

MACCONKEY SORBITOL AGAR w/ CEFIXIME and TELLURITE

INTENDED USE

Remel MacConkey Sorbitol Agar w/ Cefixime and Tellurite (CT-SMAC) is a solid medium recommended for use in qualitative procedures for selective and differential isolation of shiga toxin-producing *Escherichia coli* serotype O157:H7 from stool specimens.

SUMMARY AND EXPLANATION

Shiga toxin-producing *E. coli* is a major cause of diarrheal disease in the United States.¹ Most reported shiga toxin-producing *E. coli* (STEC) infections are caused by serotype O157:H7. Such infections can result in severe complications (i.e., hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS)) if appropriate treatment is not administered in a timely manner.² In 2009 the Centers for Disease Control and Prevention (CDC) issued guidelines for clinical laboratories with the recommendation that all stools submitted for routine testing from patients with acute community-acquired diarrhea should be cultured for *E. coli* O157:H7.³ Effective culture methods require the use of a sorbitol-containing medium to readily differentiate nonsorbitol fermenting O157:H7 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.⁴ CT-SMAC agar is a modification of SMAC agar which contains the selective agents cefixime and tellurite.^{5,6} Cefixime inhibits the growth of other enterics, such as *Proteus* spp., and potassium tellurite inhibits non-O157 STEC. The use of CT-SMAC has been shown to increase the sensitivity of culture for isolation of *E. coli* O157:H7. It is recommended by the American Society for Microbiology and the CDC for use as a primary isolation medium for recovery of *E. coli* O157:H7 from all stool specimens submitted from patients with community-acquired diarrhea.⁷

PRINCIPLE

Gelatin, casein, and meat 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 and crystal violet are selective agents which inhibit gram-positive organisms, including enterococci. Cefixime and tellurite inhibit the growth of non-O157 *E. coli* and other sorbitol nonfermenting enteric gram-negative bacilli. D-sorbitol is fermented by strains of *E. coli* other than serotype O157:H7, producing colonies that are pink; colonies of *E. coli* O157:H7 are colorless on CT-SMAC agar after overnight incubation.

REAGENTS (CLASSICAL FORMULAE)*

Gelatin Peptone	17.0 g	Neutral Red	30.0 mg
D-Sorbitol	10.0 g	Crystal Violet	1.0 mg
Sodium Chloride.....	5.0 g	Cefixime	0.05 mg
Bile Salts	1.5 g	Potassium Tellurite.....	2.5 ml
Casein Peptone.....	1.5 g	Agar	13.5 g
Meat Peptone	1.5 g	Demineralized Water.....	1000.0 ml

pH 7.1 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE

1. Consult appropriate references for the recommended procedure for sample preparation and inoculation.
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. Incubate plate in ambient air for 18-24 hours at 33-37°C.
4. Examine plate for typical colony morphology. On CT-SMAC, nonsorbitol fermenting colonies are clear or colorless.

INTERPRETATION OF THE TEST

Sorbitol-negative - Clear or colorless colonies
Sorbitol-positive - Pink colonies

QUALITY CONTROL

All lot numbers of MacConkey Sorbitol Agar w/ Cefixime and Tellurite 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

Escherichia coli serotype O157:H7 ATCC® 43888
Escherichia coli ATCC® 25922
Proteus mirabilis ATCC® 12453

INCUBATION

Ambient, 18-24 h @ 33-37°C
Ambient, 18-24 h @ 33-37°C
Ambient, 18-24 h @ 33-37°C

RESULTS

Growth, colorless colonies
Complete inhibition to growth, pink colonies
Inhibition (partial to complete)

LIMITATIONS

1. Sorbitol-negative isolates should be biochemically confirmed to be *E. coli* using standard biochemical tests or commercial automated systems. Consult appropriate references for further instructions.³
2. Non-O157 strains of STEC are sensitive to potassium tellurite and, therefore, inhibited on CT-SMAC agar.³
3. Prolonged incubation of cultures beyond 24 hours may result in STEC colonies losing their characteristic colorless appearance.³
4. *Escherichia hermannii* resembles *E. coli* biochemically, is sorbitol-negative, and positive agglutination reactions with O157 antiserum have been reported.⁷ These species can be differentiated with additional biochemical tests; *E. hermannii* ferments cellobiose and produces colonies with a yellow pigment.

BIBLIOGRAPHY

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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.

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