4. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
5. Aseptically add 12.5 mL Egg Yolk Enrichment 50% and 4.1 mL Antimicrobic Vial P rehydrated with 5 mL sterile water (25,000 units of polymyxin B). Mix thoroughly.

Difco™ Antimicrobic Vial P (Polymyxin B)

1. To rehydrate, aseptically add 5 mL sterile purified water (to achieve the desired concentration for MYP Agar).
2. Rotate in an end-over-end motion to dissolve the contents completely.

Procedure

Consult appropriate references.[4-6]

Expected Results

Consult appropriate references.[4-6]

References

1. Mossel, Koopman and Jongerius. 1967. Appl. Microbiol. *15*:650.
2. Donovan. 1958. J. Appl. Bacteriol. *21*:100.
3. Coliner. 1948. J. Bacteriol. *55*:777.
4. Rhodehamel and Harmon. 1995. FDA bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
5. Bennett and Belay. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
6. Andrews. 2000. *In* Horwitz (ed.), Official methods of analysis of AOAC International, 17th ed., vol. I. AOAC International, Gaithersburg, Md.

Availability

Difco™ MYP Agar

AOAC BAM COMPF ISO USDA

Cat. No. 281010 Dehydrated – 500 g

Europe

Cat. No. 257004 Prepared Plates – Pkg. of 20*

Japan

Cat. No. 251264 Prepared Plates – Pkg. of 20*
251265 Prepared Plates – Ctn. of 100*

Difco™ Antimicrobic Vial P

AOAC BAM COMPF ISO USDA

Cat. No. 232681 Vial – 6 × 10 mL*

Difco™ Egg Yolk Enrichment 50%

AOAC BAM COMPF ISO USDA

Cat. No. 233471 Tube – 12 × 10 mL*
233472 Bottle – 6 × 100 mL*

**Store at 2-8°C.*

MacConkey Agars
MacConkey Agar • MacConkey Agar Base
MacConkey Agar without Crystal Violet
MacConkey Agar without Crystal Violet or Salt
MacConkey Agar without Salt

Intended Use

MacConkey Agar conforms with the specifications of *The United States Pharmacopeia (USP)*.

MacConkey agars are slightly selective and differential plating media mainly used for the detection and isolation of gram-negative organisms from clinical,[1] dairy,[2] food,[3,4] water,[5] pharmaceutical[6] and industrial[7] sources.

MacConkey Agar is used for isolating and differentiating lactose-fermenting from lactose-nonfermenting gram-negative enteric bacilli.

MacConkey Agar Base is used with added carbohydrate in differentiating coliforms based on fermentation reactions.

MacConkey Agar without Crystal Violet is used for isolating and differentiating enteric microorganisms while permitting growth of staphylococci and enterococci. The medium can be used also to separate *Mycobacterium fortuitum* and *M. chelonae* from other rapidly growing mycobacteria.

MacConkey Agar without Crystal Violet or Salt and MacConkey Agar without Salt are used for isolating and differentiating gram-negative bacilli while suppressing the swarming of most *Proteus* species.

Summary and Explanation

MacConkey Agar is based on the bile salt-neutral red-lactose agar of MacConkey.[8]

The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Formula modifications improved the growth of *Shigella* and *Salmonella* strains. These modifications included the addition of 0.5% sodium chloride, decreased agar content, and altered bile salts and neutral red concentrations. The formula improvements gave improved differential reactions between these enteric pathogens and the coliform group.

MacConkey Agar contains crystal violet and bile salts that inhibit gram-positive organisms and allow gram-negative organisms to grow. Isolated colonies of coliform bacteria are brick red in color and may be surrounded by a zone of precipitated bile. This bile precipitate is due to a local pH drop around the colony due to lactose fermentation. Colonies that do not ferment lactose (such as typhoid, paratyphoid and dysentery bacilli) remain colorless. When lactose nonfermenters grow in proximity to coliform colonies, the surrounding medium appears as cleared areas. It is recommended in the *USP* for use in the performance of Microbial Limit Tests.[6]

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™ MacConkey Agar

Dehydrated Appearance:	Pink to pinkish beige, free-flowing, homogeneous.
Solution:	5.0% solution, soluble in purified water upon boiling. Solution is reddish purple, slightly opalescent.
Prepared Appearance:	Reddish purple, slightly opalescent.
Reaction of 5.0% Solution at 25°C:	pH 7.1 ± 0.2

