

## TRYPTIC SOY MEDIA

### PRODUCT:

#### Plate and Tube Media:<sup>a</sup>

Tryptic Soy Agar, item no. P2500  
 Tryptic Soy Agar With Sheep Blood, item no. P2600  
 Tryptic Soy Yeast With Rabbit Blood, item no. P2560  
 Tryptic Soy Broth, item no. (varies by volume)  
 Tryptic Soy Yeast Broth, item no. T3710, T7750

<sup>a</sup>see catalog for ordering options

### PURPOSE:

Tryptic soy is a general- purpose base medium that is used for the cultivation of fastidious microorganisms. It is a soybean-casein digest medium and meets the U.S. Pharmacopeia Standards for use in microbiological tests. Slanted tube media, with or without blood, are used for storage and/or shipment of cultures.

### PRINCIPLE:

Tryptic soy is a highly nutritious medium and is commonly used as a base medium for the cultivation of microorganisms. The addition of blood to Tryptic Soy Agar (TSA) facilitates the growth of more fastidious microorganisms and allows for the interpretation of hemolytic reactions. Hemolytic reactions can be visualized by holding the agar plate in front of a light source or by observing the area surrounding the colony under low power microscopic magnification (10X). Stabbing a loop into the agar several times at the time of media inoculation.<sup>4</sup> can enhance hemolysis

#### HEMOLYTIC REACTIONS:

Beta Hemolysis ..... Clear zone surrounding the colony (Complete lysis of the red blood cells).  
 Alpha Hemolysis ..... Green discoloration (Partial lysis of the red blood cells).  
 Alpha Prime Hemolysis ..... Green discoloration followed by a clear zone.  
 Gamma Hemolysis ..... No lysis of the red blood cells surrounding the colony.

Hemolysis may vary with the microorganism and/or with the blood source. Rabbit, horse, and sheep blood are used most frequently. Sheep blood is the generally recommended blood for addition to base agar because beta-hemolytic streptococci show a characteristic clear zone on sheep blood. In addition, *Haemophilus haemolyticus* and *Haemophilus parahaemolyticus* (normal oral flora) appear identical to beta-hemolytic streptococci on horse and rabbit blood and are inhibited on sheep blood.

HEMOLYTIC VARIANCES:	SHEEP	HORSE/RABBIT
<b>Beta-streptococci:</b>		
Groups A, B, C, F, G	Beta/None	Beta/None
<b>Enterococci:**</b>	Alpha/None	Beta/Alpha
<b>Non-enterococci:</b>	Alpha/None	Alpha/None
<b>Listeria species:</b>	Beta/None	Beta/None
<b>Haemophilus species:</b>		
<i>H. influenzae</i>	No growth	None
<i>H. parainfluenzae</i>	No growth	None
<i>H. haemolyticus</i>	No growth	Beta
<i>H. parahaemolyticus</i>	No growth	Beta

\*\* Some strains of *E. faecalis* may show beta hemolysis.<sup>1</sup>

**FORMULAS:**

Approximate, per liter of deionized filtered water.

- (1) **Tryptic Soy Agar (TSA):**  
 Pancreatic Digest of Casein ..... 15.0 g  
 Enzymatic Soy Digest ..... 5.0  
 Sodium Chloride ..... 5.0  
 Agar ..... 15.0  
 Final pH 7.3 ± 0.2 at 25°C
- (2) **Tryptic Soy Agar With Sheep Blood:**  
 Same as (1) above except it also contains various concentrations of Sheep Blood.
- (3) **Tryptic Soy Yeast Agar With 5% Rabbit Blood and 10.0g of Yeast Extract:**  
 Same as (1) above except it also contains 50.0 ml of Rabbit Blood and 10.0g of yeast extract.  
 Final pH 7.3 ± 0.2 at 25°C
- (4) **Tryptic Soy Broth:**  
 Pancreatic Digest of Casein ..... 17.0 g  
 Enzymatic Soy Digest ..... 3.0  
 Dextrose ..... 2.5  
 Sodium Chloride ..... 5.0  
 Dipotassium Phosphate ..... 2.5  
 Final pH 7.3 ± 0.2 at 25°C
- (5) **Tryptic Soy Yeast Broth:**  
 Same as (5) above except it also contains 10.0 g of Yeast Extract.  
 Final pH 7.5 ± 0.2 at 25°C

**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, evaporation, or discoloration), or if the expiration date has passed.

**Limitations:** Tryptic soy serves as a nonselective medium; biochemical and/or serological testing are necessary for the definitive identification of microorganisms isolated.

Tryptic soy media may not support the growth of some fastidious microorganisms and the addition of supplements may be required.

