

## Revisions

SO 0191-5

Rev from	Rev to	ECO #
0205	0506	4014-06

**Notes:**

1. BD Cat. Number 222226, 222227
2. Blank (Sheet) Size: Length: 11"      Width: 8.5"  
 Number of Pages: 2      Number of Sheets: 1  
 Page Size: Length 11"      Width 8.5"      Final Folded Size: N/A
3. Style (see illustrations below): # 1



4. VS Controlled by BDDS, Madison      for Material Information
5. Ink Colors: Printed two sides  Yes       No  
 No. of Colors: 1      PMS# Black
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Proofer	Date			
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Part Number: 8809991JAA		Category and Description Package Insert, Sorbitol MacConkey II Agar w/Cefixime & Tellurite	Sheet: 1 of 3 <hr/> Scale: N/A	A

# BD BBL™ Prepared Plated Medium for Detection of *Escherichia coli* Associated with Hemorrhagic Colitis

## Sorbitol MacConkey II Agar with Cefixime and Tellurite

8809991JAA  
2006/05

### INTENDED USE

Sorbitol MacConkey II Agar with Cefixime and Tellurite (SMAC-CT) is used as a selective and differential medium for the detection of *Escherichia coli* serotype O157:H7.

### SUMMARY AND EXPLANATION

*Escherichia coli* serotype O157:H7 is a human pathogen associated with hemorrhagic colitis<sup>1</sup> that results from the action of a shiga-like toxin (SLT). On standard MacConkey agar containing lactose, this strain is indistinguishable from other lactose-fermenting *E. coli*. However, unlike most *E. coli* strains, *E. coli* O157:H7 ferments sorbitol slowly or not at all. When sorbitol is substituted for lactose, the O157:H7 strain fails to ferment the sorbitol, producing colorless colonies, while other *E. coli* yield sorbitol-positive pink to red colonies.

The addition of cefixime and tellurite inhibits *Proteus mirabilis*, non-O157 *E. coli* strains, and other sorbitol nonfermenting strains.<sup>2,3</sup> This inhibition significantly reduces the number of sorbitol nonfermenters that need to be screened during the attempted isolation of *E. coli* O157:H7. Most fecal flora that are not inhibited ferment sorbitol and appear pink on this medium, permitting ready recognition of *E. coli* O157:H7.

### PRINCIPLES OF THE PROCEDURE

SMAC-CT is modified MacConkey II Agar using sorbitol instead of lactose and with cefixime and tellurite 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. Bile salts and crystal violet, which inhibit gram-positive bacteria, especially enterococci and staphylococci, are also included.

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.

### REAGENTS

#### Sorbitol MacConkey II Agar with Cefixime and Tellurite (SMAC-CT)

Approximate Formula\* Per Liter Purified Water

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
Neutral Red .....	0.03	g
Crystal Violet .....	0.001	g
Agar .....	13.5	g
Cefixime .....	0.05	mg
Potassium Tellurite .....	2.5	mg

\*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.

**Storage Instructions:** On receipt, store plates in the dark at 2 to 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 to 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. *Do not leave the medium at room temperature for more than 24 h.*

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

### SPECIMEN COLLECTION AND HANDLING

Refer to appropriate texts for details of specimen collection and handling procedures.<sup>4-7</sup>

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"<sup>8-11</sup> and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. Prior to discarding, sterilize specimen containers and other contaminated materials by autoclaving.

### PROCEDURE

**Material Provided:** Sorbitol MacConkey II Agar with Cefixime and Tellurite.

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

**Test Procedure:** Observe aseptic techniques. The agar surface should be smooth 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 a 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 for 18 to 24 h in an aerobic atmosphere without additional CO<sub>2</sub>.

### User Quality Control:

1. Examine plates for signs of deterioration as described under "Product Deterioration."
2. Check performance by inoculating a representative sample of plates with pure cultures of stable control organisms that give known, desired reactions. The following test strains are recommended:

TEST STRAIN	EXPECTED RESULTS
<i>Escherichia coli</i> O157:H7 ATCC™ 700728	Good growth, colorless to slight pale-gray colonies (negative for sorbitol fermentation).
<i>Escherichia coli</i> ATCC 25922	Inhibition (partial to complete)
<i>Proteus mirabilis</i> ATCC 12453	Inhibition (partial to complete)

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 NCLC) guidance and CLIA regulations for appropriate Quality Control practices.

### RESULTS

After 18 to 24 h of incubation, *E. coli* O157:H7 should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Most fecal flora will be partially to completely inhibited. Sorbitol fermenters that are not inhibited will 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 to confirm findings.<sup>12</sup> BBL™ provides a serological kit for confirmation of O157 and H7 antigens.

### LIMITATIONS OF THE PROCEDURE

This prepared plated medium is intended for primary isolation.

It has been reported that some *Enterobacteriaceae* and *Pseudomonas aeruginosa* are inhibited on MacConkey Agar when incubated in a CO<sub>2</sub>-enriched atmosphere (G. Mazura-Reetz, T.R. Neblett, and J.M. Galperin. Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C179, p. 339).

Prolonged incubation of the culture beyond 24 h may result in colonies of *E. coli* serotype O157:H7 losing their characteristic colorless to gray with dark gray centers appearance. There are additional species of facultatively anaerobic gram-negative rods that do not ferment sorbitol.

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. The agents in selective media may inhibit some strains of the desired species or permit the growth of a species they were designed to inhibit, especially if the species is present in large numbers in the specimen. Specimens cultured on selective media should also be cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

### PERFORMANCE CHARACTERISTICS

Thirty-two (32) *E. coli* O157:H7 isolates were tested in-house. Additionally, eleven (11) non-O157:H7 *E. coli* isolates and nineteen (19) other strains were evaluated in-house.

Pure culture suspensions were prepared in BBL Saline, Normal, and adjusted to a concentration of 10<sup>5</sup> CFU (colony-forming-units) /plate. Plates of Sorbitol MacConkey II Agar with Cefixime and Tellurite were streaked with 10 µL of each suspension, incubated for 18 h, and read for growth and reactions. One hundred percent (100%) correlation was seen between results on test agar and expected results for all 32 *E. coli* O157:H7 strains tested. Ten of the eleven non-O157 strains were inhibited. All strains grew well on Trypticase™ Soy Agar with 5% Sheep Blood (TSA II).

For the nineteen other strains tested, performance results were as expected; growth results ranged from complete to partial inhibition and, if growth occurred, reactions were correct.

Growth occurred with *Salmonella* group N and *Escherichia hermannii*, which are known to cross react with *E. coli* O157 antisera.

#### AVAILABILITY

Cat. No.	Description
222226	BBL™ Sorbitol MacConkey II Agar with Cefixime and Tellurite Pkg. of 10 plates
222227	BBL™ Sorbitol MacConkey II Agar with Cefixime and Tellurite Ctn. of 100 plates

#### REFERENCES

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