



BD™ MacConkey Agar with Sorbitol

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 specimens.

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.²⁻⁵

Usually, O157 strains differ from normal *E. coli* strains by being sorbitol and beta-glucuronidase (β -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 O11 and O55.⁸ 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

Peptones	20.0 g
D-Sorbitol	10.0
Bile Salts	1.5
Sodium Chloride	5.0
Neutral Red	0.03
Crystal Violet	0.001
Agar	15.0

pH 7.1 \pm 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.

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

* 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/qcrefssets.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.^{14,15} To isolate *E. coli* O157:H7 directly from fecal specimens, inoculate fecal specimens 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.^{6,9,12,13}

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.^{6-13,16}

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**.

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PACKAGING/AVAILABILITY

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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