BACTEROIDES BILE ESCULIN AGAR (BBEA)

PRODUCTS:

Plate Media:[a]

Bacteroides Bile Esculin Agar, item no. P1110

[a]see catalog for ordering options

PURPOSE:

The primary purpose of Bacteroides Bile Esculin Agar (BBEA) is the rapid isolation and presumptive identification of *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 medium within 48 hours.[2] Livingston et al.[6] published their work on BBEA as a primary plating medium 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 medium 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:

Ingredient	Amount
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 one millimeter in diameter compared to the two- to three-millimeter 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; 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 one milliliter must be received in the microbiology laboratory within ten minutes; sample volumes of one milliliter or more must be received within one 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 medium should be brought to room temperature. Place one drop of liquid specimen or minced tissue onto the prereduced medium 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 medium 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
	Key: See "Interpretation"

User Quality Control: Check for signs of contamination and deterioration.

BIBLIOGRAPHY:

1. Casemore, D. P., *J. Clin. Pathol.*, 20:298-299, 1967.
2. Dowell, V. R., Jr., et al., "Laboratory Methods in Anaerobic Bacteriology," *CDC Lab. Manual*, USDHEW, Washington, D. C., 1974.
3. Draper, D. L., et al., *J. Clin. Microbiol.*, 5:439-443, 1977.
4. Finegold, S. M., and E. J. Baron, *Bailey and Scott's Diagnostic Microbiology*, 7th ed., C. V. Mosby, St. Louis, 1986.
5. Lennette, E. H., et al., *Manual of Clinical Microbiology*, 6th ed., American Society for Microbiology, Washington, D. C., 1995.
6. Livingston, S. J., et al., *J. Clin. Microbiol.*, 7:448-453, 1978.
7. Sutter, V. L., and S. M. Finegold, *Appl. Microbiol.*, 21:13-20, 1971.

* For more detailed information, consult appropriate references and/or details in the preface of the PML Technical Manual.

PML MICROBIOLOGICALS, INC.
Data #150
Copyright 1989 by PML Microbiologicals, Inc.
Revision Date: January 2001

BRUCELLA ANAEROBIC MEDIA

PRODUCT:

Plate Media:

Brucella Anaerobic Agar With 5% Sheep Blood, item no. P1200
Brucella Anaerobic Agar With 5% Sheep Blood and Pyridoxal, item no. P1225
Brucella Anaerobic Agar With 5% Sheep Blood and Neomycin, item no. P1220
Brucella Anaerobic Agar With 5% Laked Sheep Blood, Kanamycin, and Vancomycin (LBA), item no. P1750

[a]see catalog for ordering options

PURPOSE:

Brucella anaerobic medium is a nutritious base medium used for the cultivation of anaerobic bacteria from clinical specimens.

PRINCIPLE:

Anaerobic bacteria habitation is extensive; they are found in the soil, in oceans, in food, and in animals. The environment in which anaerobes thrive has both low oxygen tension and low oxidation-reduction potential (Eh)[3] which in nature is a result of the metabolic activity of microbes that consume oxygen through respiration. In humans, anaerobes are normally present in the oral cavity around the teeth, the gastrointestinal tract, the orifices of the genitourinary tract, and on the skin. Anaerobic infections of humans can involve any organ of the body; the most common anaerobic infections are found in aspiration pneumonia, brain and lung abscesses, intra-abdominal/pelvic sepsis, dental infections, and chronic sinusitis. The anaerobes involved in most clinically significant infections, e.g., *Bacteroides fragilis* group and pigmented *Bacteroides* and *Porphyromonas* species, are moderately obligate anaerobes, and that means they can grow in oxygen levels from 2-8%. Strict anaerobes, e.g., *Clostridium haemolyticum* and *Clostridium novyi*, are unable to grow in oxygen levels greater than 0.5%. Moderately obligate anaerobes encountered in human infections may be killed by atmospheric oxygen when anaerobic conditions are not maintained during collection, transportation, processing, and identification. Moderately obligate anaerobes appear to produce the enzymes catalase, peroxidase, and superoxide dismutase, which protect them from toxic oxygen-reduction products.

