 1973, Appl. Microbiol., 26:138.

Revision : 2 / 2015

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