Difco™ MacConkey Agar Base

Dehydrated Appearance:	Pink to pinkish beige, free-flowing, homogeneous.
Solution:	4.0% solution, soluble in purified water upon boiling. Solution is red, very slightly to slightly opalescent.
Prepared Appearance:	Red, slightly opalescent.
Reaction of 4.0% Solution at 25°C:	pH 7.1 ± 0.2

Difco™ MacConkey Agar without Crystal Violet

Dehydrated Appearance:	Pinkish beige, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in purified water upon boiling. Solution is reddish orange, clear to very slightly opalescent.
Prepared Appearance:	Reddish orange, slightly opalescent.
Reaction of 5.2% Solution at 25°C:	pH 7.4 ± 0.2

Difco™ MacConkey Agar without Salt

Dehydrated Appearance:	Pinkish beige, free-flowing, homogeneous.
Solution:	4.7% solution, soluble in purified water upon boiling. Solution is reddish orange, slightly opalescent.
Prepared Appearance:	Reddish orange, slightly opalescent.
Reaction of 4.7% Solution at 25°C:	pH 7.4 ± 0.2

Continued

Identity Specifications

BBL™ MacConkey Agar

Dehydrated Appearance:	Fine, homogenous, may contain dark particles.
Solution:	5.0% solution, soluble in purified water upon boiling. Solution is medium to dark, rose to brown-rose with or without a trace orange tint, clear to slightly hazy.
Prepared Appearance:	Medium to dark, rose to brown-rose with or without a trace orange tint, clear to slightly hazy.
Reaction of 5.0% Solution at 25°C:	pH 7.1 ± 0.2

BBL™ MacConkey Agar without Crystal Violet

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	5.2% solution, soluble in purified water upon boiling. Solution is medium, red-orange to red-rose, slightly hazy to hazy.
Prepared Appearance:	Medium, red-orange to red-rose, slightly hazy to hazy.
Reaction of 5.2% Solution at 25°C:	pH 7.4 ± 0.2

BBL™ MacConkey Agar without Crystal Violet or Salt

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.37% solution, soluble in purified water upon boiling. Solution is medium, red-orange to red-rose, slightly hazy to hazy.
Prepared Appearance:	Medium, red-orange to red-rose, slightly hazy to hazy.
Reaction of 4.37% Solution at 25°C:	pH 7.4 ± 0.2

Continued

MacConkey Agar Base is prepared without added carbohydrates, which permits their addition either individually or in combination. It is recommended that carbohydrates such as sucrose or lactose be added in a concentration of 1% to the basal medium.

MacConkey Agar without Crystal Violet is a differential medium that is less selective than MacConkey Agar. The lack of crystal violet permits the growth of *Staphylococcus* and *Enterococcus*. Staphylococci produce pale pink to red colonies and enterococci produce compact tiny red colonies either on or beneath the surface of the medium. The medium is used also to separate *Mycobacterium fortuitum* and *M. chelonae* from other rapidly growing mycobacteria.[9,10]

MacConkey Agar without Crystal Violet or Salt and MacConkey Agar without Salt (which also lacks crystal violet) are differential media used for isolating and cultivating gram-negative enteric organisms and gram-positive cocci from waters, feces and other sources suspected of containing these organisms, as well as limiting the swarming of *Proteus* species.

Principles of the Procedure

Peptones are sources of nitrogen and other nutrients. Lactose is a fermentable carbohydrate. When lactose is fermented, a local pH drop around the colony causes a color change in the pH indicator (neutral red) and bile precipitation. Bile salts, bile salts no. 3, oxgall and crystal violet are selective agents that inhibit growth of gram-positive organisms. Agar is the solidifying agent.

