

Technical Information

Bile Esculin Agar

Product Code: DM 1972I

Application: - Bile Esculin Agar is recommended for the isolation and identification of *Yersinia enterocolitica*.

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

Principle & Interpretation

Bile Esculin Agar is recommended for the isolation and identification of *Y. enterocolitica* a causative agent of Yersiniosis, a severe form of human gastroenteritis, as per ISO 10273-1994 ⁽¹⁾. The medium 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 (DM1941) or ITC Broth (DM2220). After enrichment transfer a loopful (or 0.5ml) of culture onto Yersinia Selective Agar Base (DM1834). 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 (DM 972I). A black halo around the colonies indicates a positive reaction.

Methodology

Suspend 64.5 grams of powder media in 1000 ml distilled water. Shake well and heat 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.

Quality Control

Physical 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 Range 6.40-6.80

Cultural Response/Characteristics

DM1972I: Cultural characteristics observed after an incubation at 35-37°C for 18 -24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
<i>Enterococcus faecalis</i> ATCC 29212	50-100	Good-Luxuriant	>=50%	Positive reaction, blackening of medium
<i>Escherichia coli</i> ATCC 25922	50-100	good	40-50%	Negative reaction
<i>Enterococcus faecium</i> ATCC 27273	50-100	Good-Luxuriant	>=50%	Positive reaction, blackening of medium around the colony
<i>Yersinia enterocolitica</i> ATCC 27729	50-100	Good-Luxuriant	>=50%	Positive reaction, blackening of medium

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8⁰ in sealable plastic bags for 2-5 days.

Further Reading

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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