Recovery of anaerobes from clinical specimens requires decreased oxygen tension, low oxidation-reduction potential, and the use of both enriched and selective media. Most specimens, involving anaerobes, contains a mixture of microbes which necessitates the use of antibiotics in the media to inhibit contaminating flora. Neomycin and kanamycin inhibit aerobic, facultative anaerobic, and some anaerobic gram-negative microorganisms. Neomycin may be less inhibitory to some strains of *Fusobacterium nucleatum*, anaerobic cocci, and *Bifidobacterium* species. Vancomycin inhibits most gram-positive microorganisms. The addition of laked sheep blood to the base medium with antibiotics enhances earlier pigmentation of pigmented *Bacteroides* species.

Nonselective enriched media are also required in order to support the growth of all types of anaerobes and to facilitate morphologic characteristics necessary for identification. Brucella anaerobic base medium consists of a peptic digest of animal tissue and a pancreatic digest of casein, a vegetable protein, and provides a complementary source of peptones, carbohydrates, and vitamins. Sheep blood, yeast autolysate, menadione (Vitamin K_3), pyridoxal, and hemin are added as enrichments. Sheep blood enhances hemolysis detection and pigment production; menadione enhances the growth of pigmented *Bacteroides* and *Porphyromonas* species; hemin enhances the growth of *Bacteroides fragilis* group and other *Bacteroides* species. A reducing substance, sodium bisulfite, is added to help maintain reduced conditions and a low pH.

FORMULAS:

Approximate, per liter of deionized filtered water.

(1) Brucella Anaerobic Agar With 5% Sheep Blood:

Ingredient	Amount
Peptic Digest of Animal Tissue	10.0 g
Pancreatic Digest of Casein	10.0
Dextrose	1.0
Yeast Autolysate	2.0
Sodium Chloride	5.0
Sodium Bisulfite	0.1
Agar	15.0
Menadione	0.5 mg
Hemin	5.0
Sheep Blood	50.0 ml

Final pH 7.0 ± 0.2 at 25°C

(2) Brucella Anaerobic Agar With 5% Sheep Blood and Pyridoxal:
Same as (1) above except it also contains 10.0 mg of Pyridoxal HCL.

(3) Brucella Anaerobic Agar With 5% Sheep Blood and Neomycin:
Same as (1) above except it also contains 100.0 mg of Neomycin Sulfate.

(4) Brucella Anaerobic Agar With Laked Sheep Blood, Kanamycin, and Vancomycin:
Same as (1) above except 5% Laked Sheep Blood is substituted for 5% Sheep Blood, and also contains 100.0 mg of Kanamycin and 7.5 mg of Vancomycin.

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 (cracking, shrinking, or discoloration), or if the expiration date has passed.

Limitations: *Prophyromonas asaccharolyticus* may not grow on media containing vancomycin.

Yeasts and other kanamycin/vancomycin-resistant microbes may not be inhibited and may grow on selective media containing these antibiotics.

It may be necessary to incubate inoculated culture media for 48 hours (preferably 3-5 days) before examining them and exposing the culture to room air; 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.

The following specimens should **not** be set up routinely for anaerobic culture: throat, nasal swab, gastric contents, small bowel contents, feces, coughed sputum, voided or catheterized urine, vaginal or cervical swabs, materials from superficial wounds or abscesses not collected properly to exclude surface contaminants, and materials from abdominal wounds obviously contaminated with feces. This principle can be applied to other uncommon types of specimens which may be submitted. Exceptions will have to be made in certain instances.

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 recovery of anaerobes. Swabs are less desirable because they are easily contaminated, expose anaerobes to oxygen, allow specimens to dry out, and permit only the collection of small specimen volumes.

Immediate transportation to the microbiology laboratory is most important for 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 one milliliter must be received in the microbiology laboratory within ten minutes; sample volumes of one milliliter or more must be received within one hour**.[6] 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 material.

A direct microscopic examination (Gram stain) of the clinical material is invaluable. The unique morphology of many anaerobes will often alert the microbiologist to the possibility of infection with anaerobes and, at the same time, is a valuable guide to the selection of media.

