BACTEROIDES BILE ESCULIN AGAR (BBEA)

PRODUCT:

Plated Media:[a]

Bacteroides Bile Esculin Agar (BBEA) P1110

PURPOSE:

Bacteroides Bile Esculin Agar (BBEA) is used for the rapid isolation and presumptive identification of the *Bacteroides fragilis* group.

PRINCIPLE:

Bacteroides Bile Esculin Agar was developed to accelerate further the recognition of *Bacteroides fragilis* group by providing tentative identification from a primary plate media within 48 hours.[2] Livingston et al.[6] published their work on BBEA as a primary plating media in 1978, based on established rapid testing for presumptive identification.

Twenty percent bile stimulation, esculin hydrolysis, catalase production, and kanamycin inhibition were established tests for the presumptive identification of *Bacteroides fragilis* group. By combining the components of these tests, an effective primary plate media was found. Twenty percent bile (oxgall) allows *Bacteroides fragilis* group to grow or stimulates growth while other anaerobes are inhibited. Esculin with ferric ammonium citrate allows detection of esculin hydrolysis, and catalase testing can be performed due to the presence of hemin. The substitution of gentamicin for kanamycin, both aminoglycosides, proved to suppress facultative anaerobes while allowing *Bacteroides fragilis* group to grow. In addition, gentamicin proved to be an effective substitute because gentamicin does not lose its activity at incubation temperatures and can be incorporated into BBEA before autoclaving.[6]

FORMULAS:

Approximate, per liter deionized filtered water.

(1) Bacteroides Bile Esculin Agar:

Tryptic Soy Agar	40.0 g
Oxgall	20.0
Esculin	1.0
Ferric Ammonium Citrate	0.5
Gentamicin Sulfate	100.0 mg
Hemin	10.0

Final pH 7.0 ± 0.2 at 25°C

PRECAUTIONS: *

For *in vitro* diagnostic use. Observe approved biohazard precautions.

Storage: Upon receipt store at 2-8°C away from direct light. Media should not be used if there are signs of contamination, deterioration (shrinking, cracking, or discoloration), or if the expiration date has passed.

Limitations: Some strains of *Bacteroides vulgatus*, a member of *Bacteroides fragilis* group, can be esculin negative.

Some non-*Bacteroides fragilis* group microorganisms are bile-resistant and can hydrolyze esculin; *Bacteroides eggerthii*, *Bacteroides splanchnicus*, *Fusobacterium mortiferum*, *Klebsiella pneumoniae*, *Enterococcus* species, and yeasts are examples of such microorganisms. In general, the above-mentioned microorganisms are less than 1 mm in diameter compared to the 2-3 mm size of *Bacteroides fragilis* group.[4,5]

It may be necessary to incubate an inoculated culture for 48 hours (preferably 3-5 days) before exposing the culture to room air; as many anaerobes are more sensitive to oxygen during the log phase of growth and may be killed by exposure to oxygen before the colonies are fully developed.

PROCEDURE: *

Specimen Collection: To assure the recovery of anaerobes associated with infections, specimens need to be collected and transported properly. In general, aspirates by needle and syringe or tissue samples are more suitable for the recovery of anaerobes. Swabs are less desirable because they are easily contaminated, expose anaerobes to oxygen, allow specimens to dry out, or

permit only the collection of small specimen volumes.

Immediate transportation to the microbiology laboratory is most important for the successful recovery of significant anaerobic pathogens. Clear any air bubbles from the syringe of aspirate specimens and cap the needle with a rubber stopper. **Volumes of less than 1 ml must be received in the microbiology laboratory within 10 minutes; sample volumes of 1 ml or more must be received within 1 hour**.[5] Alternately, oxygen-free transport systems that contain a redox indicator can be used. Anaerobic transport systems must be received within two to three hours after specimen collection. Swabs, as mentioned, are less desirable. However, at times it is impossible to obtain an aspirate or tissue sample. Place the swab in an anaerobic system that will protect the specimen from drying or exposure to oxygen. Information concerning anaerobe specimen collection and commercial two-container sets are available in standard reference materials.

Method of Use: Prior to inoculation, the media should be brought to room temperature. Place one drop of liquid specimen or minced tissue onto the prereduced media or inoculate the plate with a swab, if swabs are submitted for culture. Streak the plate to obtain isolated colonies. Immediately after streaking for isolation, place the media into an anaerobic atmosphere. Incubate at 35°C for 48-72 hours.

Interpretation: *Bacteroides fragilis* group (*Bacteroides fragilis*, *Bacteroides vulgatus*, *Bacteroides ovatus*, *Bacteroides uniformis*, *Bacteroides distasonis*, and *Bacteroides thetaiotaomicron*) blacken the agar. Other microorganisms are suppressed and do not blacken the agar.

Material Required but Not Provided: Standard microbiological supplies and equipment such as loops, incubators, anaerobic transport containers, and anaerobic environments are not provided.

QUALITY CONTROL: *

Microorganisms Used (ATCC #):	**Expected Results:**
Bacteroides fragilis (25285)	Growth, blackens agar
Clostridium perfringens (13124)	Inhibition, partial to complete
Escherichia coli (25922)	Inhibition, partial to complete, no blackening
	Key: See “Interpretation”

User Quality Control: Check for signs of contamination and deterioration. Bacteroides Bile Esculin Agar should appear firm, translucent, and dark amber in color.

BIBLIOGRAPHY:

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4. Finegold, S. M., and E. J. Baron, *Bailey and Scott’s Diagnostic Microbiology*, 7th ed., C. V. Mosby, St. Louis, 1986.
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6. Livingston, S. J., et al., *J. Clin. Microbiol.*, 7:448-453, 1978.
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* For more detailed information, consult appropriate.

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Data #150

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