



QUALITY CONTROL PROCEDURES

I INTRODUCTION

MacConkey II Agar with Sorbitol is used as a selective and differential medium for the detection of *Escherichia coli* serotype O157:H7 associated with hemorrhagic colitis.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with broth cultures diluted to contain 10³–10⁴ CFU/0.01 mL.
 - a. To each plate, add 0.01 mL of the dilution and streak for isolation.
 - b. Incubate plates at 35 ± 2°C in an aerobic atmosphere.
 - c. Include **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
2. Examine plates after 18–24 h for growth, colony size, pigmentation and selectivity.
3. Expected Results

Organisms	ATCC™	Recovery	Colony Color
* <i>Escherichia coli</i>	700728	Growth	Colorless (negative for sorbitol fermentation)
* <i>Escherichia coli</i>	25922	Growth	Red
* <i>Enterococcus faecalis</i>	29212	Inhibition (partial)	
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	Growth	Pink to red

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.1 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 35 ± 2°C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

MacConkey II Agar with Sorbitol is used as a selective and differential medium for the detection of *Escherichia coli* serotype O157:H7 associated with hemorrhagic colitis. It is also used for the isolation of O157:H7 from foods.¹

V SUMMARY AND EXPLANATION

Escherichia coli serotype O157:H7 is a human pathogen associated with hemorrhagic colitis.² 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 of the fecal flora ferment sorbitol and appear pink on this medium. The MacConkey Agar with Sorbitol, therefore, permits ready recognition of *E. coli* O157:H7 in stool cultures.^{2,3}

VI PRINCIPLES OF THE PROCEDURE

MacConkey II Agar with Sorbitol is modified MacConkey II Agar using sorbitol instead of lactose. It is a selective and differential medium, 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.

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.

MacConkey II Agar with Sorbitol is also formulated to reduce swarming of *Proteus* species.

VII REAGENTS

MacConkey II Agar with Sorbitol

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Gelatin	17.0 g	Sodium Chloride	5.0 g
Pancreatic Digest of Casein	1.5 g	Neutral Red	0.03 g
Peptic Digest of Animal Tissue	1.5 g	Crystal Violet.....	0.001 g
D-Sorbitol	10.0 g	Agar.....	13.5 g
Bile Salts	1.5 g		

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"⁴⁻⁷ and institutional guidelines should be followed in handling all items contaminated with

blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2–8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2–8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{8,9} Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: MacConkey II Agar with Sorbitol

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth, firm and moist, but without excessive moisture.

Inoculate the medium as soon as possible after the specimen arrives at the laboratory. To culture a specimen from a swab, inoculate the medium by rolling the swab over one third of the agar and streak the remainder of the agar with a sterilized inoculating loop to obtain isolated colonies. Material not being cultured directly from swabs may be streaked onto the medium with a sterilized inoculating loop. The streak plate technique is used primarily to obtain isolated colonies from specimens containing mixed flora.

Incubate plates, protected from light, in an inverted position (agar side up) at 35 ± 2°C in an aerobic atmosphere for 18–24 h.

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

After 18–24 h 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.

Gram staining, biochemical tests and serological procedures should be performed on colorless colonies to confirm *E. coli* serotype O157:H7.

XI LIMITATIONS OF THE PROCEDURE

It has been reported that some *Enterobacteriaceae* and *Pseudomonas aeruginosa* are inhibited on MacConkey Agar when incubated in a CO₂-enriched atmosphere.¹⁰

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. Therefore, suspect colonies of *E. coli* serotype O157:H7 must be confirmed.

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.^{8,9,11-14}

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

XII PERFORMANCE CHARACTERISTICS

Ninety nine (99) clinical isolates of *E. coli* O157:H7 were tested externally.¹⁵

Pure culture suspensions were prepared in BBL Normal Saline and adjusted to a concentration of 10⁵ CFU (colony forming units)/mL. Plates of MacConkey II Agar with Sorbitol and Trypticase Soy Agar with 5% Sheep Blood (TSA II, control) were streaked with 10 µL of each suspension using the 4-quadrant streak method. Plates were incubated aerobically at 35 ± 2°C for 18–24 h and read for growth and reactions. All 99 isolates produced colorless colonies on MacConkey II Agar with Sorbitol. Furthermore, the amount of growth on the MacConkey II Agar with Sorbitol plates was comparable to that produced on the TSA II agar plates (control).

XIII AVAILABILITY

Cat. No.	Description
297953	BBL™ MacConkey II Agar with Sorbitol, Pkg. of 10 plates
298519	BBL™ MacConkey II Agar with Sorbitol, Ctn. of 100 plates

XIV REFERENCES

- Downes, F.P. and K. Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods. 4th ed. American Public Health Association, Washington, D.C.
- March, S.B., and S. Ratnam. 1986. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol. 23:869-872.
- Bopp, C.A., F.W. Brenner, P.I. Fields, J.G. Wells, and N.A. Strockbine. 2003. *Escherichia, Shigella, and Salmonella*, p. 654-671. In Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Tenover (ed.), Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.

4. National Committee for Clinical Laboratory Standards. 2001. Approved Guideline M29-A2. Protection of laboratory workers from occupationally acquired infections, 2nd ed. NCCLS, Wayne, PA.
5. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. *Infect. Control Hospital Epidemiol.* 17:53-80.
6. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, D.C.
7. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). *Official Journal L262*, 17/10/2000, p. 0021-0045.
8. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Tenover (ed.). 2003. *Manual of clinical microbiology*, 8th ed. American Society for Microbiology, Washington, D.C.
9. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. *Bailey and Scott's diagnostic microbiology*, 11th ed. Mosby, Inc., St. Louis.
10. Mazura-Reetz, G., T.R. Neblett, and J.M. Galperin. 1979. MacConkey agar: CO₂ vs. ambient incubation, abstr. C 179, p. 339. *Abstr. 79th Annu. Meet. Am. Soc. Microbiol.*
11. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. *Bergey's Manual™ of determinative bacteriology*, 9th ed. Williams & Wilkins, Baltimore.
12. MacFaddin, J.F. 2000. *Biochemical tests for identification of medical bacteria*, 3rd ed. Lippincott Williams & Wilkins, Baltimore.
13. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn, Jr. 1997. *Color atlas and textbook of diagnostic microbiology*, 5th ed. Lippincott-Raven, Philadelphia.
14. Isenberg, H.D. (ed.). 2004. *Clinical microbiology procedures handbook*, vol. 1, 2 and 3, 2nd ed. American Society for Microbiology, Washington, D.C.
15. Data on file, BD Diagnostics.

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