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# Columbia Agars

## Columbia Agar Base • Columbia Blood Agar Base Columbia Blood Agar Base EH • Columbia Agar with 5% Sheep Blood • Columbia Agar with Fildes Enrichment and Bacitracin

### Intended Use

Columbia Agar Base, without or with the addition of 5% (or 10%) sheep blood, is a highly nutritious, general-purpose medium for the isolation and cultivation of nonfastidious and fastidious microorganisms from a variety of clinical and non-clinical materials.

Columbia Blood Agar Base EH (Enhanced Hemolysis) is used with blood in isolating and cultivating fastidious microorganisms.

Columbia Agar with Fildes Enrichment and Bacitracin is used in qualitative procedures for isolation and cultivation of *Haemophilus* species from clinical specimens.

Columbia Agar Base meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*<sup>1-3</sup> performance specifications, where applicable.

### Summary and Explanation

Ellner et al.,<sup>4</sup> in 1966, reported the development of a blood agar formulation, which has been designated as Columbia Agar. The base achieves the more rapid and luxuriant growth obtained from casein hydrolysate media with the sharply defined hemolytic reactions, more typical colonial morphology and improved pigment production achieved with media containing infusion peptone.

Columbia Agar Base is utilized as the base for media containing blood and for selective media formulations in which various combinations of antimicrobial agents are used as additives.

Sheep blood allows detection of hemolytic reactions and supplies the X factor (heme) necessary for the growth of many bacterial species but lacks V factor (nicotinamide adenine dinucleotide), since it contains NADase which destroys the NAD. For this reason, *Haemophilus influenzae*, which requires both the X and V factors, will not grow on this medium. Fildes found that supplementing nutrient agar with a digest of sheep blood supplied both of these factors and the medium would support the growth of *H. influenzae*.<sup>5,6</sup> The inclusion of bacitracin makes

## User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™** and **BBL™** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

### Identity Specifications

#### Difco™ Columbia Blood Agar Base

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 4.4% solution, soluble in purified water upon boiling. Solution is light to medium amber, opalescent with fine precipitate.

Prepared Appearance: Plain – Light to medium amber, slightly opalescent to opalescent with fine precipitate.  
With sheep blood – Cherry red, opaque, no hemolysis.

Reaction of 4.4% Solution at 25°C: pH 7.3 ± 0.2

### Identity Specifications

#### Difco™ Columbia Blood Agar Base EH

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 3.9% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear to slightly opalescent.

Prepared Appearance: Plain – Light to medium amber, clear to slightly opalescent.  
With sheep blood – Medium to bright cherry red, opaque, no hemolysis.

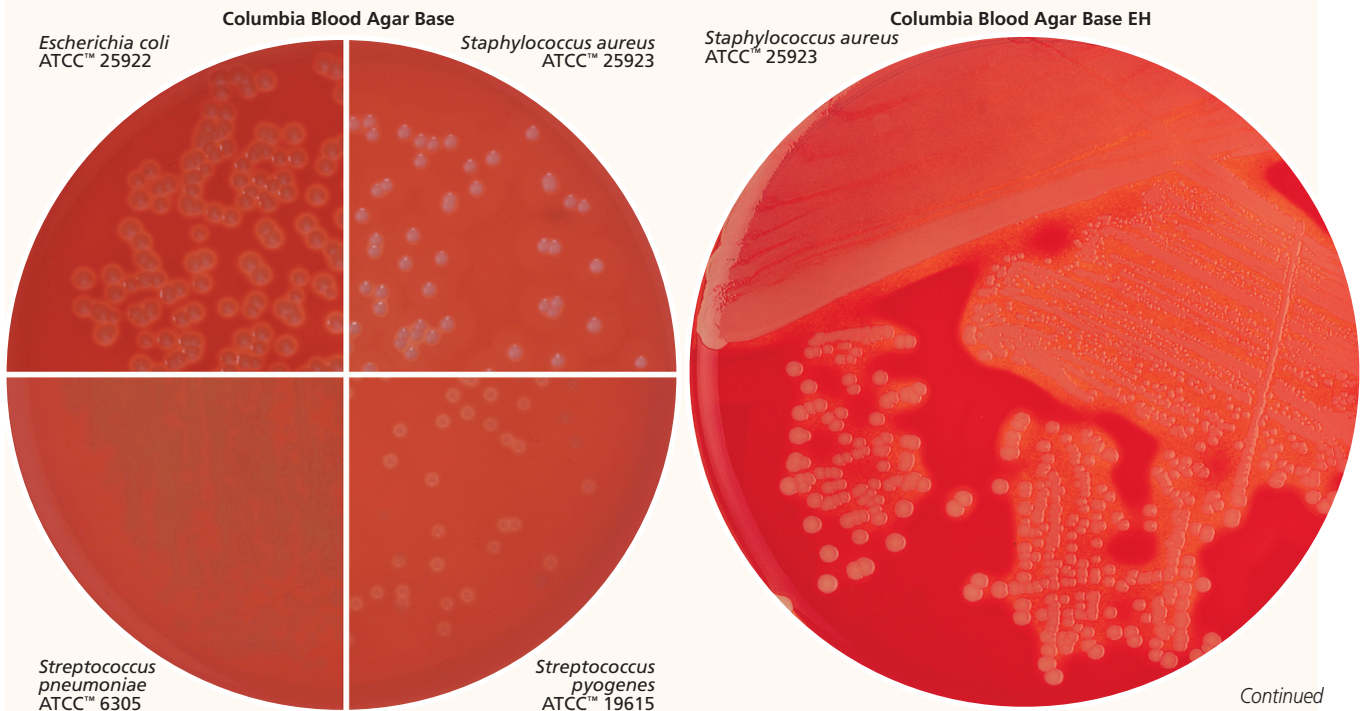
Reaction of 3.9% Solution at 25°C: pH 7.3 ± 0.2

