COLUMBIA AGAR BASE MEDIA

PRODUCT:

Plate Media:[a]

Columbia Agar With 5% Sheep Blood, item no. P1350
Columbia Agar With 5% Horse Blood,item no. P1355
Columbia CNA Agar With 5% Sheep Blood,item no. P1400
Columbia CNA Agar With 5% Sheep Blood Plus 5 mcg/ml Vancomycin and 8 mcg/ml Amphotericin B, item no. P1410

[a]see catalog for ordering options

PURPOSE:

Columbia agar is a nutrient agar used for the cultivation of fastidious and nonfastidious microorganisms. Sheep or horse blood is added to enhance further the growth of fastidious microorganisms and to assist in the preliminary identification of hemolytic strains. The media becomes selective for streptococci and staphylococci with the addition of colistin and nalidixic acid (CNA).

PRINCIPLE:

General purpose media, like columbia agar, supply the basic ingredients necessary for microbes to replicate and grow. The nutrients required for growth include nitrogen, hydrogen, carbon, mineral salts, sulfur, vitamins, and other growth factors. In order for the microbes to synthesize the nutrients, the nutrients must not only be present in the proper proportions but must also be in a form usable by the microorganisms. Heterotrophs, which most clinical microorganisms are, require a preformed food supply because it is believed they have lost the ability to synthesize energy from simple organic compounds.[2] Preformed food supplies come in the form of amino acids, carbohydrates, peptides, lipids, and bacterial vitamins and can be added to agar to form a highly nutritious culture medium.

Peptones, digestive products of protein, consist of a mixture of nitrogenous compounds: proteoses, polypeptides, and amino acids. Enzymes (pancreatic, papaic, and trypsin) digest animal and vegetable proteins, while preserving the vitamins and keeping the amino acids intact. Yeast and meat extracts provide additional nitrogenous compounds, carbohydrates, and vitamins.

Ellner et al.[3] discovered peptones from both animal and vegetable protein to be complementary, and the growth of the microorganisms to be better than on the then more frequently used base media (casein hydrolysate or meat infusion media). In addition, yeast and beef extracts were added and appeared to increase the growth of *Neisseria* species, while cornstarch, by neutralizing the inhibitory effects of glucose, decreased the formation of a green coloration (alpha hemolysis) by beta-hemolytic streptococci. Columbia agar was made a selective medium by adding colistin and nalidixic acid (CNA), which inhibit gram-negative microorganisms. CNA was found to be more effective in suppressing *Proteus*, *Klebsiella*, and *Pseudomonas* species than Phenylethyl Alcohol Agar (PEA) and did not restrict the growth of gram-positive cocci as did PEA.

Green, et al.,[10] described a media for screening stool specimens for Vancomycin Resistant Gram-Positive Cocci (VRGPC) in children that incorporates vancomycin (5 mcg/ml) and amphotericin B (8 mcg/ml) into Columbia CNA Agar. There are increasing reports of infections due to VRGPC that include *Streptococcus*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Pediococcus* species. Currently Vancomycin Resistant Enterococcus (VRE) is the most widely recognized Vancomycin Resistant Gram-Positive Cocci (VRGPC). Because of the emergence of other VRGPC, there is a need to screen patients for these organisms. Therefore, the addition of vancomycin and amphotericin B (fungal inhibitor) allows the laboratory to screen contaminated specimens for possible VRGPC while holding down the overgrowth of normal flora organisms.

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FORMULAS:

Approximate, per liter of deionized filtered water.

(1) Columbia Agar With 5% Sheep Blood:

Peptic Digest of Animal Tissue	5.0 g
Pancreatic Digest of Casein	5.0
Yeast Enriched Peptone	10.0
Pancreatic Digest of Heart Muscle	3.0
Cornstarch	1.0
Sodium Chloride	5.0
Agar	14.0
Sheep Blood	50.0 ml

Final pH 7.3 ± 0.2 at 25°C

(2) Columbia Agar With 5% Horse Blood:
Same as (1) above except it also contains 50.0 ml ofHorse Blood.

