In contrast, a coagulase-negative species that does not utilize mannitol, such as *Staphylococcus epidermidis*, does not change the color of the medium and it remains clear. Other coagulase-negative species may utilize mannitol and produce a yellow zone around the colonies, but an opaque zone will not be produced.

Formula

BBL™ Coagulase Mannitol Agar

Approximate Formula* Per Liter

Brain Heart Infusion	5.0	g
Pancreatic Digest of Casein	10.5	g
Papaic Digest of Soybean Meal	3.5	g
Sodium Chloride	3.5	g
D-Mannitol	10.0	g
Agar	14.5	g
Bromcresol Purple	0.02	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 47 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 15 minutes.
4. Cool to 50°C and add 7-15% pretested, undiluted rabbit coagulase plasma with EDTA. Mix gently and pour into plates, approximately 18 mL per plate.
5. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: The use of **BBL** Coagulase Plasma, Rabbit with EDTA, in place of citrated plasma, prevents false-positive coagulase reactions by citrate-utilizing microorganisms.

Procedure

Inoculate and incubate the plates in an inverted position (agar side up) at 35 ± 2°C, and examine for growth after 18-24 hours. Avoid prolonged incubation because it may cause the opaque zones surrounding coagulase-positive organisms to become clear.

Expected Results

After 18-24 hours of incubation, coagulase-positive organisms will produce opaque zones; coagulase-negative organisms will produce no opacity. Organisms that utilize mannitol produce yellow zones. *S. aureus* may be presumptively identified as those colonies with opaque, yellow zones around them.

Limitations of the Procedure

Some old or mutant strains of *S. aureus* may be weak coagulase producers or exhibit negative coagulase reactions and should be subcultured and retested if in doubt. *Escherichia coli* also uses mannitol and may be weakly coagulase-positive. Colonial morphology and a Gram stain should readily allow for differentiation from *S. aureus*.

References

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Availability

BBL™ Coagulase Mannitol Agar

Cat. No. 211116 Dehydrated – 500 g

C

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)*[1-3] performance specifications, where applicable.

Summary and Explanation

Ellner et al.,[4] 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*.[5,6] 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.

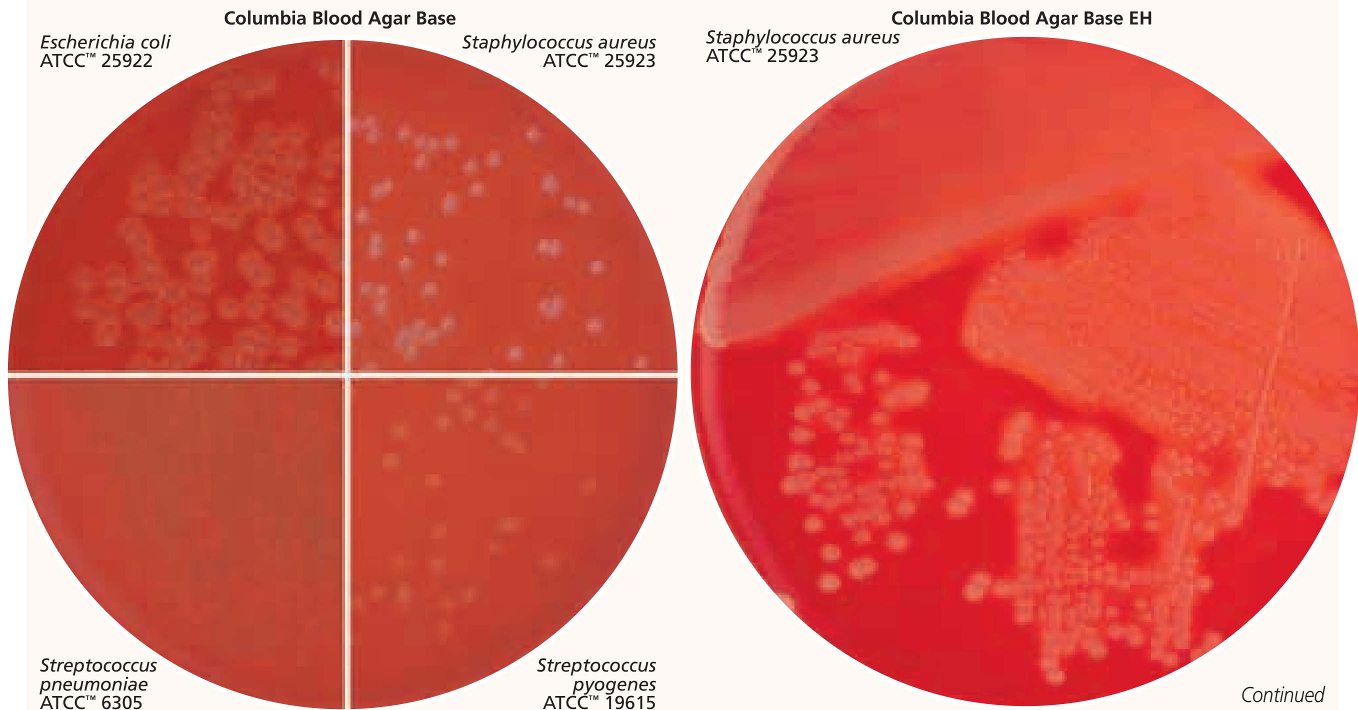
Reaction of 3.9% Solution at 25°C: pH 7.3 ± 0.2

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_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



Continued

the enriched Columbia Agar medium selective for the isolation of *Haemophilus* species from clinical specimens, especially from the upper respiratory tract.[7]

