TRYPTIC SOY ANAEROBIC MEDIA

PRODUCTS:

Plated Media:

CDC Anaerobic Blood Agar	P1243
CDC Anaerobic + Kanamycin and Vancomycin (KV) Agar	P1242
CDC Anaerobic + Gentamicin Agar	P1244
Anaerobes Gentamicin Agar	303445
Phenylethyl Alcohol (PEA) Anaerobic Agar with 5% Sheep Blood	P2220

PURPOSE:

Tryptic Soy Anaerobic media are nutritional base media used for the cultivation of anaerobic bacteria from clinical specimens.

PRINCIPLE:

Anaerobic bacteria are found in the soil, oceans, food, and animals. In humans, anaerobes normally inhabit the oral cavities around the teeth, gastrointestinal tract, genitourinary tract, and skin. Anaerobic infections of humans can involve any organ of the body; the most common infections are found in aspiration pneumonia, lung abscesses, intra-abdominal/pelvic sepsis, dental infections, chronic sinusitis, and brain abscesses. The anaerobes involved in clinically significant infections are usually moderately obligate anaerobes and can grow in oxygen levels from 2-8%.[7] Moderately obligate anaerobes appear to produce the enzymes catalase, peroxidase, or superoxide dismutase which protect them from toxic oxygen and oxygen derivatives.[6,7] Recovery of anaerobes from clinical specimens requires decreased oxygen tension, low oxidation-reduction potential (Eh), and the use of both enriched and selective media. Anaerobic conditions must be maintained during the collection, transportation, processing, and identification of specimens.

Tryptic Soy Anaerobic media are nutritional media consisting of pancreatic digest of casein and enzymatic digest of soybean meal which provide a complementary source of amino acids, carbohydrates, and vitamins. Sheep blood, yeast extract, and menadione add supplementary enrichments. Sheep blood enhances hemolysis and pigment production; yeast extract is rich in vitamins and enhances the growth of fastidious microbes; menadione (vitamin K_3) enhances the growth of pigmented *Bacteroides* species. CDC Anaerobic Blood Agar contains the additional supplements of hemin and L-cystine and improves the growth of *Clostridium haemolyticum*, *Fusobacterium necrophorum*, certain strains of *Actinomyces israelii* and *Bacteroides thetaiotaomicron*, and certain strains of thiol-dependent streptococci.[7]

The addition of antibiotics such as kanamycin and vancomycin enables the selective isolation of gram-negative anaerobic bacilli by inhibiting facultatively and obligately anaerobic gram-positive bacteria.[4] Gentamicin improves the recovery of obligately anaerobic bacteria from specimens containing mixed flora.

Recovery of anaerobes from clinical specimens requires the use of nonselective and selective media. Tryptic Soy Anaerobic media is made selective by the addition of phenylethyl alcohol. Phenylethyl alcohol inhibits facultative anaerobic gram-negative bacilli, especially *Proteus* species.

FORMULAS:

Approximate, per liter of deionized filtered water.

(1) CDC Anaerobic Blood Agar:

Pancreatic Digest of Casein	15.0 g
Enzymatic Soy Digest	5.0
Sodium Chloride	5.0
Yeast Extract	5.0
L-cystine	0.4
Agar	15.0
Hemin	5.0 mg
Menadione	0.5
Sheep Blood	50.0 ml

Final pH 7.4 ± 0.2 at 25°C

(2) CDC Anaerobic + KV Agar:
Same as **(1)** above with the addition of 100.0 mg of Kanamycin and 7.5 mg of Vancomycin.

(3) CDC Anaerobic + Gentamicin Agar:
Same as **(1)** above with the addition of 0.075 mg of Gentamicin
Final pH 7.3 ± 0.2 at 25°C

(4) Anaerobes Gentamicin Agar:
Same as **(3)** above with an increased volume of yeast extract to 10.0 g and without the addition of L-cystine.

(5) Phenylethyl Alcohol Anaerobic Agar with 5% Sheep Blood:
Same as **(1)** above with the addition of 2.5 g of ß-Phenylethyl alcohol, and without the addition of L-cystine and yeast extract.
Final pH 7.3 ± 0.2 at 25°C

PRECAUTIONS:*

For *in vitro* diagnostic use only. Not intended for use in the diagnosis of disease or other conditions. 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: Media may need to be pre-reduced in an oxygen-free holding jar in order to recover some anaerobic microorganisms.

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 selective nature of PEA Anaerobic Agar with 5% Sheep Blood may inhibit specific strains of anaerobes for which it is designed to isolate.