### Cultural Response

#### Difco™ Columbia Blood Agar Base or Columbia Blood Agar Base EH

Prepare the medium per label directions without (plain) and with 5% sheep blood (SB) for Columbia Blood Agar Base and with 5% sheep blood for Columbia Blood Agar Base EH. Inoculate and incubate at 35 ± 2°C with 5-10% CO<sub>2</sub> for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB	HEMOLYSIS
<i>Escherichia coli</i>	25922	30-300	Good	Good	Beta
<i>Neisseria meningitidis</i>	13090	30-300	Good	Good	Gamma (none)
<i>Staphylococcus aureus</i>	25923	30-300	Good	Good	Beta
<i>Streptococcus pneumoniae</i>	6305	30-300	Good	Good	Alpha
<i>Streptococcus pyogenes</i>	19615	30-300	Good	Good	Beta



the enriched Columbia Agar medium selective for the isolation of *Haemophilus* species from clinical specimens, especially from the upper respiratory tract.<sup>7</sup>

Columbia Agar with 5% sheep blood is a general all-purpose enriched primary isolation medium that allows growth of all clinically significant anaerobes and facultative anaerobes.<sup>8,9</sup> Columbia Agar supplemented with 5% sheep blood is recommended when processing clinical specimens for unusual organisms, such as *Bartonella bacilliformis*, the causative agent of Oroya fever and Peruvian wart.<sup>8</sup> Columbia Agar supplemented with 5% sheep blood and 20 µg of ampicillin per mL is used in

isolating *Aeromonas* sp. from stool samples of patients showing clinical symptoms of gastroenteritis.<sup>10</sup>

Columbia Agar Base is used to prepare Modified Butzler Agar, which is a selective isolation medium for the detection of thermotolerant *Campylobacter* in food and animal feed.<sup>11</sup> Columbia Agar Base is a component of Oxford Medium and Columbia Blood Agar Base is a component of Modified Oxford Medium, both of which are used to detect *Listeria monocytogenes* in food and milk samples.<sup>11-14</sup> Columbia Agar is listed as one of the recommended media for the isolation of *Clostridia* sp. from nonsterile pharmaceutical products.<sup>1</sup>

### Identity Specifications

#### BBL™ Columbia Agar Base

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

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

Prepared Appearance: Plain – Medium, yellow to tan, hazy.  
With sheep blood – Cherry red, opaque, no hemolysis.

Reaction of 4.25%

Solution at 25°C: pH 7.3 ± 0.2

#### BBL™ Columbia Agar (prepared)

Appearance: Light to dark yellow and hazy with small cream particles in sediment; may appear as flocculation and contain small suspended insolubles.