(3) Columbia CNA Agar With 5% Sheep Blood:
Same as (1) above except it also contains 10.0 mg of Colistin and 10.0 mg of Nalidixic Acid.

(4) Columbia CNA Agar With 5% Sheep Blood Plus 5 mcg/ml Vancomycin and 8 mcg/ml Amphotericin B:
Same as (3) above except it also contains 5.0 mg of Vancomycin and 8.0 mg Amphotericin B.

PRECAUTIONS:*

For in vitro diagnostic use. Observe approved biohazard precautions.

Storage: Upon receipt store at 2-8°C away from direct light. Media should not be used if there are signs of contamination, deterioration (shrinking, cracking, or discoloration), or if the expiration date has passed.

Limitations: On columbia agar with blood, colonial morphology and hemolysis may not be typical; small amounts of reducing sugars inhibit the expression of beta hemolysis. Columbia CNA Agar With 5% Sheep Blood inhibits many strains of coagulase-negative staphylococci, especially *Staphylococcus saprophyticus*.[7]

Columbia agar with 5% horse blood is very sensitive to light, heat, and temperature fluctuations. Medium will perform satisfactorily if storage instructions on each package label are followed.

Columbia agar with 5% blood is a nonselective media; biochemical and/or serologic testing are necessary for definitive identification of microorganisms.

Haemophilus hemolyticus and *Haemophilus parahaemolyticus* appear beta-hemolytic on horse blood agar and are indistinguishable from group A streptococci; additional tests are required for definitive identification.

Sheep blood contains V factor-destroying enzyme (nucleotidase) which prevents the growth of *Haemophilus* species on sheep blood agar, unless another microorganism provides the V factor (satellitism).

Over inoculation of contaminated specimens (stool, rectal, etc.) onto selective media may decrease the inhibitory performance of the media.

Organisms isolated on selective media must be identified using appropriate biochemical tests and tested for antibiotic sensitivity if appropriate using approved NCCLS methods.

PROCEDURE:*

Specimen Collection: Information on specimen collection is found in standard reference material. In general, specimens should be protected from extremes of heat and cold and should be delivered to the laboratory without delay.

Method of Use: Prior to inoculation, the medium should be brought to room temperature. Inoculate according to standard microbiological procedures and streak the inoculum so as to obtain isolated microbial colonies. Incubate under conditions that will permit growth. In general, incubate at 35°C for 18-72 hours with adequate moisture in either aerobic, capnophilic or anaerobic atmosphere depending on the specific microbes to be cultured.

Interpretation:

Organism	Colonial Morphology
Streptococcus pyogenes	Small, beta-hemolytic, transparent to opaque, domed, smooth and entire edge.
Streptococcus viridans	Small, alpha-hemolytic, transparent to opaque, domed, smooth and entire edge.
Streptococcus pneumoniae	Small, alpha-hemolytic, round and mucoid with entire edge.
Staphylococcus aureus	Average, ± hemolysis, opaque, circular, smooth, raised, white to golden yellow pigent.
Staphylococcus epidermidis	Average, ± hemolysis, opaque, circular, smooth, raised, usually white to colorless.
Corynebacteria	Small, grayish colonies.
Listeria monocytogenes	Small, beta-hemolytic, transparent, gray to white.
Yeast	Small, white to gray in 48-72 hours.
Escherichia coli	Large, grayish colonies.

For other clinically significant organisms, a reference such as Lennette et al.[9] should be consulted.

Material Required but Not Provided: Standard microbiological supplies and equipment such as loops, needles, incubator, and incinerator are not provided.