Throat, gingival, sputum, gastric contents, small bowel, feces, rectal swabs, surfaces of decubitus ulcers, encrusted walls of abscesses, mucosal lining, eschar, voided urine, vagina or cervix, skin and adjacent mucous should not be cultured for anaerobic bacteria due to large number of anaerobic bacteria present in these sites as normal flora.

PROCEDURE:*

Specimen Collection: Information on specimen collection is found in standard reference materials. In general, collecting specimens from mucous membranes or skin requires decontamination of the surface area. Specimen collection using a normal swab is not recommended because the swab is likely to dry out, expose anaerobes to ambient air, and may be contaminated by indigenous flora. Aspiration with a needle and syringe is preferred for anaerobe specimen collection. Once collected, the specimen should be protected from oxygen exposure by using an anaerobic transport system, protected from extreme heat and cold, and delivered to the laboratory without delay.

Method of Use: Prior to inoculation, the media should be brought to room temperature. Inoculate the media with a large amount of inoculum; use of large inocula serves to minimize the toxic effects of oxygen.[6] Streak the inoculum so as to obtain isolated colonies. Incubate at 35°C in an anaerobic environment using an anaerobic jar, anaerobic bag, or a glove box chamber without disturbing the environment for 48 hours. Media incubated in a glove box chamber or an anaerobic bag allows inspection of the media earlier because these systems allow examination without exposure to oxygen. Continue incubation for up to 7 days if no growth occurs. Examine colony morphology, perform a 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 Tryptic Soy Anaerobic Media:

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, media, 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.

Materials 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:
CDC Anaerobic Blood Agar	*Clostridium perfringens* (13124)	Growth, double zone hemolysis
	Bacteroides fragilis (25285)	Growth
	Fusobacterium nucleatum (25586)	Growth
	Peptostreptococcus anaerobius (27337)	Growth
	Prevotella melaninogenica (25845)	Growth
CDC Anaerobic + KV Agar	*Bacteroides fragilis* (25285)	Growth
	Clostridium perfringens (13124)	Inhibition, partial to complete (p/c)
	Staphylococcus aureus (25923)	Inhibition, p/c
	Escherichia coli (25922)	Inhibition, p/c
CDC Anaerobic + Gentamicin, Anaerobes Gentamicin Agar	*Clostridium perfringens* (13124)	Growth, double zone hemolysis
	Bacteroides fragilis (25285)	Growth
	Fusobacterium nucleatum (25586)	Growth
	Peptostreptococcus anaerobius (27337)	Growth
	Bacteroides levii (29147)	Growth (Anaerobes Gent. only)
	Escherichia coli (25922)	Inhibition, p/c
	Proteus mirabilis (12453)	Inhibition, p/c (CDC Gent. only)
	Staphylococcus aureus (25923)	Inhibition, p/c (Anaer. Gent. only)
Phenylethyl Alcohol Anaerobic Agar	*Clostridium perfringens* (13124)	Growth, double zone hemolysis
	Bacteroides fragilis (25285)	Growth
	Proteus mirabilis (12453)	Inhibition, p/c

User Quality Control: Check for signs of contamination and deterioration. Tryptic Soy Anaerobic media should appear opaque, and cherry red in color.

BIBLIOGRAPHY:

1. Balows, A., et al., *Anaerobic Bacteria: Role in Disease*, Charles C. Thomas, Springfield, Ill., 1974.
2. Dowell, V. R., Jr., et al., *J. Bacteriol.*, 88:1811, 1964.
3. Dowell, V. R., Jr., and T. M., Hawkins, *Laboratory Methods in Anaerobic Bacteriology*, CDC Manual, USHEW Publ. No. 74-8272, Washington, D. C., 1974.
4. Finegold, S. M., et al., *Ernaehrungsforschung*, 10:517, 1965.
5. Forbes, B. A., et al., Bailey and Scott's Diagnostic Microbiology, 12th ed., C. V. Mosby, St. Louis, 2007.
6. Koneman, E. W., et al., *Color Atlas and Textbook of Diagnostic Microbiology, 6th ed.*, J. B. Lippincott, Philadelphia, 2005.
7. Murray, P.R., et al., *Manual of Clinical Microbiology, 9th ed*., American Society for Microbiology, Washington, D. C., 2007.
8. Sutter, V. L., et al., *Wadsworth Anaerobic Bacteriology Manual*, 3rd ed., C. V. Mosby, St. Louis, 1980.

* For more detailed information, consult appropriate references.

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