

MacConkey Agars with Sorbitol

MacConkey Sorbitol Agar • MacConkey II Agar with Sorbitol • Sorbitol MacConkey II Agar with Cefixime and Tellurite

Intended Use

MacConkey Sorbitol Agar and MacConkey II Agar with Sorbitol are selective and differential media for the detection of sorbitol-nonfermenting *Escherichia coli* serotype O157:H7 associated with hemorrhagic colitis. These media are also referred to as “Sorbitol MacConkey Agar.”

Sorbitol MacConkey II Agar with Cefixime and Tellurite (SMAC-CT) is a more selective and differential medium designed to inhibit *Proteus mirabilis*, non-O157 *E. coli* strains and other sorbitol-nonfermenting strains.

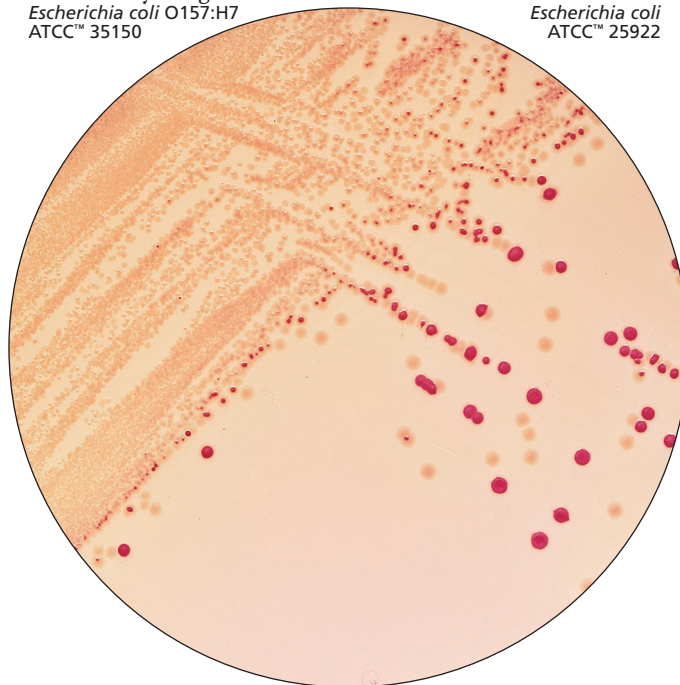
Summary and Explanation

Escherichia coli serotype O157:H7 is a human pathogen associated with hemorrhagic colitis that results from the action of a shiga-like toxin (SLT).¹ On standard MacConkey Agar containing lactose, this strain is indistinguishable from other lactose-fermenting *E. coli*. Unlike most *E. coli* strains, *E. coli* O157:H7 ferments sorbitol slowly or not at all. Therefore, the efficacy of MacConkey Agar containing sorbitol instead of lactose as a differential medium for the detection of *E. coli* O157:H7 in stool cultures was determined. Field trial results showed that the growth of *E. coli* O157:H7 on MacConkey Agar with Sorbitol was heavy and occurred in almost pure culture as colorless sorbitol-nonfermenting colonies. Most organisms

MacConkey II Agar with Sorbitol

Escherichia coli O157:H7
ATCC™ 35150

Escherichia coli
ATCC™ 25922



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 Sorbitol Agar

Dehydrated Appearance: Pinkish beige, free-flowing, homogeneous (may contain dark particles).

Solution: 5.0% solution, soluble in purified water upon boiling. Solution is reddish-purple, very slightly to slightly opalescent.

Prepared Appearance: Reddish-purple, slightly opalescent.

Reaction of 5.0%

Solution at 25°C: pH 7.1 ± 0.2

Cultural Response

Difco™ MacConkey Sorbitol Agar

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

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
<i>Enterococcus faecalis</i>	29212	10 ³ -2×10 ³	Marked to complete inhibition	—	—
<i>Escherichia coli</i>	25922	10 ² -10 ³	Good	Pink-red	+
<i>Escherichia coli</i> O157:H7	35150	10 ² -10 ³	Good	Colorless	—

Identity Specifications

BBL™ MacConkey II Agar with Sorbitol

Dehydrated Appearance: Fine, homogeneous, 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

Cultural Response

BBL™ MacConkey II Agar with Sorbitol

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours (42-48 hours for *E. coli* ATCC™ 25922).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
<i>Escherichia coli</i>	25922	10 ³ -10 ⁴	Good	Pink to red to rose-red	+
<i>Escherichia coli</i> O157:H7	35150	10 ³ -10 ⁴	Good	Colorless	—
<i>Proteus mirabilis</i>	12453	10 ³ -10 ⁴	Good	Colorless	—

of the fecal flora ferment sorbitol and appear pink on this medium. MacConkey Agar with Sorbitol, therefore, permits ready recognition of *E. coli* O157:H7 in stool cultures.¹⁻³

The addition of cefixime and tellurite significantly reduces the number of sorbitol nonfermenters that need to be screened during the attempted isolation of *E. coli* O157:H7.^{4,5}

Principles of the Procedure

MacConkey Sorbitol Agar and MacConkey II Agar with Sorbitol, modified MacConkey agars using sorbitol instead of lactose, are only slightly selective, since the concentration of bile salts, which inhibits 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. MacConkey II Agar with Sorbitol is also formulated to reduce swarming of *Proteus* species.