QUALITY CONTROL:*

Microorganisms Used (ATCC #):	Columbia Agar +5% Sheep Blood	Expected Results: Columbia Agar + 5% Horse Blood	Columbia CNA Agar
Staphylococcus aureus (25923)	Growth	Growth	Growth
Escherichia coli (25922)	Growth	Growth	inhibition
Streptococcus pyogenes (19615)	Growth, beta hemolysis	Growth, beta hemolysis	Growth, beta hemolysis
Streptococcus pneumoniae (6305)	Growth, alpha hemolysis	Growth, alpha hemolysis	Growth, alpha hemolysis
Haemophilus parahaemolyticus (10014)		Growth, beta hemolysis	

Columbia CNA Agar Plus Vancomycin and Amphotericin B	Expected Results
Enterococcus faecalis (51299)	Growth
Enterococcus faecalis (29212)	Inhibition
Escherichia coli (25922)	Inhibition
Candida albicans (10231)	Inhibition

User Quality Control: Check for signs of contamination and deterioration.

BIBLIOGRAPHY:

1. Baker, F. J., and M. R. Breach, *Med. Microbiol. Techniques,* 1980.
2. Casman, E. P., *Am. J. Clin. Pathol.* 17:281-289, 1947.
3. Ellner, P. D., et al., *Am. J. Clin. Pathol.* 45:502-504, 1966.
4. Facklam, R. R., *Isolation and Identification of Streptococci*, USHEW, PHS, Centers for Disease Control, Atlanta, 1980.
5. Finegold, S. M., and E. J. Baron, *Bailey and Scott's Diagnostic Microbiology*, 7th ed., C. V. Mosby, St. Louis, 1986.
6. Jawetz, E. L., et al., *Review of Medical Microbiology*, 17th ed., Appleton and Lange, Norwalk, Conn., 1987.
7. Koneman, E. W., et al., *Color Atlas and Textbook of Diagnostic Microbiology*, 3rd ed., J. B. Lippincott, Philadelphia, 1988.
8. Lennette, E. H., et al., *Manual of Clinical Microbiology*, 3rd ed., American Society for Microbiology, Washington, D. C., 1980.
9. Lennette, E.H., et al., *Manual of Clinical Microbiology*, 4th ed., American Society for Microbiology, Washington, D.C., 1985.
10. Green, M., et al., *Journal of Clinical Microbiology*, 28:484-488, 1990.

*For more detailed information, consult appropriate references and/or details in the preface of the PML Technical Manual.

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MACCONKEY MEDIA

PRODUCT:

Plate and Tube Media:[a]

MacConkey Agar, item No. P1800
MacConkey-Sorbitol Agar, Item no. P1863
MacConkey Agar Without Crystal Violet, item no. P1850
MacConkey Agar No. 2, item no. P1852
MacConkey Agar Plus 8 mcg/ml Ceftazidime, item no. P1867

[a]see catalog for ordering options

PURPOSE:

MacConkey media are selective, differential media used to isolate enteric microorganisms from mixtures of bacteria. In addition, MacConkey Agar Without Crystal Violet is used to distinguish *Mycobacterium fortuitum-chelonei* complex from other rapidly growing mycobacteria. MacConkey Agar No. 2 is a modification of the original MacConkey medium and is especially useful for the recognition of enterococci in the presence of coliforms and nonlactose-fermenting organisms.

PRINCIPLE:

In 1905 MacConkey[8] first described the selective, differential medium that he used to isolate enteric gram-negative bacilli. It consisted of a nutritious base medium that also contained crystal violet and bile salts which inhibited the growth of gram-positive microorganisms. The original formula has been modified by an addition of sodium chloride and a modification of the concentration of bile salts, agar, and neutral red. These changes have enhanced the recovery of *Shigella* and *Salmonella* species, the differentiation of coliforms from enteric pathogens, and the inhibition of the swarming of *Proteus* species. Microorganisms capable of growing on MacConkey Agar and capable of metabolizing lactose, produce acid by-products that lower the pH of the medium close to the colony. The lowering of the pH causes the neutral red indicator to turn red, and if sufficient acid is produced, a zone of precipitated bile develops around the colony. Microbes that do not metabolize lactose appear colorless and translucent.