Reaction at 25°C: pH 7.3 ± 0.2

### Cultural Response

#### BBL™ Columbia Agar Base

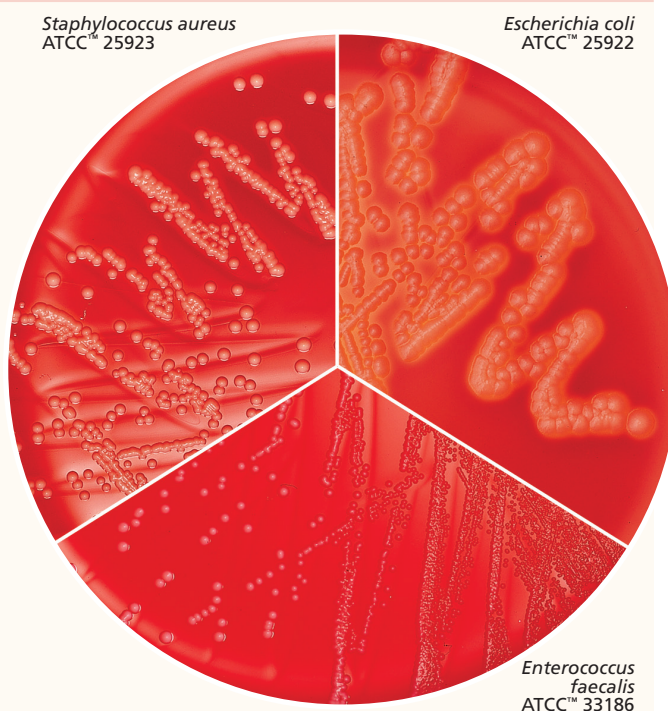
Prepare the medium per label directions without (plain) and with 5% sheep blood (SB). Inoculate and incubate at 35 ± 2°C under appropriate atmospheric conditions for 48 hours (incubate *C. jejuni* at 42 ± 2°C for 48-72 hours). For *Clostridium sporogenes* (both strains), inoculate with fresh 24-48 hour Reinforced Clostridial Medium cultures, in duplicate, and incubate one set at 30-35°C and the other set at 35-37°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB
<i>Campylobacter jejuni</i>	33291	10 <sup>3</sup>	N/A	Good
<i>Campylobacter jejuni</i>	33292	10 <sup>3</sup>	N/A	Good
<i>Candida albicans</i>	10231	10 <sup>3</sup> -10 <sup>4</sup>	N/A	Good
<i>Escherichia coli</i>	25922	10 <sup>3</sup> -10 <sup>4</sup>	N/A	Good
<i>Listeria monocytogenes</i>	19115	10 <sup>3</sup> -10 <sup>4</sup>	N/A	Good
<i>Pseudomonas aeruginosa</i>	10145	10 <sup>3</sup> -10 <sup>4</sup>	Good	N/A
<i>Shigella flexneri</i>	12022	10 <sup>3</sup> -10 <sup>4</sup>	Good	N/A
<i>Staphylococcus aureus</i>	25923	10 <sup>3</sup> -10 <sup>4</sup>	Good	N/A
<i>Streptococcus pneumoniae</i>	6305	10 <sup>3</sup> -10 <sup>4</sup>	Good	N/A
<i>Clostridium sporogenes</i>	11437	<100	Growth (at 30-35°C)	N/A
<i>Clostridium sporogenes</i>	11437	<100	Growth (at 35-37°C)	N/A
<i>Clostridium sporogenes</i>	19404	<100	Growth (at 30-35°C)	N/A
<i>Clostridium sporogenes</i>	19404	<100	Growth (at 35-37°C)	N/A

#### BBL™ Columbia Agar (prepared)

Inoculate and incubate under anaerobic conditions at 30-35°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Clostridium sporogenes</i>	11437	10-100	Growth
<i>Clostridium sporogenes</i>	19404	10-100	Growth



## Principles of the Procedure

Columbia Agar Base supplemented with sheep, rabbit or horse blood derives its superior growth-supporting properties from the combination of peptones prepared from pancreatic digest of casein, meat peptic digest and heart pancreatic digest. 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. Sodium chloride maintains osmotic balance in the medium.

It should be noted that Columbia Sheep Blood Agar has a relatively high carbohydrate content and, therefore, beta-hemolytic streptococci may produce a greenish hemolytic reaction that may be mistaken for alpha hemolysis.

