

## 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.<sup>2</sup>
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.<sup>1</sup> 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.<sup>2</sup>

## 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.<sup>3</sup> Colistin and polymyxin B disrupt the cell membrane of gram-negative organisms, whereas the nalidixic acid blocks DNA replication in susceptible gram-negative bacteria.<sup>4</sup>

## 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.

## User Quality Control

*Enterococcus faecalis*  
ATCC™ 29212

### Identity Specifications

#### BBL™ Columbia CNA Agar

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 4.25% solution, soluble in purified water upon boiling. Solution is medium, tan to yellow, hazy.

Prepared Appearance: Tan to yellow, hazy.

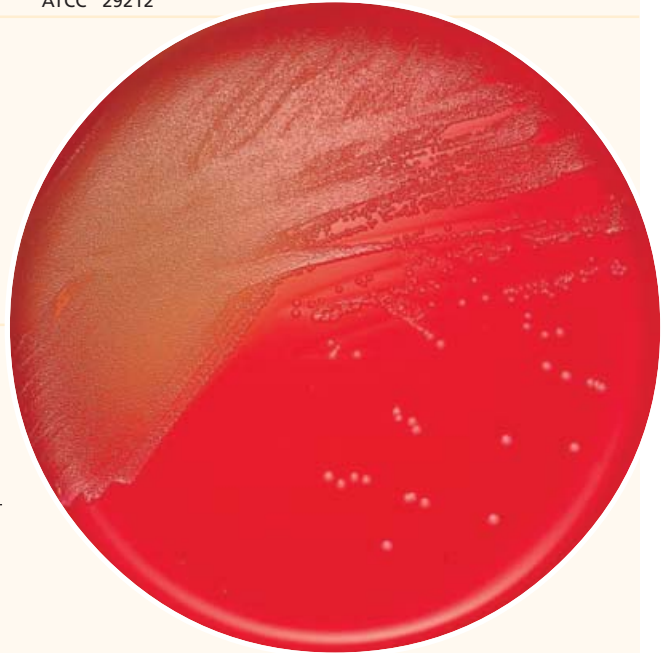
Reaction of 4.25% Solution at 25°C: pH 7.3 ± 0.2

### Cultural Response

#### BBL™ Columbia CNA Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C with 3-5% CO<sub>2</sub> for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	HEMOLYSIS
<i>Proteus mirabilis</i>	12453	10 <sup>4</sup> -10 <sup>5</sup>	Partial to complete inhibition	–
<i>Staphylococcus aureus</i>	25923	10 <sup>3</sup> -10 <sup>4</sup>	Good	Beta
<i>Streptococcus pneumoniae</i>	6305	10 <sup>3</sup> -10 <sup>4</sup>	Good	Alpha
<i>Streptococcus pyogenes</i>	19615	10 <sup>3</sup> -10 <sup>4</sup>	Good	Beta, slight greening may be present



## Expected Results

Typical colonial morphology on Columbia CNA Agar with 5% Sheep Blood is as follows:

- Streptococci (non-group D) ... Small, white to grayish. Beta or alpha hemolysis.
- Enterococci (group D) ..... Small, but larger than group A streptococci, blue-gray. Beta or alpha hemolysis.
- Staphylococci ..... Large, white to gray or cream to yellow, with or without hemolysis.
- Micrococci ..... Large, white to gray or yellow to orange, with or without hemolysis.
- Corynebacteria ..... Small to large, white to gray or yellow, with or without hemolysis.
- Candida* ..... Small, white.
- Listeria monocytogenes* ..... Small to large, blue-gray, with beta hemolysis.
- Gram-negative bacteria ..... No growth to trace growth.

## References

- Ellner, Stoessel, Drakeford and Vasi. 1966. *Am. J. Clin. Pathol.* 45:502.
- Garrod and O'Grady. 1971. *In Antibiotics and chemotherapy*, 3rd ed. Williams & Wilkins, Baltimore, Md.
- Chapin and Murray. 1999. *In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology*, 7th ed. American Society for Microbiology, Washington, D.C.
- Estevez. 1984. *Lab. Med.* 15:258.

## Availability

### BBL™ Columbia CNA Agar

Cat. No. 212104 Dehydrated – 500 g  
294221 Dehydrated – 5 lb (2.3 kg)  
212249 Dehydrated – 25 lb (11.3 kg)

### BBL™ Columbia CNA Agar with 5% Sheep Blood

**BS10 CMPH MCM7**

*United States and Canada*

Cat. No. 221352 Prepared Plates – Pkg. of 20\*  
221353 Prepared Plates – Ctn. of 100\*

*Europe*

Cat. No. 254007 Prepared Plates – Pkg. of 20\*  
254072 Prepared Plates – Ctn. of 120\*

*Japan*

Cat. No. 251352 Prepared Plates – Pkg. of 20\*

### BBL™ Columbia CNA Agar with 5% Sheep Blood// MacConkey II Agar

**BS10 CMPH MCM7**

*United States and Canada*

Cat. No. 221600 Prepared **I Plate™** Dishes – Pkg. of 20\*  
221601 Prepared **I Plate™** Dishes – Ctn. of 100\*

*Japan*

Cat. No. 251600 Prepared **I Plate™** Dishes – Pkg. of 20\*

### BBL™ Columbia CNA Agar, Modified, with Sheep Blood// Enterococcosel™ Agar

Cat. No. 297413 Prepared **I Plate™** Dishes – Ctn. of 100\*

### BBL™ Columbia CNA Agar with 5% Sheep Blood// Levine EMB Agar

Cat. No. 295618 Prepared **I Plate™** Dishes – Ctn. of 100\*

### BBL™ Columbia CNA Agar with 5% Sheep Blood// EMB Agar, Modified (Holt-Harris and Teague)

Cat. No. 221941 Prepared **I Plate™** Dishes – Pkg. of 20\*

### BBL™ Columbia PNA Agar with 5% Sheep Blood// MacConkey II Agar

Cat. No. 297272 Prepared **I Plate™** Dishes – Ctn. of 100\*

\*Store at 2-8°C.