Cultural Response

Difco™ MacConkey Agar or Difco™ MacConkey Agar Base

Prepare the medium per label directions. For MacConkey Agar Base, prepare without and with 1% added lactose. Inoculate and incubate at 35 ± 2°C for 18-24 hours (and 40-48 hours for *E. coli*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	29212	10^3-2×10^3	Partial to complete inhibition	–	–
Escherichia coli	25922	10^2-10^3	Good	Pink to red; w/o lactose: Colorless	+ –
Proteus mirabilis	12453	10^2-10^3	Good	Colorless	–
Salmonella choleraesuis subsp. *choleraesuis* serotype Typhimurium	14028	10^2-10^3	Good	Colorless	–

Difco™ MacConkey Agar without Crystal Violet

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	29212	10^2-10^3	Good	Red	–
Escherichia coli	25922	10^2-10^3	Good	Pink to red	–
Proteus mirabilis	12453	10^2-10^3	Good	Colorless	–
Salmonella choleraesuis subsp. *choleraesuis* serotype Typhimurium	14028	10^2-10^3	Good	Colorless	–

Difco™ MacConkey Agar without Salt

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	33186	10^2-10^3	Good	Red	–
Escherichia coli	25922	10^2-10^3	Good	Pink to red	–
Proteus mirabilis	12453	10^2-10^3	Good	Colorless, no swarming	–
Salmonella choleraesuis subsp. *choleraesuis* serotype Typhimurium	14028	10^2-10^3	Good	Colorless	–
Shigella flexneri	12022	10^2-10^3	Good	Colorless	–

Cultural Response

BBL™ MacConkey Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours (and 40-48 hours for *E. coli*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	29212	10^3-2×10^3	Partial to complete inhibition	–	–
Escherichia coli	25922	10^2-10^3	Good	Pink to rose-red	+
Proteus mirabilis	12453	10^2-10^3	Good	Colorless	–
Salmonella choleraesuis subsp. *choleraesuis* serotype Typhimurium	14028	10^2-10^3	Good	Colorless	–

BBL™ MacConkey Agar without Crystal Violet

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours and up to 48 hours if necessary (up to 11 days for *M. fortuitum*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	29212	10^3-10^4	Good	Rose-red	–
Escherichia coli	25922	10^3-10^4	Good	Pink to rose-red	–
Mycobacterium fortuitum	6841	10^3-10^4	Good	Rose-red	–
Salmonella choleraesuis subsp. *choleraesuis* serotype Typhimurium	14028	10^3-10^4	Good	Colorless	–
Staphylococcus aureus	25923	10^3-10^4	Good	Pink to rose-red	–

BBL™ MacConkey Agar without Crystal Violet or Salt

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours and up to 48 hours if necessary.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	29212	10^3-10^4	Good	Rose-red	–
Escherichia coli	25922	10^3-10^4	Good	Pink to rose-red	–
Proteus mirabilis	12453	10^3-10^4	Good	Colorless, no swarming	–
Salmonella choleraesuis subsp. *choleraesuis* serotype Typhimurium	14028	10^3-10^4	Good	Colorless	–

Formulae

Difco™ MacConkey Agar

Approximate Formula* Per Liter

Peptone	17.0 g
Proteose Peptone	3.0 g
Lactose	10.0 g
Bile Salts No. 3	1.5 g
Sodium Chloride	5.0 g
Agar	13.5 g
Neutral Red	0.03 g
Crystal Violet	1.0 mg

Difco™ MacConkey Agar Base

Consists of the same ingredients without the lactose.

BBL™ MacConkey Agar

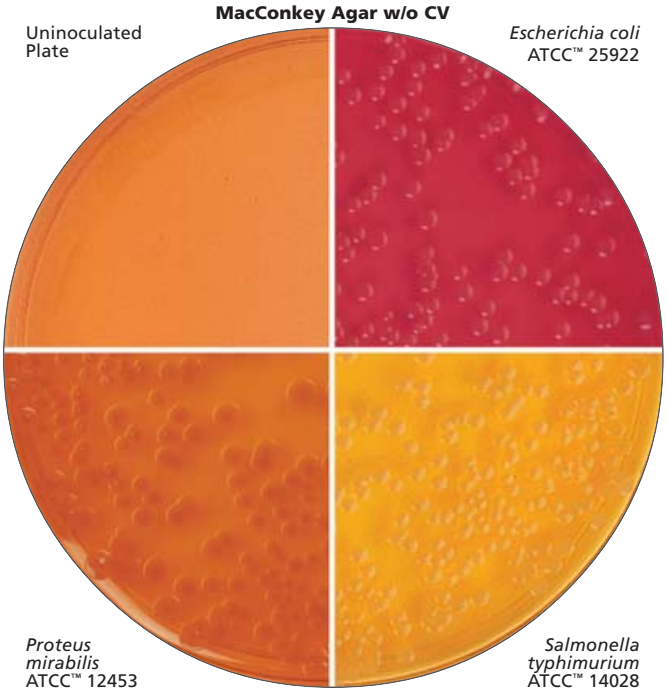
Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	17.0 g
Pancreatic Digest of Casein	1.5 g
Peptic Digest of Animal Tissue	1.5 g
Lactose	10.0 g
Bile Salts	1.5 g
Sodium Chloride	5.0 g
Agar	13.5 g
Neutral Red	0.03 g
Crystal Violet	1.0 mg