Approximately 2% of group A streptococci may be missed if incubated aerobically unless a provision is made to reduce the oxygen tension. It is recommended that several stabs be made into the blood agar at the time of media inoculation. These group A streptococci produce streptolysin O only, an oxygen-labile hemolysin.<sup>3</sup>

Incubation in increased CO<sub>2</sub> is less than ideal for determining streptococcal hemolysis because an increased concentration of CO<sub>2</sub> in the presence of oxygen increases the streptococcal production of peroxide which makes the red blood cells resistant to the lysins of beta-streptococci. Preferably, incubation should be in an anaerobic atmosphere,<sup>1</sup> or aerobically with stabs (see above).

The presence of dextrose in Tryptic Soy Broth (TSB) and Tryptic Soy Yeast Broth (TSYB) make these broths unsuitable for maintaining stock cultures; the fermentation of dextrose by the microorganisms may acidify the media and lead to the destruction of the microbes. These broths are not typically used for direct inoculation of specimens but are used for the growth enhancement of isolated organisms.

## MACCONKEY MEDIA

### PRODUCT:

#### Plate and Tube Media:<sup>3</sup>

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

<sup>3</sup>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-chelonae* 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<sup>3</sup> 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.<sup>4</sup> MacConkey Agar Without Crystal Violet also serves as a useful aid in the identification of rapidly growing mycobacteria. *Mycobacterium fortuitum-chelonae* complex will grow on this medium while other rapid growers do not.

A further modification of MacConkey Agar was made by Farmer and Davis;<sup>1</sup> 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.<sup>9</sup> *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  $\beta$ -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.<sup>15</sup> 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:**

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:**

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:**

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:**

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 Cefotaxime:**  
 Same as (1) above except it also contains 8 mg of Cefotaxime.

**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.<sup>5,10,12,13</sup>

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-chelonae* 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:**

**MacConkey Agar**

<i>Escherichia coli</i>	Red, smooth, circular, with zone of precipitation and entire edge.
<i>Salmonella</i> species	Circular, moist, smooth, translucent to opaque, colorless, with entire edge.
<i>Shigella</i> species	Circular, colorless, moderately transparent, smooth, entire edge.
<i>Proteus</i> species	Circular, smooth, translucent, colorless; some strains will show signs of spreading, but spreading is usually inhibited.
<i>Enterobacter</i> species	Colorless to pink with pink centers, mucoid, thick, smooth, with entire edge.
<i>Pseudomonas</i> species	Large, spreading, colorless to grayish-green with dark centers, translucent, with irregular edge.

**MacConkey Sorbitol Agar**

<i>Escherichia coli</i> O157:H7	Colorless, smooth, circular and entire edge.
<i>Escherichia coli</i>	Red, smooth, circular, with zone of precipitation and entire edge.

**MacConkey Agar without Crystal Violet**

<i>Enterococcus faecalis</i>	Small, red, smooth, domed, circular.
<i>Staphylococcus aureus</i>	Pale pink-red, smooth, domed, circular.

**MacConkey Agar No. 2**

<i>Enterococcus faecalis</i>	Small red, smooth, domed, circular.
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**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	<i>Escherichia coli</i> (25922)	Growth
	<i>Proteus mirabilis</i> (12453)	Growth
	<i>Salmonella typhimurium</i> (14028)	Growth
	<i>Enterococcus faecalis</i> (29212)	Inhibition
MacConkey-Sorbitol Agar	<i>Escherichia coli</i> O157:H7 (35150)	Growth
	<i>Escherichia coli</i> (25922)	Growth
	<i>Enterococcus faecalis</i> (29212)	Inhibition
MacConkey Agar Without Crystal Violet	<i>Escherichia coli</i> (25922)	Growth
	<i>Proteus mirabilis</i> (12453)	Growth
	<i>Staphylococcus aureus</i> (25923)	Growth
	<i>Enterococcus faecalis</i> (29212)	Growth
MacConkey Agar No. 2	<i>Escherichia coli</i> (25922)	Growth
	<i>Proteus mirabilis</i> (12453)	Growth
	<i>Staphylococcus aureus</i> (25923)	Inhibition
	<i>Enterococcus faecalis</i> (29212)	Growth
MacConkey Agar Plus 8 mcg/ml Cefazidime	<i>Klebsiella pneumoniae</i> (PML #425)	Growth
	<i>Klebsiella pneumoniae</i> (PML #427)	Growth
	<i>Escherichia coli</i> (25922)	Inhibited
	<i>Pseudomonas aeruginosa</i> (27853)	Inhibited

Key: See "Interpretation"

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

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