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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 nonclinical 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.

Summary and Explanation

Ellner et al.,[1] 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.

The 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*.[2,3] The inclusion of bacitracin makes the enriched Columbia Agar medium selective for the isolation of *Haemophilus* species from clinical specimens, especially from the upper respiratory tract.[4]

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, 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.

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 Digest	3.0	g
Corn Starch	1.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

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

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

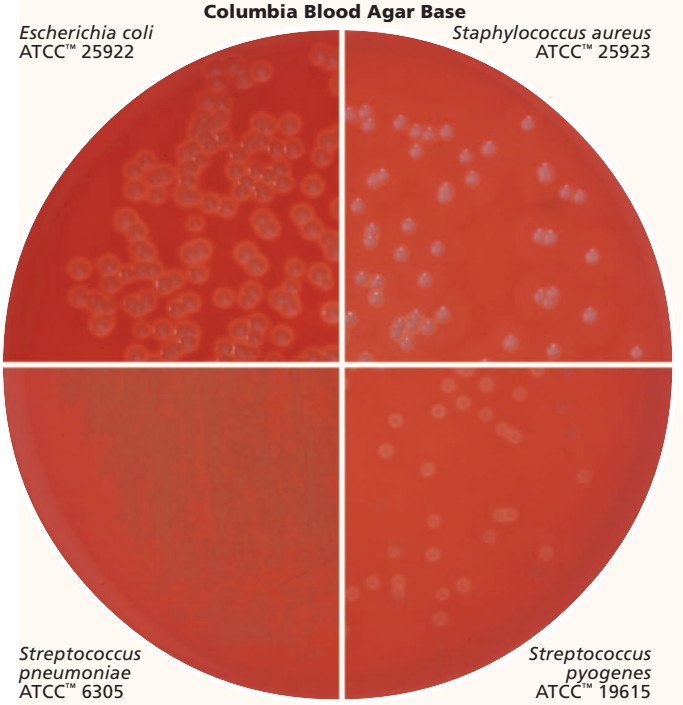
Cultural Response

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_2 for 18-48 hours.

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

Columbia Blood Agar Base



Columbia Blood Agar Base EH

Continued

Identity Specifications

BBL™ Columbia Agar Base

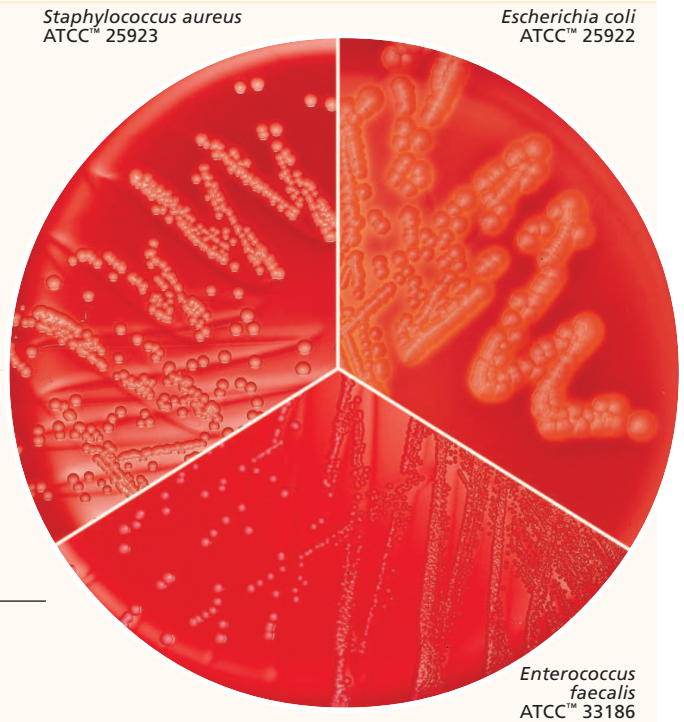
Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.25% solution, soluble in purified water upon boiling. Solution is medium, yellow to tan, hazy.
Prepared Appearance:	Plain – Medium, yellow to tan, hazy. With sheep blood – Cherry red, opaque, no hemolysis.
Reaction of 4.25% Solution at 25°C:	pH 7.3 ± 0.2

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).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB
Campylobacter jejuni	33291	10^3	N/A	Good
Campylobacter jejuni	33292	10^3	N/A	Good
Candida albicans	10231	10^3-10^4	N/A	Good
Escherichia coli	25922	10^3-10^4	N/A	Good
Listeria monocytogenes	19115	10^3-10^4	N/A	Good
Pseudomonas aeruginosa	10145	10^3-10^4	Good	N/A
Shigella flexneri	12022	10^3-10^4	Good	N/A
Staphylococcus aureus	25923	10^3-10^4	Good	Good, beta hemolysis
Streptococcus pneumoniae	6305	10^3-10^4	Good	Good, alpha hemolysis
Streptococcus pyogenes	19615	10^3-10^4	N/A	Good, beta hemolysis



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BBL™ Columbia Agar Base

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

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.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate plates at 35 ± 2°C for 18-72 hours.

Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 3-10% CO_2.

Expected Results

After incubation 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 semiquantitatively scored on the basis of growth in each of the streaked areas.

References

1. Ellner, Stoessel, Drakeford and Vasi. 1966. Am. J. Clin. Pathol. *45*:502.
2. Fildes. 1920. Br. J. Exp. Pathol. *1*:129.
3. Fildes. 1921. Br. J. Exp. Pathol. *2*:16.
4. Chapin and Doern. 1983. J. Clin. Microbiol. *17*:163.

Availability

Difco™ Columbia Blood Agar Base

EP ISO

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

BBL™ Columbia Agar Base

EP ISO

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

BBL™ Columbia Agar with 5% Sheep Blood

BS10 CMPH

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*

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*

**Store at 2-8°C.*