Technical Information

Bile Esculin Agar

Product Code: DM 1972

Application: - Bile Esculin Agar 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
Esculin	1.000
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. These Group D species, are the predominant normal inhabitants of the human gastrointestinal tract, are termed as faecal Streptococci or Enterococci [1]. They have ability of to split esculin [2] and hydrolyse esculin to esculetin and dextrose, to reacts with ferric citrate producing brownish black precipitate [3]. This property 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 on the basis of esculin hydrolysis can help in the differentiation of *Enterobacteriaceae, Klebsiella, Enterobacter, Serratia* from other *Enterobacteriaceae* genera [9]. However, other tests such as salt tolerance should be performed for further identification & confirmation of *Enterococci* [5]. The medium is highly nutritious. 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 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 exhibit 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 64.5 grams of powder media in 1000 ml distilled water. Shake well & 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.

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 6.5% w/v aqueous solution at 25°C. pH : 6.6±0.2

pH range 6.40-6.80

Cultural Response/Characteristics

DM1972: Cultural characteristics observed in an increased atmosphere of Carbon dioxide after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
Enterococcus faecalis *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. Lippinccott 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. Swan, 1954, J. Clin. Pathol., 7:160.
6. Facklam R., 1972, Appl. Microbiol., 23:113 1.
7. Facklam R. R and Moody M. D., 1970, Appl. Microbiol., 20(2):245.
8. Edberg S. C., Pittman S., and Singer J. M., 1977, J. Clin. Microbiol., 6:111.
9. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H.,(Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.

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