Difco™ MacConkey Agar without Crystal Violet

Approximate Formula* Per Liter

Peptone	20.0 g
Lactose	10.0 g
Bile Salts	5.0 g
Sodium Chloride	5.0 g
Agar	12.0 g
Neutral Red	0.05 g



BBL™ MacConkey Agar without Crystal Violet

Approximate Formula* Per Liter

Pancreatic Digest of Casein	10.0	g
Peptic Digest of Animal Tissue	10.0	g
Lactose	10.0	g
Bile Salts	5.0	g
Sodium Chloride	5.0	g
Agar	12.0	g
Neutral Red	0.05	g

Difco™ MacConkey Agar without Salt

Approximate Formula* Per Liter

Peptone	20.0	g
Lactose	10.0	g
Bile Salts	5.0	g
Agar	12.0	g
Neutral Red	75.0	mg

BBL™ MacConkey Agar without Crystal Violet or Salt

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	10.0	g
Yeast Extract	10.0	g
Lactose	10.0	g
Oxgall	5.0	g
Magnesium Sulfate	0.2	g
Agar	12.0	g
Neutral Red	75.0	mg

**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™ MacConkey Agar – 50 g;
   BBL™ MacConkey Agar – 50 g;
   Difco™ MacConkey Agar Base – 40 g;
   Difco™ MacConkey Agar without Crystal Violet – 52 g;
   BBL™ MacConkey Agar without Crystal Violet – 52 g;
   BBL™ MacConkey Agar without Crystal Violet or Salt – 47.3 g;
   Difco™ MacConkey Agar without Salt – 47 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.
   NOTE: If MacConkey Agar Base is to be used within 12 hours, omit autoclaving and gently boil medium for 5 minutes. Add 1% carbohydrate before or after autoclaving, depending upon heat lability. The surface of MacConkey agars without salt should be thoroughly air-dried prior to inoculation.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For procedures on the isolation and identification of enteric organisms consult the appropriate references.

Expected Results

Lactose-fermenting organisms grow as pink to brick-red colonies with or without a zone of precipitated bile. Lactose-nonfermenting organisms grow as colorless or clear colonies.

Swarming by *Proteus* spp. is reduced on MacConkey agars without salt.

On MacConkey Agar without Crystal Violet and MacConkey agars without salt, staphylococci produce pale pink to red colonies and enterococci produce tiny red colonies; these organisms are inhibited on MacConkey Agar.

On MacConkey Agar without Crystal Violet, potentially pathogenic rapid growers of the *M. fortuitum* complex usually grow in 5-11 days, while the commonly saprophytic species are inhibited.[9,10]

On MacConkey agars without salt, the swarming of *Proteus* is reduced.

Limitations of the Procedure

1. Although MacConkey media are selective primarily for gram-negative enteric bacilli, biochemical and, if indicated, serological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.[1,3]
2. Incubation of MacConkey Agar plates under increased CO_2 has been reported to reduce the growth and recovery of a number of strains of gram-negative bacilli.[11]
3. Some strains of *M. smegmatis* from humans may grow on MacConkey Agar without Crystal Violet, but these strains can be differentiated from *M. fortuitum* complex by the 3-day arylsulfatase test.[9]

References

1. Bopp, Brenner, Wells and Strockbine. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. Flowers, Andrews, Donnelly and Koenig. 1993. *In* Marshall (ed.), Standard methods for the examination of dairy products. 16th ed., American Public Health Association, Washington, D.C.
3. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington. D.C.
4. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
5. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
6. United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.
7. Horwitz (ed.). 2000. Official methods of analysis of AOAC International, 17th ed. AOAC International, Gaithersburg, Md.
8. MacConkey. 1905. J. Hyg. 5:333.
9. Kent and Kubica. 1985. Public health mycobacteriology: a guide for the level III laboratory. USDHHS, Centers for Disease Control, Atlanta, Ga.
10. Master. 1994. *In* Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1, suppl. 1. American Society for Microbiology, Washington, D.C.
11. Mazura-Reetz, Neblett and Galperin. 1979. Abstr. C179, p. 339. Abstr. Annu. Meet. American Society for Microbiology 1979.

