Technical Information

Bile Esculin MiVeg Agar w/ Kanamycin

Product Code : VM2035

Application:- Bile Esculin MiVeg Agar w/ Kanamycin is recommended for the selective isolation and presumptive identification of *Bacteroides fragilis* group of bacteria from mixed flora.

Composition

Ingredients	Gms / Litre
MiVeg peptone No. 2	17.000
MiVeg extract	6.000
Synthetic detergent	5.000
Ferric citrate	0.500
Esculin	1.000
Kanamycin	0.100
Ferric pyrophosphate	0.010
Vitamin	0.010
Agar	15.000
Final pH (at 25°C)	7.1±0.2

** Formula adjusted, standardized to suit performance parameters.

Principle & Interpretation

Bile Esculin MiVeg Agar w/ Kanamycin is prepared by using Miveg peptones instead of animal based peptones in Bile Esculin Agar so the medium becomes BSE/TSE risk free. Bile Esculin Agar was originally formulated by Swan (1) for the isolation and identification of Group D Streptococci from food. Facklam and Moody (2,3) further reported that using Bile Esculin Agar, Group D Streptococci could be differentiated from non Group D Streptococci. Bile Esculin Agar was also shown to aid differentiation of *Enterobacteriaceae, Klebsiella, Enterobacter, Serratia* from other *Enterobacteriaceae* genera (4) on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci (5). Group D Streptococci possess the group D lipoteichoic acid antigen in their cell walls. Former Group D species, which are predominant normal inhabitants of the human gastrointestinal tract, are termed as faecal Streptococci or Enterococci (6). The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld (7). Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate (8). The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix (9).

Bile Esculin Agar with Kanamycin is used for the selective isolation and presumptive identification of *Bacteroides fragilis* group of bacteria from mixed flora. This medium is a modification of the original formulation of Swan (1). In this medium kanamycin is added to an enriched Bile Esculin Agar, enriched with hemin and vitamin K1. Hemin and vitamin K1 enriches and enhances the growth of *Bacteroides* species. Kanamycin selectively promotes the growth of *Bacteroides fragilis* while inhibiting the growth of facultative anaerobic and aerobic gram-negative bacilli. Anaerobes that are incapable of hydrolyzing esculin do not form brown or black pigmented colonies on this medium. The plates should be reduced by keeping in anaerobic jar for 18-24 hours, just before incubation (10).

Ingredients like MiVeg peptone no. 2 and MiVeg extract supplies carbon, nitrogen, amino acids, vitamins and all essential growth nutrients. Synthetic detergent and kanamycin present in the medium 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. Viridans Streptococci sometimes exhibit a weak positive reaction. Also, *Leuconostoc, Pediococcus, Lactococcus* species causing human infections give a positive bile esculin test (11). To enhance the growth of Enterococci, Bile Esculin Agar can be supplemented with 50ml/l horse serum (8).

The test specimens can be directly streaked on the surface of the plate. The inoculated plates should be immediately incubated under anaerobic conditions at 35-37°C. Incubation for 18-24 hours, but in case of no. growth in 18-24 hours then incubation should be continued upto 7 days.

Methodology

Suspend 44.6 grams of powder media in 1000 ml purified/distilled water. Mix thoroughly. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45 - 50°C and pour into sterile Petri plates. DO NOT OVERHEAT.

Quality Control

Physical Appearance

Cream to yellow coloured homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Medium amber coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.46 % w/v aqueous solution pH: 7.1±0.2 at 25°C

pH range

6.9-7.3

Cultural Response/Characteristics

Cultural characteristics observed under anaerobic condition, after an incubation at 35-37°C for 18-24 hours (in case of no growth, incubation continued upto 7 days).

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Esculin hydrolysis
Bacteroides fragilis ATCC25285	50-100	good-luxuriant	>=50%	Positive reaction, blackening of medium around the colony
Escherichia coli ATCC25922	50-100	none-poor	>=10%	Negative reaction
Fusobacterium necrophorum ATCC 25286	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

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4. Edberg S. C., Pittman S., and Singer J. M., 1977, J. Clin. Microbiol., 6:111.
5.Facklam R., 1973, Appl. Microbiol., 26:138.
6. 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
7. Meyer and Schonfeld, 1926, Zentralbl. Bakeriol, Parasitenk. Infectionskr. Hyg. Abt. Orig. 99:402.
8. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
9. Rochaix, 1924, Comt. Rend. Soc. Biol., 90:771.
10.Dowell, 1975, Am. J. Med. Technol., 41:402.
11.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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