Bacteroides Bile Esculin Agar Base (BBE)

M805

Bacteroides Bile Esculin Agar is used for selective isolation, identification and cultivation of *Bacteroides fragilis* group.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	15.000
Papaic digest of soyabean meal	5.000
Sodium chloride	5.000
Oxgall	20.000
Esculin	1.000
Ferric ammonium citrate	0.500
Hemin	0.010
Vitamin K1	0.010
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 61.52 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add rehydrated contents of two vials of Bacteroides Selective Supplement (FD062). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Bacteroides is the most common member of the normal gut flora and can cause serious infections if the normal GI mucosal barrier is breached. In the bloodstream, the organism can be carried to virtually any organ of the body. The *Bacteroides fragilis* group is more resistant to antimicrobial agents than most other anaerobes (1) and therefore immediate identification and prompt treatment is of vital importance. Bacteroides Bile Esculin Agar formulated by Livingston, Kominos and Yee (2) is used as a primary isolation medium for the selective and presumptive identification of *Bacteroides fragilis* group (1). Accompanying gram-negative organisms can be totally inhibited due to the presence of oxgall and gentamicin, with the latter added as a supplement. The medium is differential for *B. fragilis* group because of esculin hydrolysis test.

B. fragilis hydrolyses the esculin present in the medium to esculetin and dextrose. The esculetin thus produced reacts with ferric ammonium citrate, present in the medium to form a brown-black coloured complex that is deposited around the colonies as a black halo.

The medium contains highly nutritious casein enzymic hydrolysate, papaic digest of soyabean meal and hemin, which support growth of fastidious anaerobic bacteria like Bacteroides species. Oxgall inhibits almost all anaerobic gram-negative bacilli except *Bacteroides fragilis* (3). Gentamicin inhibits most organisms other than esculin positive *Bacteroides* that can tolerate bile. The minimum inhibitory concentration of 80 mcg/ml or greater is required for *Bacteroides fragilis* group organisms (4).

The medium can be directly inoculated with the specimen. Since the medium is highly selective, a non-selective medium should be also inoculated with the specimen. *B. fragilis* group grow well on this medium. However, *B. vulgatus* usually does not hydrolyze esculin thus no discoloration to the surrounding medium is observed (5).

Quality Control

Appearance

Cream to yellow 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 with a bluish tinge forms in Petri plates.

Reaction
Reaction of 6.15% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed with added Bacteroides Selective Supplement (FD062) when incubated anaerobically at 35-37°C for 40-48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Esculin hydrolysis
Cultural Response				
Bacteroides fragilis ATCC 23745	50-100	good-luxuriant	>=50%	positive reaction,blackening of the medium
Bacteroides vulgatus ATCC 8482	50-100	good-luxuriant	>=50%	negative reaction
Clostridium perfringens ATCC 13124	>=10^3	inhibited	0%	
Proteus mirabilis ATCC 12453	50-100	none -poor	<=10%	negative reaction

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. Murray P. R., Baren E. J, Jorgensen J. H., Pfaller M. A., YolkenR. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
2. Livingston, Kominos and Yee, 1978, J. Clin. Microbiol., 7:448.
3. Shimade K., Sutler V.L. and Finegold S.M., 1970, Appl. Microbiol.1.345138889
4. Finegold S.M. and Sutler V.L., 1971, J. Infect. Dis., 124:556.
5. MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medial Bacteria, Vol. I, Williams and Wilkins, Baltimore.

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