PRODUCT SPECIFICATION SHEET

MacConkey Sorbitol Agar (Sorbitol Agar) (DM154)

Intended Use

MacConkey Sorbitol Agar (Sorbitol Agar) (DM154) is recommended for isolation and identification of enteropathogenic *Escherichia coli* strains associated with infant diarrhoea.

Product Summary and Explanation

MacConkey Sorbitol Agar (Sorbitol Agar) is based on the formula by Rappaport and Henig.[1] This medium is originally developed for isolating enteropathogenic (EPEC) *serotypes, O* 11 and O55, this medium is also recommended for the isolation and differentiation of enterohemorrhagic *E. coli* O157:H7. This organism causes hemorrhagic colitis, that result from the action of a shiga-like toxin (SLT),[2] which results in bloody diarrhea and can lead to kidney failure and death.[3] Serotype O157 has been implicated in serious foodborne diseases. On standard MacConkey Agar containing lactose, this strain is indistinguishable from other lactose-fermenting *E. coli*. Unlike most *E. coli* strains, *E. coli* O157:H7 ferments sorbitol slowly or not at all. Therefore, the efficacy of MacConkey Agar containing sorbitol instead of lactose as a differential medium for the detection of *E. coli* O157:H7 in stool cultures was determined. Field trial results showed that the growth of *E. coli* O157:H7 on MacConkey Sorbitol Agar (Sorbitol Agar) was heavy and occurred in almost pure culture as colorless sorbitol-nonfermenting colonies. Most organisms of the fecal flora ferment sorbitol and appear pink on this medium. MacConkey Sorbitol Agar (Sorbitol Agar), therefore, permits ready recognition of *E. coli* O157:H7 in stool cultures.[3-5] The addition of cefixime and tellurite significantly reduces the number of sorbitol non-fermenters that need to be screened during the attempted isolation of *E. coli* O157:H7.[6, 7]

Principles of the Procedure

Peptic digest of animal tissue and proteose peptone in MacConkey Sorbitol Agar, supply necessary nutrients like nitrogenous and carbonaceous compounds, minerals, vitamins and trace ingredients for the growth of organisms. Crystal violet and bile salt mixture present in the medium inhibit growth of gram-positive bacteria, especially enterococci and staphylococci. Sodium chloride maintains osmotic equilibrium. Differentiation of enteric microorganisms is achieved by the combination of D-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.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	17.00
Proteose peptone	3.00
D-Sorbitol	10.00
Bile salts mixture	1.50
Sodium chloride	5.00
Neutral red	0.03
Crystal violet	0.001
Agar	13.50
Final pH: 7.1 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 50.03 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

4. AVOID OVERHEATING.
5. Cool to 40-50°C and pour into sterile petri plates.

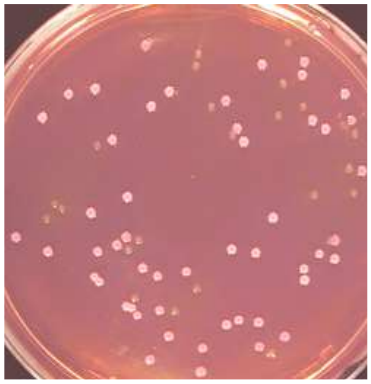
Quality Control Specifications

Dehydrated Appearance	Light yellow to pink homogeneous free flowing powder
Prepared Medium	Purplish red coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 5.0% Solution	pH : 7.1 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.35% Agar gel

Expected Cultural Response: Cultural characteristics observed after an incubation 35 - 37°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Colour of colony
1.	*Escherichia coli O157:H7 NCTC 29900*	50 -100	good-luxuriant	>=50 %	Colourless
2.	*Escherichia coli ATCC 25922*	50 -100	good-luxuriant	>=50 %	Pink
3.	*Escherichia coli serotype O11 and O55*	50-100	fair to good	>=50 %	Colourless
4.	*Salmonella Typhi ATCC 6539*	50 -100	good-luxuriant	>=50 %	Pink
5.	*Shigella flexneri ATCC 12022*	50 -100	good-luxuriant	>=50 %	Colourless

The organisms listed are the minimum that should be used for quality control testing.



(Enlarged view)

MacConkey Sorbitol agar (DM154)
1. E. coli O157:H7 : smaller, colorless colonies sometimes with an orange halo.
2. E. coli non-O157:H7 : larger, Pink colonies.

Test Procedure
Refer to appropriate references for specific procedures, for isolation and identification of enteropathogenic *Escherichia coli* strains associated with infant diarrhoea.

Results
1. *E. coli* O157:H7, and other organisms that do not ferment sorbitol, are colorless on MacConkey Sorbitol Agar.
2. Sorbitol-fermenting organisms produce pink colonies.
3. Confirmatory biochemical and serological testing should be performed on suspected colonies.

Storage
Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration
Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Colonies that are sorbitol positive can revert, and can be mistaken for sorbitol negative.
2. Prolonged incubation of the culture may result in colonies of *E. coli* serotype O157:H7 losing their characteristic colorless appearance. There are additional species of facultative anaerobic gram-negative rods that do not ferment sorbitol.

Packaging

Product Name : MacConkey Sorbitol Agar (Sorbitol Agar).
Product Code : DM154
Available Pack sizes : 100gm / 500gm

References

1. Rappaport, F., and E. Henig. 1952. Media for the isolation and differentiation of pathogenic *Escherichia coli* (serotypes 0111 and 055). J. Clin. Pathol. 5:361-362.
2. March and Ratnam. 1986. J. Clin. Microbiol. *23*:869.
3. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
4. Centers for Disease Control. 1991. Morbid. Mortal. Weekly Rep. *40*:265.
5. Bopp, Brenner, Wells and Stockbine. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
6. Zadik, Chapman and Siddons. 1993. J. Med. Microbiol. *39*:155.
7. Sanderson, Gay, Hancock, Gay, Fox and Besser. 1995. J. Clin. Microbiol. *33*:2616.

Further Information

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DM154PSS,QAD/FR/024,Rev.00