Fildes enrichment is prepared by the action of the enzyme pepsin on defibrinated sheep blood. Bacitracin is a polypeptide antibiotic that is active mainly against gram-positive bacteria.

## Formulae

### Difco™ Columbia Blood Agar Base

Approximate Formula* Per Liter	
Pancreatic Digest of Casein .....	10.0 g
Proteose Peptone No. 3.....	5.0 g
Yeast Extract .....	5.0 g
Beef Heart, Infusion from 500 g .....	3.0 g
Corn Starch.....	1.0 g
Sodium Chloride .....	5.0 g
Agar .....	15.0 g

### BBL™ Columbia Agar Base

Approximate Formula* Per Liter	
Pancreatic Digest of Casein .....	10.0 g
Meat Peptic Digest.....	5.0 g
Yeast Extract .....	5.0 g
Heart Pancreatic Digest .....	3.0 g
Corn Starch.....	1.0 g
Sodium Chloride .....	5.0 g
Agar .....	13.5 g

### Difco™ Columbia Blood Agar Base EH

Approximate Formula* Per Liter	
Pantone .....	12.0 g
Bitone H Plus.....	6.0 g
Enzymatic Digest of Animal Tissue .....	3.0 g
Starch .....	1.0 g
Sodium Chloride .....	5.0 g
Agar .....	12.0 g

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

## Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:  
Difco™ Columbia Blood Agar Base – 44 g;  
BBL™ Columbia Agar Base – 42.5 g;  
Difco™ Columbia Blood Agar Base EH – 39 g.  
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. For preparation of blood agar, cool the base to 45-50°C and add 5% sterile, defibrinated blood. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

## Sample Collection and Handling

For clinical specimens, refer to laboratory procedures for details on specimen collection and handling.<sup>8-10</sup>

For food or milk samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.<sup>11-14</sup>

For pharmaceutical samples, refer to *USP* General Chapter <62> for details on the examination of nonsterile products and tests for isolating *Clostridium* sp. using Columbia Agar.<sup>1</sup>

## Procedure

Refer to appropriate standard references for details on test methods to obtain isolated colonies from specimens or samples using Columbia Agar.<sup>1,8-14</sup> Incubate the plates at 35 ± 2°C for 18-72 hours under appropriate atmospheric conditions, or as instructed in the standard reference.<sup>1,8-14</sup>

Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 3-10% CO<sub>2</sub>.

## Expected Results

After the recommended incubation period, most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a “dilution” technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Further, growth of each organism may be semi-quantitatively scored on the basis of growth in each of the streaked areas.

## References

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## Availability

### Difco™ Columbia Blood Agar Base

**AOAC** **BAM** **EP** **ISO**

Cat. No.	279240	Dehydrated – 500 g
	279220	Dehydrated – 2 kg
	279230	Dehydrated – 10 kg

### BBL™ Columbia Agar Base

**AOAC** **COMPF** **EP** **ISO** **JP** **USP**

Cat. No.	211124	Dehydrated – 500 g <sup>†</sup>
	211125	Dehydrated – 5 lb (2.3 kg) <sup>†</sup>
	211126	Dehydrated – 25 lb (11.3 kg) <sup>†</sup>
	215191	Prepared Plates – Pkg. of 20* <sup>†</sup>
	295661	Prepared Plates with Fildes Enrichment and Bacitracin – Pkg. of 20*

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

**BS12** **CMPH2** **MCM9**

#### United States and Canada

Cat. No.	221165	Prepared Plates – Pkg. of 20*
	221263	Prepared Plates – Ctn. of 100*

#### Europe

Cat. No.	254005	Prepared Plates – Pkg. of 20*
	254071	Prepared Plates – Ctn. of 120*

#### Japan

Cat. No.	251165	Prepared Plates – Pkg. of 20*
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### Difco™ Columbia Blood Agar Base EH

Cat. No.	279030	Dehydrated – 500 g
	279010	Dehydrated – 2 kg
	279020	Dehydrated – 10 kg

### BBL™ Fildes Enrichment

Cat. No.	211866	Prepared Tubes, 5 mL (K Tubes) – Pkg of 10*
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\*Store at 2-8°C.

<sup>†</sup>QC testing performed according to USPIE/JP performance specifications.