Technical Information

Bile Esculin Agar Base

Product Code: DM 1340

Application: - Bile Esculin Agar Base is a differential medium recommended for isolation and presumptive identification of group D Streptococci from food and pharmaceutical products.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Beef extract	3.000
Oxgall	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

Group D Streptococci possess the group D lipoteichoic acid antigen in their cell walls, which are predominant normal inhabitants of the human gastrointestinal tract, also termed as faecal Streptococci or Enterococci [1]. The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld [2]. Both 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 reported 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 used for differentiation of *Enterobacteriaceae, Klebsiella, Enterobacter, Serratia* from other *Enterobacteriaceae* genera [9]. However, other tests such as salt tolerance should be performed for identifying Enterococci [5].

Bile Esculin Agar Base with added supplements is recommended for selective isolation and presumptive identification of group D streptococci from food and pharmaceutical products.

Peptic digest of animal tissue and beef extract serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Oxgall inhibits most of the other accompyning bacteria. Esculin when added as a supplement 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 shows a weak positive reaction. Also, *Leuconostoc, Pediococcus, Lactococcus* species causing human infections give a positive bile esculin test [10]. To enhance the growth of Enterococci, Bile Esculin Agar can be supplemented with 50ml/l horse serum [3].

Inoculate and incubate the test sample in Todd Hewitt Broth (DM1313). After 24 hours incubation add two drops of the culture onto the surface of slant or plate media [3, 5].

Methodology

Suspend 63.5 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Add 1 gram of Esculin (MS2050). 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.

Quality Control

Physical Appearance
Cream 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 or in tubes as slants.

Reaction
Reaction of 6.3 5% w/v aqueous solution at 25°C. pH : 6.6±0.2

pH Range 6.40-6.80

Cultural Response/Characteristics
DM1340: Cultural characteristics observed with added Esculin (FD050) in an increased atmosphere of Carbon dioxide, after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Esculine Hydrolysis
Proteus mirabilis ATCC 29212	50-100	luxuriant	>=50%	positive reaction, blackening of medium around the colony
Proteus mirabilis ATCC 25933	50-100	luxuriant	>=50%	Negative reaction
Streptococcus pyogenes ATCC 19615	50-100	none-poor	<=10%	Negative reaction

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: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr. , 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4 th Ed., J. B. Lippincott Company
2. Meyer and Schonfeld, 1926, Zentralbl. Bakeriol, Parasitenk. Infectionskr. Hyg. Abt. Orig. 99:402.
3. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore..
4. Rochaix, 1924, Comt. Rend. Soc. Biol., 90:771.
5. Facklam R., 1973, Appl. Microbiol., 26:138.
6. Swan, 1954, J. Clin. Pathol., 7:160.
7. Facklam R., 1972, Appl. Microbiol., 23:113 1.

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