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

SMAC-CT is modified MacConkey II Agar using sorbitol instead of lactose and with cefixime (0.05 mg/L) and potassium tellurite (2.5 mg/L) added. Cefixime inhibits *Proteus* spp. and tellurite inhibits non-O157 *E. coli* and other organisms, thus improving the selectivity of SMAC-CT for *E. coli* O157:H7.

Formulae

Difco™ MacConkey Sorbitol Agar

Approximate Formula* Per Liter	
Peptone	15.5 g
Proteose Peptone	3.0 g
D-Sorbitol	10.0 g
Bile Salts	1.5 g
Sodium Chloride	5.0 g
Agar	15.0 g
Neutral Red.....	0.03 g
Crystal Violet.....	1.0 mg

BBL™ MacConkey II Agar with Sorbitol

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
D-Sorbitol	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.

Procedure

Prepare plated medium from tubed agar deeps by liquefying the medium in boiling water, cooling to 45-50°C and pouring into sterile Petri dishes.

Use standard procedures to obtain isolated colonies from specimens. Incubate plates, protected from light, in an inverted position (agar side up) at 35 ± 2°C for 18-24 hours in an aerobic atmosphere without additional CO₂.

Expected Results

After 18-24 hours of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Sorbitol fermenters produce pink to red colonies, some surrounded by zones of precipitated bile, while sorbitol nonfermenters produce colorless colonies.

Most fecal flora will be partially to completely inhibited on SMAC-CT.

Gram staining, biochemical tests and serological procedures should be performed to confirm findings.

Limitations of the Procedure

1. It has been reported that some *Enterobacteriaceae* and *Pseudomonas aeruginosa* are inhibited on MacConkey Agar when incubated in a CO₂-enriched atmosphere.⁶
2. Prolonged incubation of the culture may result in colonies of *E. coli* serotype O157:H7 losing their characteristic colorless appearance. There are additional species of facultatively anaerobic gram-negative rods that do not ferment sorbitol.
3. The color of sorbitol-positive colonies can fade, making them hard to distinguish from sorbitol-negative colonies.⁷

References

1. March and Ratnam. 1986. J. Clin. Microbiol. 23:869.
2. Centers for Disease Control. 1991. Morbid. Mortal. Weekly Rep. 40:265.
3. Bopp, Brenner, Wells and Stockbine. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
4. Zadik, Chapman and Siddons. 1993. J. Med. Microbiol. 39:155.
5. Sanderson, Gay, Hancock, Gay, Fox and Besser. 1995. J. Clin. Microbiol. 33:2616.
6. Mazura-Reetz, Neblett and Galperin. 1979. Abstr. C 179, p. 339. Abstr. Annu. Meet. Am. Soc. Microbiol. 1979.
7. Adams. 1991. Clin. Lab. Sci. 4:19.

Availability

Difco™ MacConkey Sorbitol Agar

BAM **BS12** **CCAM** **CMPH2** **COMPF** **ISO** **MCM9** **SMWW**

Cat. No. 279100 Dehydrated – 500 g

BBL™ MacConkey II Agar with Sorbitol

BAM **BS12** **CCAM** **CMPH2** **COMPF** **ISO** **MCM9** **SMWW**

Cat. No. 299769 Dehydrated – 500 g

United States and Canada

Cat. No. 297684 Prepared Deeps (Pour Tubes) – Pkg. of 10
297953 Prepared Plates – Pkg. of 10*
298519 Prepared Plates – Ctn. of 100*

Europe

Cat. No. 254455 Prepared Plates – Pkg. of 20*

Japan

Cat. No. 251147 Prepared Plates – Pkg. of 20*
251767 Prepared Plates – Ctn. of 100*

Mexico

Cat. No. 252637 Prepared Plates – Pkg. of 10*

BBL™ MacConkey II Agar with Sorbitol//SS Agar

Japan

Cat. No. 251163 Prepared **I Plate™** Dishes – Ctn. of 100*

BBL™ Sorbitol MacConkey II Agar with Cefixime and Tellurite

BAM **CCAM** **ISO** **SMWW**

United States and Canada

Cat. No. 222226 Prepared Plates – Pkg. of 10*
222227 Prepared Plates – Ctn. of 100*

Japan

Cat. No. 251156 Prepared Plates – Pkg. of 20*
251153 Prepared Plates – Ctn. of 100*

*Store at 2-8°C.