Columbia Broth

Intended Use

Columbia Broth is used for cultivating fastidious microorganisms.

Summary and Explanation

Columbia Broth is prepared according to the formulation described by Morello and Ellner.[1] In their study Columbia Broth, a medium developed for blood cultures, was superior to a commonly used general purpose broth for faster growth of *Staphylococcus aureus*, *Escherichia coli* and streptococci (viridans and enterococcus groups). Columbia Broth, in the presence of CO_2 and supplemented with SPS, is an excellent blood culture medium.[2] In the study by Morello and Ellner,[1] the addition of sodium polyanetholsulfonate (SPS) in Columbia Broth was emphasized. SPS is an anticoagulant that inhibits serum bactericidal activity against many bacteria, inhibits phagocytosis, inactivates complement, and neutralizes lysozymes and the aminoglycoside class of antibiotics.[2]

Principles of the Procedure

Peptones and yeast extract provide nitrogen, carbon, vitamins and trace nutrients essential for growth. Dextrose is added to the formula as a carbon energy source. The medium is buffered with Tris. Corn starch is omitted to reduce opalescence.[1] Cysteine is the reducing agent. Magnesium and iron are added to facilitate organism growth.

Formula

Difco™ Columbia Broth

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	10.0	g
Yeast Extract	5.0	g
Proteose Peptone No. 3	5.0	g
Tryptic Digest of Beef Heart	3.0	g
L-Cysteine HCl	0.1	g
Dextrose	2.5	g
Sodium Chloride	5.0	g
Magnesium Sulfate (anhydrous)	0.1	g
Ferrous Sulfate	0.02	g
Sodium Carbonate	0.6	g
Tris (Hydroxymethyl) Aminomethane	0.83	g
Tris (Hydroxymethyl) Aminomethane HCl	2.86	g

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

User Quality Control

Identity Specifications

Difco™ Columbia Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in purified water upon warming. Solution is light amber, clear to very slightly opalescent, may have a slight amount of fine precipitate.
Prepared Appearance:	Light amber, clear to very slightly opalescent, may have a slight amount of fine precipitate.
Reaction of 3.5% Solution at 25°C:	pH 7.5 ± 0.2

Cultural Response

Difco™ Columbia Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under appropriate conditions for 18-48 hours. Incubate *Bacteroides fragilis* anaerobically.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacteroides fragilis	25285	10^2-10^3	Good
Neisseria meningitidis	13090	10^2-10^3	Good
Pseudomonas aeruginosa	27853	10^2-10^3	Good
Staphylococcus aureus	25923	10^2-10^3	Good
Streptococcus pyogenes	19615	10^2-10^3	Good

Directions for Preparation from Dehydrated Product

1. Suspend 35 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. OPTIONAL: Sodium polyanetholesulfonate (SPS) may be added at this time with agitation to ensure a uniform solution. The culture medium should contain 0.025 to 0.05% SPS.
4. Autoclave at 121˚C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Process clinical specimens from different body sites as described in *Clinical Microbiology Procedures Handbook*,[2] *Manual of Clinical Microbiology*[3] or according to laboratory procedures.

Expected Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. *Neisseria* spp. may be inhibited by SPS in Columbia Broth. The addition of 1.2% gelatin may counteract the inhibitory effect, but SPS may also inhibit other organisms.[2]
2. Opalescence in Columbia Broth cannot always be relied upon as evidence of bacterial growth in the bottle.
3. It is possible for significant numbers of viable bacteria to be present in an inoculated and incubated blood culture bottle without the usual signs of bacterial growth.

References

1. Morello and Ellner. 1969. Appl. Microbiol. *17*:68.
2. Isenberg and Garcia (ed). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

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Cat. No. 294420 Dehydrated – 500 g