Method of Use: Prior to inoculation, the medium should be brought to room temperature. Inoculate the media with a large amount of inoculum; use of a large inoculum serves to minimize the harmful effects of toxic oxygen growth-limiting factors. Streak the inoculum so as to obtain isolated colonies. Incubate at 35°C in an anaerobic environment using an anaerobic jar, anaerobic bag, or glove box chamber without disturbing the environment for 48 hours. Media incubated in a chamber or anaerobic bag allows inspection of the culture earlier because these systems allow examination without exposure to oxygen.

Examine for characteristic morphologic growth and Gram stain and identify anaerobes using aerotolerance testing, biochemical testing, and/or gas-liquid chromatography. See references for further details.

Interpretation: The following growth characteristics are typical for organisms appearing on nonselective Brucella Anaerobic Agar:

Organism	Colonial Morphology
Peptococcus	Convex, gray to white, opaque, shiny, entire edge.
Peptostreptococcus	Convex, gray to white, opaque, shiny or dull, entire edge.
Veillonella	Convex, translucent, glistening, entire edge.
Bacteroides fragilis	Convex, entire edge, glistening or dull, yellow-beige-brown to black depending upon length of incubation, medium, etc. Will fluoresce red if young colonies are viewed with UV illumination.
Fusobacterium nucleatum	Convex, glistening with internal iridescent flecking or raised opaque "bread crumb" colonies.
Clostridium perfringens	Low convex, semi-opaque, shiny, entire, with double zone of hemolysis; narrow zone of complete hemolysis surrounded by a larger zone of incomplete hemolysis.

Material Required but Not Provided: Standard microbiological supplies and equipment such as anaerobic holding jars, anaerobic incubation systems, incinerators, and inoculating loops are not provided.

QUALITY CONTROL:*

Media Used:	Microorganisms Used (ATCC #):	Expected Results:
Brucella Anaerobic Agar, Brucella Anaerobic Agar with Pyridoxal	*Clostridium perfringens (13124)*	Growth/double zone hemolysis
	Bacteroides fragilis (25285)	Growth
	Fusobacterium nucleatum (25586)	Growth
	Bacteroides levii (29147)	Growth
	Peptostreptococcus anaerobius (27337)	Growth
Brucella Anaerobic Agar with Neomycin	*Clostridium perfringens (13124)*	Growth/double zone hemolysis
	Bacteroides fragilis (25285)	Growth
	Proteus mirabilis (12453)	Inhibition
Laked Blood Agar	*Clostridium perfringens (13124)*	Inhibition
	Bacteroides fragilis (25285)	Growth
	Staphylococcus aureus (25923)	Inhibition, partial to complete
	Escherichia coli (25922)	Inhibition

User Quality Control: Check for signs of contamination and deterioration.

BIBLIOGRAPHY:

1. Balows, A., et al., *Anaerobic Bacteria: Role in Disease,* Charles C. Thomas, Springfield, Ill., 1974.
2. Blair, J. E., et al., *Manual of Clinical Microbiology,* 1st ed., American Society for Microbiology, Bethesda, Md., 1970.
3. Dowell, V. R., Jr., and T. M. Hawkins, *Laboratory Methods in Anaerobic Bacteriology,* CDC Lab. Manual, USHEW Publ. No. 74-8272, Washington, D. C., 1974.
4. Dowell, V. R., Jr., et al., *J. Bacteriol.*, 88:1811, 1964.
5. Finegold, S. M., et al., *Ernaehrungsforschung*, 10:517, 1965.
6. Finegold, S. M., and E. J. Baron, *Bailey and Scott's Diagnostic Microbiology*, 7th ed., C. V. Mosby, St. Louis, 1986.
7. Koneman, E. W., et al., *Color Atlas and Textbook of Diagnostic Microbiology,* 3rd ed., J. B. Lippincott, Philadelphia, 1988.
8. Lennette, E. H., et al., *Manual of Clinical Microbiology,* 6th ed., American Society for Microbiology, Washington, D. C., 1995.
9. Sutter, V. L., et al., *Wadsworth Anaerobic Bacteriology Manual,* 3rd ed., C. V. Mosby, St. Louis, 1980.

* For more detailed information, consult appropriate references and/or details in the preface of the PML Technical Manual.

PML MICROBIOLOGICALS, INC.
Data #210
Copyright 1989 by PML Microbiologicals, Inc.
Revision Date: January 2001