MACCONKEY AGAR w/ SORBITOL and MUG

INTENDED USE
Remel MacConkey Agar w/ Sorbitol and MUG is a solid medium recommended for use in qualitative procedures for selective and differential isolation of sorbitol-negative *Escherichia coli*.

SUMMARY AND EXPLANATION
Shiga toxin-producing *E. coli* (STEC) serotype O157:H7 was first identified as a cause of hemorrhagic colitis in 1982.[1] Since then it has been recovered from patients with diseases ranging from mild diarrhea to hemorrhagic colitis and hemolytic uremic syndrome.[2] The pathogenesis of these diseases results from shigqa toxin-producing serotypes of *E. coli* (STEC), which are indistinguishable from other serotypes of *E. coli* on routine isolation media. Effective culture methods require the use of a sorbitol-containing medium to readily differentiate nonsorbitol fermenting STEC colonies from those of commensal strains of *E. coli* (sorbitol fermenters). MacConkey Sorbitol Agar (SMAC) was introduced in 1986 as a primary plating medium for detection of STEC in stool specimens.[3] MacConkey agar with MUG was developed in 1984 by Trepeta and Edberg.[4] They incorporated the substrate 4-methylumbelliferyl-β-D-glucuronide (MUG) into MacConkey Agar obviating the need for a second medium. Most strains of *E. coli* hydrolyze MUG and produce β-glucuronidase, a blue-green fluorescent end product. STEC strains are usually MUG-negative.[5] In 1987, Krishnan et al. incorporated the MUG test directly into SMAC agar to facilitate detection of STEC after 1 to 2 days of incubation.[6]

PRINCIPLE
Gelatin, meat, and casein peptones supply nitrogenous compounds and amino acids necessary for bacterial growth. Sodium chloride is a source of essential electrolytes and maintains osmotic equilibrium. Bile salts inhibit gram-positive organisms and crystal violet inhibits enterococci. D-sorbitol is fermented by commensal non-pathogenic strains of *E. coli* producing pink colonies; STEC does not ferment sorbitol and produces clear or colorless colonies. The substrate MUG is hydrolyzed by most commensal *E. coli* strains but not by most strains of STEC. Most STEC strains ferment lactose after overnight incubation but not D-sorbitol, allowing for differentiation between STEC strains and most fecal strains of sorbitol-positive *E. coli.* STEC strains of *E. coli* are typically MUG-negative.

REAGENTS (CLASSICAL FORMULA)*

Gelatin Peptone	17.0 g	Meat Peptone	1.5 g
D-Sorbitol	10.0 g	4-methylumbelliferyl-β-D-glucuronide (MUG)	0.1 g
Sodium Chloride	5.0 g	Neutral Red	30.0mg
Bile Salts	1.5 g	Crystal Violet	1.0mg
Casein Peptone	1.5 g	Agar	13.5 g
		Demineralized Water	1000.0 ml

pH 7.1 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Inoculate and streak the specimen as soon as possible after it is received in the laboratory.
2. If material is being cultured directly from a swab, roll the swab over a small area of the agar surface and streak for isolation.
3. A nonselective medium should also be inoculated to increase the chance of recovering enteric pathogens when the number is low and to provide isolation of other organisms present in the specimen.
4. Incubate plates in ambient air at 35-37°C for 18-24 hours.
5. After incubation, use a longwave ultraviolet light (360 nm) to observe sorbitol nonfermenting colonies for blue-green fluorescence, in a darkened room.

QUALITY CONTROL
All lot numbers of MacConkey Agar w/ Sorbitol and MUG have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Escherichia coli Serotype O157:H7 ATCC® 43888	Ambient, 18-24 h @ 33-37°C	Growth, clear colonies
Escherichia coli ATCC® 25922	Ambient, 18-24 h @ 33-37°C	Growth, pink colonies, blue fluorescence
Proteus mirabilis ATCC® 12453	Ambient, 18-24 h @ 33-37°C	Growth, clear colonies
Salmonella enterica serovar Typhimurium ATCC® 14028	Ambient, 18-24 h @ 33-37°C	Growth, pink colonies
Staphylococcus aureus ATCC® 25923	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)
Enterococcus faecalis ATCC®29212	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)

LIMITATIONS
1. Sorbitol-negative isolates should be confirmed as *E. coli* by current accepted methodologies.[7]
2. Shiga toxin-producing *E. coli* are reportable to State Public Health Departments and recommended procedures for further identification may differ from location to location.[7]
3. Confirmed *E. coli* isolates may be serotyped using *E. coli* O157 and H7 antisera.[7]

(Continued on back)

BIBLIOGRAPHY

1. Wells, J.G., B.R. Davis, I.K. Wachsmuth, L.W. Riley, R.S. Remis, R. Sokolow, and G.K. Morris. 1983. J. Clin. Microbiol. 18:512-520.
2. Haldane, D.J. M., M.A.S. Damn, and J.D. Anderson. 1986. J. Clin. Microbiol. 24:652-653.
3. March, S.B. and S. Ratnam. 1986. J. Clin. Microbiol. 23:869-872.
4. Trepeta, R.W. and S.C. Edberg. 1984. J. Clin. Microbiol. 19:172-174.
5. Doyle, M.P. and J.L. Schoeni. 1984. Appl. Microbiol. 48:855-856.
6. Krishnan, C., V.A. Fitzgerald, S.J. Dakin, and R.J. Behme. 1987. J. Clin. Microbiol. 25:1043-1047.
7. Versalovic, J., K.C. Carroll, G. Funke, J.H. Jorgensen, M.L. Landry, and D.W. Warnock. 2011. Manual of Clinical Microbiology. 10th ed. ASM Press, Washington, D.C.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

ATCC® is a registered trademark of American Type Culture Collection.

IFU 1563, Revised June 13, 2012 Printed in U.S.A.

remel

12076 Santa Fe Drive, Lenexa, KS 66215, USA
General Information: (800) 255-6730 Website: www.remel.com Email: remel@remel.com
Local/International Phone: (913) 888-0939 International Fax: (913) 895-4128