Substituting a less selective bile salts mixture in the MacConkey Agar No. 2 formula permits the growth of enterococci as well as members of the family *Enterobacteriaceae*. While it is selective, it does not suppress a mixed bacterial flora to the same extent as other inhibitory media, including other MacConkey agars. Enterococci are frequently sought as an index of fecal pollution. Nonlactose-fermenting organisms are colorless on this medium; bio-tolerant micrococci such as staphylococci and nonfecal streptococci are inhibited.

Excluding crystal violet and sodium chloride from the MacConkey Agar formula permits the growth of staphylococci, enteric cocci, and mycobacteria, as well as members of the *Enterobacteriaceae* while restricting the swarming of most *Proteus* species. Due to its ability to support the growth of gram-positive cocci, this medium is recommended for the cultivation of pathogens that may be present in a variety of specimens such as urine, feces, and wounds. MacConkey Agar Without Crystal Violet can also be used in determining the fecal contamination of food and water. *Escherichia coli* and other coliforms serve as indicators of probable contamination; the presence of fecal streptococci confirms fecal pollution.[4] MacConkey Agar Without Crystal Violet also serves as a useful aid in the identification of rapidly growing mycobacteria. *Mycobacterium fortuitum-chelonei* complex will grow on this medium while other rapid growers do not.

A further modification of MacConkey Agar was made by Farmer and Davis;[1] they substituted sorbitol for lactose and made possible a screening method for the detection of *Escherichia coli* O157:H7 strains which have been implicated as a causative agent of hemorrhagic colitis.[9] *Escherichia coli* O157:H7 strains are unable to ferment sorbitol within 48 hours and remain colorless and translucent; 95% of other *Escherichia coli* strains ferment sorbitol and appear red due to the production of acid by-products which cause the neutral red indicator to turn red. Other gram-negative microbes that do not ferment sorbitol will grow on MacConkey-Sorbitol Agar and necessitate biochemical and/or serologic testing for definitive identification.

There have been recent reports describing Extended-Spectrum ß-Lactamase (ESBL) in certain Enterobacteriaceae isolates. These isolates include Escherichia, Klebsiella, and Citrobacter species that have acquired a plasmid-mediated resistance to broad-spectrum cephalosporins with the probability of spreading to other Enterobacteriaceae members.[15] The addition of ceftazidime to the MacConkey medium allows for the screening of ESBL organisms from clinical specimens. Because ESBL resistance is carried on a transmittable plasmid, screening for possible carriers would be of value for epidemiological and infectious disease tracking.

FORMULAS:

Approximate, per liter of deionized filtered water.

(1) MacConkey Agar:

Ingredient	Amount
Pancreatic Digest of Gelatin	17.0 g
Peptic Digest of Animal Tissue	1.5
Pancreatic Digest of Casein	1.5
Lactose	10.0
Bile Salts Mixture	1.5
Sodium Chloride	5.0
Agar	13.5
Neutral Red	30.0 mg
Crystal Violet	1.0

Final pH 7.1 ± 0.2 at 25°C

(2) MacConkey-Sorbitol Agar:

Ingredient	Amount
Pancreatic Digest of Gelatin	17.0 g
Peptic Digest of Animal Tissue	1.5
Pancreatic Digest of Casein	1.5
Sorbitol	10.0
Bile Salts Mixture	1.5
Sodium Chloride	5.0
Agar	13.5
Neutral Red	30.0 mg
Crystal Violet	1.0

Final pH 7.1 ± 0.2 at 25°C

(3) MacConkey Agar Without Crystal Violet:

Ingredient	Amount
Pancreatic Digest of Gelatin	20.0 g
Lactose	10.0
Bile Salts Mixture	5.0
Agar	12.0
Neutral Red	75.0 mg

Final pH 7.4 ± 0.2 at 25°C

(4) MacConkey Agar No. 2:

Ingredient	Amount
Pancreatic Digest of Gelatin	20.0 g
Lactose	10.0
Bile Salts No. 2	1.5
Sodium Chloride	5.0
Agar	15.0
Neutral Red	50.0 mg
Crystal Violet	1.0

Final pH 7.2 ± 0.2 at 25°C

(5) MacConkey Agar Plus 8 mcg/ml Ceftazidime:
Same as (1) above except it also contains 8 mg of Ceftazidime.