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.[8,9] 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.[8] 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.[10]

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.[11] 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.[11-14] Columbia Agar is listed as one of the recommended media for the isolation of *Clostridia* sp. from nonsterile pharmaceutical products.[1]

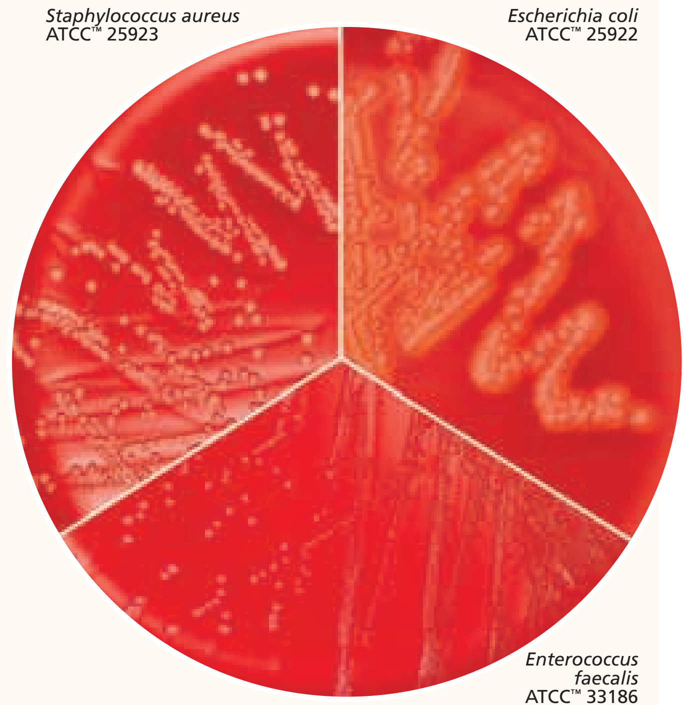
Identity Specifications

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
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	N/A
Streptococcus pneumoniae	6305	10^3-10^4	Good	N/A
Clostridium sporogenes	11437	<100	Growth (at 30-35°C)	N/A
Clostridium sporogenes	11437	<100	Growth (at 35-37°C)	N/A
Clostridium sporogenes	19404	<100	Growth (at 30-35°C)	N/A
Clostridium sporogenes	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
Clostridium sporogenes	11437	10-100	Growth
Clostridium sporogenes	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

Ingredient	Amount	Unit
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

Ingredient	Amount	Unit
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

Ingredient	Amount	Unit
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.[8-10]

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

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.[1]

Procedure

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

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 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†
211125 Dehydrated – 5 lb (2.3 kg)†
211126 Dehydrated – 25 lb (11.3 kg)†
215191 Prepared Plates – Pkg. of 20*†
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*

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.*
†QC testing performed according to USP/EP/JP performance specifications.

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Columbia Anaerobe 5% Sheep Blood Agar

Intended Use

Columbia Anaerobe 5% Sheep Blood Agar is recommended for the general cultivation of anaerobes.

Summary and Explanation

Ellner[1], using Columbia Agar Base, formulated reducible anaerobic media designed to improve recovery of anaerobes with minimal difficulty. The reducing agents used were cysteine, palladium chloride and dithiothreitol. The presence of “organic” peroxides (or peroxide-like compounds) and the redox potential (Eh) of media are important factors in the determination of whether anaerobic organisms will grow in, or on, a particular medium. The addition of reducing agents to the medium reduces the inhibitory effects of the peroxides.

Principles of the Procedure

Columbia Anaerobe Sheep Blood Agar is a highly nutritious medium due to its content of peptones, yeast extract, beef extract, hemin, vitamin K_1 and sheep blood. The peptones provide nitrogenous growth factors, carbon, sulfur and trace ingredients. Yeast extract is an important source of B vitamins. Sodium chloride maintains osmotic equilibrium. Sheep blood constituents, hemin and vitamin K_1 provide growth factors required by certain obligate anaerobes.[2-5] The addition of L-cysteine HCl and dithiothreitol facilitates the lowering of the redox potential of medium.

Procedure

This medium should be reduced at room temperature immediately prior to inoculation by placing under anaerobic conditions for 18-24 hours.[4]

Use standard procedures to obtain isolated colonies from specimens. Inoculate an enrichment broth, such as BBL™ Enriched Thioglycollate Medium, at the same time as the primary plates to detect small numbers of anaerobes.

Incubate the plates and tubes immediately after inoculation, with plates in an inverted position (agar side up), under anaerobic conditions at 35°C, or place the media in a holding jar flushed with oxygen-free gas(es) until a sufficient number of plates and tubes is accumulated (no longer than 3 hours).[6] An efficient and easy way to obtain suitable anaerobic conditions is through the use of a **BD GasPak**™ EZ anaerobic system or an alternative anaerobic system. Incubate for at least 48 hours and, if no growth occurs, continue incubation for up to 7 days.

Expected Results

After sufficient incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Growth in liquid media is indicated by the presence of turbidity compared with uninoculated control.

Examine colonies using a dissecting microscope and with a long-wave UV lamp to detect fluorescence. Colonies of the pigmenting *Porphyromonas-Prevotella* species should fluoresce orange to brick red under long-wave UV light. Fluorescence is visible before pigmentation.

In order to determine the relationship to oxygen of each colony type present on the medium, follow established procedures.[7] Those colony types that prove to contain obligate anaerobes can be further studied using appropriate identification methods.

References

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