Bile Esculin Agar

M972I

Bile Esculin Agar is recommended for the isolation and identification of *Yersinia enterocolitica* from food and animal feeding stuffs.The composition and performance criteria of this medium are as per the specifications laid down in ISO 10273:1994.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Beef extract	3.000
Esculin	1.000
Bile salts	40.000
Ferric citrate	0.500
Agar	15.000
Final pH (at 25°C)	6.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 64.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense into tubes or flasks. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubed medium to solidify in a slanted position with a butt of 2.5cm deep or pour into sterile Petri plates.

Principle And Interpretation

Yersinia enterocolitica is the causative agent of Yersiniosis, a severe form of human gastroenteritis. Bile Esculin Agar (M972I) is recommended for the isolation and identification of *Y. enterocolitica* , as per ISO 10273-1994 (1). Bile Esculin Agar containing 4% bile salts was formulated by Swan (2) and modified by Facklam and Moody (3). Bile Esculin Agar is also recommended by APHA for identification of Group D Streptococci (4). Organisms hydrolyze esculin to esculetin and dextrose. Esculetin further reacts with ferric citrate to form a dark brown or black complex (5).

Peptic digest of animal tissue and beef extract serve as source of carbon, nitrogen and essential growth factors. Bile salts inhibit the accompanying gram-positive bacteria.

The sample under test is enriched in either PSB Broth (M941) or ITC Broth (M1220). After enrichment transfer a loopful (or 0.5ml) of culture onto Yersinia Selective Agar Base (M834). Incubate at 30°C for 24 hours. Typical red centered colonies are further tested for biochemicals. For studying fermentation of esculin, a loopful is streaked on Bile Esculin Agar (M972I). A black halo around the colonies indicates a positive reaction.

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 bluish tinge forms in Petri plates or in tubes as slants.

Reaction
Reaction of 6.45% w/v aqueous solution at 25°C. pH : 6.6±0.2

pH
6.40-6.80

Cultural Response
M972I: Cultural characteristics observed after an incubation at 35-37°C for 18 -24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
Cultural Response				

Enterococcus faecalis ATCC 29212	50-100	good-luxuriant	>=50%	positive reaction, blackening of medium
Escherichia coli ATCC 25922	50-100	good	40-50%	negative reaction
Enterococcus faecium ATCC 27273	50-100	good-luxuriant	>=50%	positive reaction, blackening of medium around the colony
Yersinia enterocolitica ATCC 27729	50-100	good-luxuriant	>=50%	positive reaction, blackening of medium

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. International Organization for Standardization (ISO), 1994, Draft ISO /DIS 10273.
2. Swan A., 1954, J. Clin. Pathol., 7:160.
3. Facklam R.R. and Moody M.D., 1970, Appl. Microbiol., 20(2):245.
4. MacFaddin J.F., 1980, Biochemical Tests for Identification of Medical Bacteria, 2nd Ed., Williams and Wilkins, Baltimore.
5. Downes F. P. and Ito K., 2001, Compendium of Methods for the Microbiological Examination of Foods. 4th Ed., APHA, Washington.

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