PRECAUTIONS:*

For in vitro diagnostic use. Observe approved biohazard precautions.

Storage: Upon receipt, store at 2-8°C in the dark. Media should not be used if there are signs of contamination, deterioration (cracking, shrinking, or discoloration), or if the expiration date has passed.

Limitations: Some strains of gram-negative enteric bacilli may not grow or grow slowly on selective media, and some strains of gram-positive microbes will not be inhibited or will be only partially inhibited.

On MacConkey-Sorbitol Agar, microbes other than *Escherichia coli* O157:H7 may grow, not ferment sorbitol, and produce colorless, translucent colonies. Biochemical and/or serologic testing must be performed for definitive identification.

MacConkey Agar No. 2 is less selective and streptococci will grow.

MacConkey Without Crystal Violet is less selective and staphylococci and streptococci will grow.

All formulations are light sensitive; exposure will cause the pH to become acid, thus invalidating growth and biochemical reactions. Protect media from light.

Over inoculation of contaminated specimens (stool, rectal, etc.) onto selective media may decrease the inhibitory performance of the media.

Organisms isolated on selective media must be identified using appropriate biochemical tests and tested for antibiotic sensitivity if appropriate using approved NCCLS methods.

PROCEDURE:*

Specimen Collection: Information on specimen collection can be found in standard reference material. In general, specimens should be protected from extremes of heat and cold, should be delivered to the laboratory without delay, and should be obtained before the initiation of antimicrobial therapy. If there is a delay, a suitable transport medium such as Cary-Blair or Amies should be used.

Method of Use for MacConkey Agar, MacConkey No. 2, and MacConkey-Sorbitol Agar: Prior to inoculation, the medium should be brought to room temperature. Directly inoculate the specimen or inoculate a sampling from a transport medium onto the selective agar using standard microbiological procedures. Streak the inoculum so as to obtain isolated colonies. Incubate for 18-48 hours and examine for growth and fermentation. Strong lactose and sorbitol fermenters will form deep red colonies on the respective medium. Weak fermenters will form light pink colonies or colonies that have pink centers with a clear periphery. Nonfermenters will form colorless, translucent colonies.

Method of Use for MacConkey Without Crystal Violet: For water, waste, and food procedures see references listed in the bibliography.[5,10,12,13]

For rapidly growing mycobacteria, use a broth culture of the mycobacteria, such as 7H9 Broth, and inoculate a three-millimeter loopful of the broth onto the plated medium. Streak for isolation and incubate at 35°C. Examine for growth at 5 days and 11 days. *Mycobacterium fortuitum-chelonei* complex will grow to the end of the streak line; other rapidly growing mycobacteria will not grow or will grow only where the inoculum is heavy.

Interpretation:

Medium / Organism	Colony appearance
MacConkey Agar	
Escherichia coli	Red, smooth, circular, with zone of precipitation and entire edge.
Salmonella species	Circular, moist, smooth, translucent to opaque, colorless, with entire edge.
Shigella species	Circular, colorless, moderately transparent, smooth, entire edge.
Proteus species	Circular, smooth, translucent, colorless; some strains will show signs of spreading, but spreading is usually inhibited.
Enterobacter species	Colorless to pink with pink centers, mucoid, thick, smooth, with entire edge.
Pseudomonas species	Large, spreading, colorless to grayish-green with dark centers, translucent, with irregular edge.
MacConkey Sorbitol Agar	
Escherichia coli O157:H7	Colorless, smooth, circular and entire edge.
Escherichia coli	Red, smooth, circular, with zone of precipitation and entire edge.
MacConkey Agar without Crystal Violet	
Enterococcus faecalis	Small, red, smooth, domed, circular.
Staphylococcus aureus	Pale pink-red, smooth, domed, circular.
MacConkey Agar No. 2	
Enterococcus faecalis	Small red, smooth, domed, circular.

