INTENDED USE

BD MacConkey Agar with Sorbitol, also known as Sorbitol MacConkey Agar (SMAC), is a partially selective differential medium for the isolation of *E. coli* O157:H7 from clinical, veterinary, food, and environmental sources.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Enterohemorrhagic *E. coli* (EHEC) O157:H7 was first recognized as a human pathogen in 1982.\(^1\) Until today, the serotype O157:H7 is by far the most frequent one that is responsible for this disease, although occasionally other serotypes of *E. coli* may be involved in this or similar types of infection.\(^2\)

Due to the production of Shiga-like Toxins (SLT, verocytotoxin), the serotype O157:H7 of *E. coli* is known to be involved in cases of diarrhea, severe enterohemorrhagic colitis, and the Hemolytic Uremic Syndrome (HUS). Epidemiologically, the syndrome is a foodborne disease, often related to the consumption of undercooked beef or other food derived from animal sources such as raw milk.\(^3,4\)

Usually, O157 strains differ from normal *E. coli* strains by being sorbitol and beta-glucuronidase (ß-gluc) negative. Thus, they can be differentiated from normal *E. coli* by biochemical means when the appropriate substrates are included in bacteriological media. Sorbitol-MacConkey Agar (= SMAC) was one of the media first used to isolate these organisms.\(^6,7\)

MacConkey Agar with sorbitol is a modification of the formula given by Rappaport and Henig for isolating enteropathogenic *Escherichia coli* serotypes 011 and 055.\(^8\) The usefulness of this medium in detecting *E. coli* O157:H7, a human pathogen associated with hemorrhagic colitis, has been described.\(^9-11\) This medium employs D-sorbitol rather than lactose for isolating and differentiating the enteropathogenic *E. coli* serotypes which tend to be sorbitol negative. It can be used for clinical and food testing.\(^8-13\)

In BD MacConkey Agar with Sorbitol, peptones are nitrogen sources. D-Sorbitol is a fermentable carbohydrate. Most hemorrhagic *E. coli* strains will not ferment D-sorbitol and appear as colorless colonies on MacConkey Sorbitol Agar. Bile salts and crystal violet are selective agents that inhibit growth of gram-positive organisms. Neutral red is a pH indicator.

REAGENTS

**BD MacConkey Agar with Sorbitol**

Approximate Formula* Per Liter Purified Water

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptones</td>
<td>20.0 g</td>
</tr>
<tr>
<td>D-Sorbitol</td>
<td>10.0</td>
</tr>
<tr>
<td>Bile Salts</td>
<td>1.5</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Neutral Red</td>
<td>0.03</td>
</tr>
<tr>
<td>Crystal Violet</td>
<td>0.001</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
</tr>
</tbody>
</table>

pH 7.1 ± 0.2

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

PRECAUTIONS

[IVD]. For professional use only.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.
Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

**STORAGE AND SHELF LIFE**
On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times. Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

**USER QUALITY CONTROL**
Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate for 18 to 24 hours at 35 to 37° C.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Growth Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> O157:H7</td>
<td>Growth good to excellent; colonies colorless or beige</td>
</tr>
<tr>
<td>NCTC 12900* (sorbitol negative)</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC™ 25922</td>
<td>Growth; colonies rose to pink</td>
</tr>
<tr>
<td>(sorbitol positive)</td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 29212</td>
<td>Inhibition partial to complete</td>
</tr>
</tbody>
</table>

* NCTC 12900 is recommended for routine quality control since it does not produce toxins. The strain is available from the National Collection of Type Cultures, London, UK. For information, refer to www.phls.co.uk/labservices/nctc/qcrefsets.htm

**PROCEDURE**

**Materials Provided**
BD MacConkey Agar With Sorbitol (90 mm Stacker™ plates). Microbiologically controlled.

**Materials Not Provided**
Ancillary culture media, reagents and laboratory equipment as required.

**Specimen Types**
This medium is used for the isolation of *Escherichia coli* O157:H7 (and other sorbitol negative serotypes) from stool specimens of patients suspected to be infected with this agent, and for food, veterinary, and environmental samples (see also PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE).

**Test Procedure**
Either plate the samples directly on the medium, or use a preenrichment such as selective modified Tryptic Soy Broth or immunomagnetic separation (IMS) with Dynabeads™, following the instructions of the manufacturer, and subculture onto BD MacConkey Agar with Sorbitol. Preenrichment techniques are especially helpful if the specimens or samples are contaminated with normal flora. To isolate *E. coli* O157:H7 directly from fecal specimens, inoculate fecal samples or rectal swabs on a small area of one quadrant and streak for isolation. This will permit development of discrete colonies. It is recommended to also inoculate a medium with a higher degree of selectivity, e.g., BD CHROMagar O157™. Incubate in an aerobic atmosphere at 36 +/- 2° C for 18 to 24 hours.

**Results**
Sorbitol-fermenting organisms produce pink colonies on BD MacConkey Agar with Sorbitol. Organisms that do not ferment sorbitol, such as *E. coli* O157:H7, are colorless. Colonies presumptively identified by their colony color must be confirmed as *E. coli* O157:H7 using serological or molecular methods for the detection of the serotype and/or of the toxins.

**PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**
BD MacConkey Agar with Sorbitol is a standard medium for the isolation of sorbitol negative *E. coli* serotypes, especially O157:H7, from clinical specimens and other materials.
Upon extended incubation, strains of *E. coli* O157:H7 can ferment sorbitol. The color of sorbitol-positive colonies can fade, making them hard to distinguish from sorbitol-negative colonies. There exist sorbitol negative strains of serotypes other than O157:H7 which may or may not produce toxins and clinical symptoms.² **BD MacConkey Agar with Sorbitol** does not differentiate between toxin producing and nonproducing strains of *E. coli* O157. Strains of other organisms that do not ferment sorbitol (such as *Escherichia hermannii*) may grow on MacConkey Sorbitol Agar. Confirmatory tests such as serological or molecular methods for the detection of the serotype and/or the toxins are mandatory for the final identification of *E. coli* O157 strains isolated from **BD MacConkey Agar with Sorbitol** or other isolation media for this organism.²,⁶,⁹,¹²,¹³ A single medium is rarely adequate for detecting all potential pathogens involved. It is, therefore, recommended to cultivate appropriate specimens also on **BD™ CHROMagar O157**.

REFERENCES

PACKAGING/AVALIABILITY
BD MacConkey Agar with Sorbitol
Cat. No. 254455 Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION
For further information please contact your local BD representative.

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