Anaerobic Agar

Intended Use

Anaerobic Agar is used for cultivating anaerobic microorganisms.

Summary and Explanation

Brewer[1] described a special Petri dish cover that allowed surface growth of anaerobes and microaerophiles without anaerobic equipment. The microorganisms were grown on an agar-based medium having a low oxidation-reduction potential. Anaerobic Agar is a modification of Brewer's original formula. This medium is suitable for standard plating procedures used in cultivating anaerobic bacteria.[2-4]

Anaerobic bacteria cause a variety of infections in humans, including otitis media, oral infections, endocarditis, meningitis, wound infections following bowel surgery or trauma, and bacteremia.[5,6] Anaerobic bacteria are the predominant flora colonizing the skin and mucous membranes of the body.[3] Anaerobes vary in their sensitivity to oxygen and nutritional requirements.[2] Anaerobic bacteria lack cytochromes and thus are unable to use oxygen as a terminal electron acceptor.[3]

Principles of the Procedure

Peptone provides the nitrogen, vitamins and amino acids in Anaerobic Agar. Dextrose is a carbon source. Sodium chloride maintains the osmotic equilibrium. Sodium thioglycollate and sodium formaldehyde sulfoxylate are reducing agents. Methylene blue serves as an indicator of anaerobiosis with a blue color indicating the presence of oxygen. Agar is the solidifying agent.

Formula

Difco™ Anaerobic Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	20.0	g
Sodium Chloride	5.0	g
Dextrose	10.0	g
Agar	20.0	g
Sodium Thioglycollate	2.0	g
Sodium Formaldehyde Sulfoxylate	1.0	g
Methylene Blue	2.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 58 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Standard Petri Dishes[2]

1. Inoculate a properly obtained specimen onto the medium using the pour plate technique.
2. Immediately incubate anaerobically at 35°C.
3. Examine at 24 hours if incubating plates in an anaerobic chamber. Examine at 48 hours if incubating plates in an anaerobic jar or anaerobic pouch.
4. Extended incubation may be necessary to recover some anaerobes.

User Quality Control

Identity Specifications

Difco™ Anaerobic Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	5.8% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent when hot, changing to green when cooled.
Prepared Appearance:	Light green, slightly opalescent.
Reaction of 5.8% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Anaerobic Agar

Prepare the medium per label directions. Inoculate using the pour plate technique and incubate at 35 ± 2°C under anaerobic conditions for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacteroides fragilis	25285	10^2-10^3	Good
Clostridium perfringens	13124	10^2-10^3	Good
Fusobacterium mortiferum	9817	10^2-10^3	Good

Brewer Anaerobic Agar Plates

1. Dispense 50-60 mL of Anaerobic Agar into a standard Petri dish. For best results use porous tops to obtain a dry surface.
2. Inoculate the surface of the medium by streaking; avoid the edges of the plates.
3. Replace the standard Petri dish lid with a sterile Brewer anaerobic Petri dish cover. The cover should not rest on the Petri dish bottom. The inner glass ridge should seal against the uninoculated periphery of the agar. It is essential that the sealing ring inside the cover is in contact with the medium. This seal must not be broken before the end of the incubation period. A small amount of air is caught over the surface of the medium; however, the oxygen in this space reacts with reducing agents in the medium to form an anaerobic environment.
4. Incubate aerobically as desired.

For a complete discussion on anaerobic and microaerophilic bacteria from clinical specimens, refer to the appropriate procedures outlined in the references.[2-5] For the examination of anaerobic bacteria in food, refer to standard methods.[7-9]

Expected Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Clinical specimens must be obtained properly and transported to the laboratory in a suitable anaerobic transport container.[2]
2. The microbiologist must be able to verify quality control of the medium and determine whether the environment is anaerobic.[2]
3. The microbiologist must perform aerotolerance testing on each isolate recovered to ensure that the organism is an anaerobe.[2]
4. Methylene blue is toxic to some anaerobic bacteria.

References

1. Brewer. 1942. Science 95:587.
2. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
3. Baron, Peterson and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, Mo.
4. Murray, Baron, Pfaller, Tenover and Yolken (ed.). 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
5. Allen, Siders and Marler. 1985. *In* Lennette, Balows, Hausler and Shadomy (ed.). Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
6. Smith. 1975. The pathogenic anaerobic bacteria, 2nd ed. Charles C. Thomas, Springfield, Ill.
7. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
8. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
9. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Anaerobic Agar

Cat. No. 253610 Dehydrated – 500 g