Material Required but Not Provided: Standard microbiological supplies and equipment such as loops, incinerators, incubators, and transport media are not provided.

QUALITY CONTROL:*

Medium Used:	Microorganisms Used (ATCC#):	Expected Results:
MacConkey Agar	*Escherichia coli* (25922)	Growth
	Proteus mirabilis (12453)	Growth
	Salmonella typhimurium (14028)	Growth
	Enterococcus faecalis (29212)	Inhibition
MacConkey-Sorbitol Agar	*Escherichia coli* O157:H7 (35150)	Growth
	Escherichia coli (25922)	Growth
	Enterococcus faecalis (29212)	Inhibition
MacConkey Agar Without Crystal Violet	*Escherichia coli* (25922)	Growth
	Proteus mirabilis (12453)	Growth
	Staphylococcus aureus (25923)	Growth
	Enterococcus faecalis (29212)	Growth
MacConkey Agar No. 2	*Escherichia coli* (25922)	Growth
	Proteus mirabilis (12453)	Growth
	Staphylococcus aureus (25923)	Inhibition
	Enterococcus faecalis (29212)	Growth
MacConkey Agar Plus 8 mcg/ml Ceftazidime	*Klebsiella pneumoniae* (PML #425)	Growth
	Klebsiella pneumoniae (PML #427)	Growth
	Escherichia coli (25922)	Inhibited
	Pseudomonas aeruginosa (27853)	Inhibited

Key: See "Interpretation"

User Quality Control: Check for signs of contamination and deterioration.

BIBLIOGRAPHY:

1. Farmer, J. J., III, and B. R. Davis, *J. Clin. Microbiol*., 22:620, 1985.
2. Finegold, S. M., and E. J. Baron, *Bailey and Scott's Diagnostic Microbiology*, 7th ed., C. V. Mosby, St. Louis, 1986.
3. *International Standards for Drinking Water Quality* vol. 1, World Health Organization, Washington, D. C., 1984.
4. Jawetz, E. L., et al., *Review of Medical Microbiology*, 17th ed., Appleton and Lange, Norwalk, Conn., 1987.
5. Jones, W. D., Jr., and G. D. Kubica, *Am. J. Med. Tech*., 30:1, 1964.
6. Koneman, E. W., et al., *Color Atlas and Textbook of Diagnostic Microbiology*, 3rd ed., J. B. Lippincott, Philadelphia, 1988.
7. Lennette, E. H., et al., *Manual of Clinical Microbiology*, 4th ed., American Society for Microbiology, Washington, D. C., 1985.
8. MacConkey, A. T., *J. Hyg*., 5:333, 1905.
9. March, S. B., and S. J. Ratman, *Clin. Microbiol*., 23:869, 1986.
10. *Recommended Methods for Microbiological Examination of Foods,* 2nd ed., American Public Health Association, Washington, D. C., 1984.
11. Riley, L. W., et al., *New Engl. J. Med*., 308:681, 1983.
12. *Standard Methods for Examination of Dairy Products* 15th ed., American Public Health Association, Washington, D. C., 1985.
13. *Standard Methods for Examination of Water and Wastewater* 15th ed., American Public Health Association, Washington, D. C., 1985.
14. Wells, J. G., et al., *J. Clin. Microbiol.*, 18:512, 1983.
15. Philippon, A., et al., *Antimicrobial Agents and Chemotherapy*, 33:1131-1136, 1989.

*For more detailed information, consult appropriate references and/or details in the preface of the PML Technical Manual.

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