Availability

Difco™ MacConkey Agar

AOAC | BAM | CCAM | COMPF | EP | SMD | SMWW | USP

Cat. No.	212123	Dehydrated – 500 g
	212122	Dehydrated – 2 kg
	275300	Dehydrated – 10 kg

BBL™ MacConkey Agar

AOAC | BAM | CCAM | COMPF | EP | SMD | SMWW | USP

Cat. No.	211387	Dehydrated – 500 g
	211390	Dehydrated – 5 lb (2.3 kg)
	211391	Dehydrated – 25 lb (11.3 kg)

Difco™ MacConkey Agar Base

Cat. No.	281810	Dehydrated – 500 g

Difco™ MacConkey Agar without Crystal Violet

Cat. No.	247010	Dehydrated – 500 g

BBL™ MacConkey Agar without Crystal Violet

Cat. No.	211393	Dehydrated – 500 g

Europe

Cat. No.	256008	Prepared Plates – Pkg. of 20*

BBL™ MacConkey Agar without Crystal Violet or Salt

Cat. No.	294584	Dehydrated – 500 g
	297901	Prepared Plates – Ctn. of 100*

Difco™ MacConkey Agar without Salt

Cat. No.	233120	Dehydrated – 500 g
	233110	Dehydrated – 10 kg

Europe

Cat. No.	256009	Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

MacConkey II Agar • MacConkey II Agar with MUG

Intended Use

MacConkey II Agar conforms with specifications of *The United States Pharmacopeia* (*USP*).

MacConkey II Agar is a slightly selective and differential medium for the detection of coliform organisms and enteric pathogens.

MacConkey II Agar with MUG is used for the presumptive identification of *Escherichia coli*.

Summary and Explanation

The BBL™ MacConkey II Agar formulation was made available in 1983. It was specially designed to improve the inhibition of swarming *Proteus* species, to achieve more definitive differentiation of lactose fermenters and nonfermenters, and for the promotion of superior growth of enteric pathogens.

Trepeta and Edberg[1] modified MacConkey Agar by the incorporation of MUG (4-methylumbelliferyl-β-D-glucuronide). The resulting medium allowed the authors to presumptively identify *E. coli* from the primary plating medium within 5 minutes.

Principles of the Procedure

MacConkey II Agar is a selective and differential medium. It is only slightly selective since the concentration of bile salts, which inhibit gram-positive microorganisms, is low in comparison with other enteric plating media. Crystal violet also is included in the medium to inhibit the growth of gram-positive bacteria, especially enterococci and staphylococci.

Differentiation of enteric microorganisms is achieved by the combination of lactose and the neutral red indicator. Colorless or pink to red colonies are produced depending upon the ability of the isolate to ferment the carbohydrate.

Most strains (96-97%) of *E. coli* produce β-D-glucuronidase.[2] The enzyme hydrolyzes MUG to yield 4-methylumbelliferone, a compound that fluoresces under long-wave (366 nm) UV light. The addition of MUG to the formulation allows β-D-glucuronidase-positive strains of *E. coli* to fluoresce blue-green when examined under UV light.

BBL MacConkey II Agar with MUG contains 0.1 g of MUG per liter of MacConkey II Agar.

Formula

BBL™ MacConkey II Agar

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	17.0	g
Pancreatic Digest of Casein	1.5	g
Peptic Digest of Animal Tissue	1.5	g
Lactose	10.0	g
Bile Salts	1.5	g
Sodium Chloride	5.0	g
Agar	13.5	g
Neutral Red	0.03	g
Crystal Violet	1.0	mg

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

Directions for Preparation from Dehydrated Product

1. Suspend 50 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. Test samples of the finished product for performance using stable, typical control cultures.