Brewer Anaerobic Agar

Intended Use

Brewer Anaerobic Agar is used for cultivating anaerobic and microaerophilic bacteria.

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 agar with a low oxidation-reduction potential. Brewer Anaerobic Agar was originally formulated and modified for the procedure described by Brewer.[1] This medium is suitable for standard plating procedures used in cultivating anaerobic bacteria.

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.[2-5] 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]

User Quality Control

Identity Specifications

Difco™ Brewer 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 while hot, turning red on aeration and cooling.
Prepared Appearance:	Light pink ring at outer edge, light amber in center, slightly opalescent.
Reaction of 5.8% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Brewer Anaerobic Agar

Prepare the medium per label directions. Inoculate Brewer plates with the test organisms. Replace the porous covers with Brewer covers and seal. Incubate plates at 35 ± 2°C aerobically for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacteroides fragilis	25285	10^2-10^3	Good
Clostridium beijerinckii	17795	10^2-10^3	Good
Clostridium perfringens	12924	10^2-10^3	Good

Principles of the Procedure

Peptones and yeast extract provide the nitrogen, vitamins and amino acids in Brewer Anaerobic Agar. Dextrose is the carbon source, and sodium chloride maintains osmotic equilibrium. Sodium thioglycollate and sodium formaldehyde sulfoxylate are the reducing agents. Resazurin serves as an indicator of anaerobiosis with a pink color indicating the presence of oxygen. Agar is the solidifying agent.

Formula

Difco™ Brewer Anaerobic Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	5.0	g
Proteose Peptone No. 3	10.0	g
Yeast Extract	5.0	g
Dextrose	10.0	g
Sodium Chloride	5.0	g
Agar	20.0	g
Sodium Thioglycollate	2.0	g
Sodium Formaldehyde Sulfoxylate	1.0	g
Resazurin	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, and streak to obtain isolated colonies.
2. Immediately incubate anaerobically at 35 ± 2°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 pouch, or if using Brewer anaerobic dish cover.
4. Extended incubation may be necessary to recover some anaerobes.

Brewer Anaerobic Agar Plates

1. Dispense 50-60 mL of Brewer 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 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, and the oxygen in this space reacts with the reducing agents 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.[2-4] For the examination of anaerobic bacteria in food refer to standard methods.[6-8]

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 the organism is an anaerobe.[2]

References

1. Brewer. 1942. Science *95*:587.
2. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. 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, Jorgensen, Landry and Pfaller, (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
5. Smith. 1975. The pathogenic anaerobic bacteria, 2nd ed. Charles C. Thomas, Springfield, Ill.
6. Wehr and Frank (ed.). 2004. Standard methods for the microbiological examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
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.

Availability

Difco™ Brewer Anaerobic Agar

Cat. No. 227920 Dehydrated – 500 g