

References

1. Ellner, Granato and May. 1973. *Appl. Microbiol.* 26:904.
2. Jousimies-Somer, Summanen and Finegold. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), *Manual of clinical microbiology*, 7th ed. American Society for Microbiology, Washington, D.C.
3. Gibbons and MacDonald. 1960. *J. Bacteriol.* 80:164.
4. Dowell. 1975. In Balows (ed.), *Clinical microbiology. How to start and when to stop.* Charles C. Thomas, Springfield, Ill.
5. Isenberg, Schoenkecht and von Graevenitz. 1979. Cumitech 9, Collection and processing of bacteriological specimens. Coord. ed., Rubin. American Society for Microbiology, Washington, D.C.
6. Martin. 1971. *Appl. Microbiol.* 22:1168.
7. 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.

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 CO₂ 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

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
<i>Bacteroides fragilis</i>	25285	10 ² -10 ³	Good
<i>Neisseria meningitidis</i>	13090	10 ² -10 ³	Good
<i>Pseudomonas aeruginosa</i>	27853	10 ² -10 ³	Good
<i>Staphylococcus aureus</i>	25923	10 ² -10 ³	Good
<i>Streptococcus pyogenes</i>	19615	10 ² -10 ³	Good

Availability

BBL™ Columbia Anaerobe 5% Sheep Blood Agar

Cat. No.	221928	Prepared Plates – Pkg. of 20*
	221929	Prepared Plates – Ctn. of 100*

*Store at 2-8°C.

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.

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 polyanetholsulfonate (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 (ed). 1992. Clinical microbiology procedures handbook, vol.1. American Society for Microbiology, Washington, D.C.
3. Murray, Baron, Pfaller, Tenover and Tenover (ed.). 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Columbia Broth

Cat. No. 294420 Dehydrated – 500 g

Columbia CNA Agar • Columbia CNA Agar, Modified Columbia PNA Agar

Intended Use

Columbia CNA Agar, Columbia CNA Agar, Modified, and Columbia PNA Agar, all supplemented with 5% sheep blood, are selective and differential media used for the isolation and differentiation of gram-positive microorganisms from clinical and nonclinical materials.

Summary and Explanation

Ellner et. al., in 1966, reported the development of a blood agar formulation, which has been designated as Columbia Agar.¹ The Columbia Agar base, which achieves rapid and luxuriant growth and sharply defined hemolytic reactions, is utilized as the base for media containing blood and for selective formulations in which various combinations of antimicrobial agents are used as additives.

Ellner and his colleagues found that a medium consisting of 10 mg of colistin and 15 mg of nalidixic acid per liter in a Columbia Agar Base enriched with 5% sheep blood would support the growth of staphylococci, hemolytic streptococci and enterococci while inhibiting the growth of *Proteus*, *Klebsiella* and *Pseudomonas* species. In BBL™ Columbia CNA Agar with 5% Sheep Blood, the concentration of nalidixic acid has been reduced to 10 mg/L to increase the recovery of gram-positive cocci from clinical specimens. The concentration of nalidixic acid has been further reduced in Columbia CNA Agar, Modified to 5 mg/L.

In the Columbia PNA version of Ellner's medium, polymyxin B has been substituted for colistin (10 mg). Although the antimicrobial properties of the two agents are nearly the same, some species of gram-negative bacteria are more sensitive to polymyxin B than colistin.²

Principles of the Procedure

These media derive their superior growth-supporting properties from the combination of peptones prepared from pancreatic digest of casein, peptic digest of animal tissue and beef extract. Yeast extract and corn starch are also included in the formulation and serve as energy sources, with yeast extract being a supplier of the B-complex vitamins.

Sheep blood supports the growth of fastidious organisms and allows detection of hemolytic reactions. It should be noted that this medium has a relatively high carbohydrate content and, therefore, beta-hemolytic streptococci may produce a greenish hemolytic reaction that may be mistaken for alpha hemolysis.

The addition of the antimicrobial agents, colistin (or polymyxin B) and nalidixic acid, renders the medium selective for gram-positive microorganisms.³ Colistin and polymyxin B disrupt the cell membrane of gram-negative organisms, whereas the nalidixic acid blocks DNA replication in susceptible gram-negative bacteria.⁴

Formula

BBL™ Columbia CNA Agar

Approximate Formula* Per Liter	
Pancreatic Digest of Casein	12.0 g
Peptic Digest of Animal Tissue	5.0 g
Yeast Extract	3.0 g
Beef Extract	3.0 g
Corn Starch	1.0 g
Sodium Chloride	5.0 g
Agar	13.5 g
Colistin	10.0 mg
Nalidixic Acid	10.0 mg

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

Directions for Preparation from Dehydrated Product

1. Suspend 42.5 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 12 minutes. Cool to 45-50°C.
4. Add 5% sterile, defibrinated sheep blood.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate plates at 35 ± 2°C for 24-48 hours in an aerobic atmosphere supplemented with carbon dioxide.