Sorbitol Iron Agar

M299

Sorbitol Iron Agar is used for the cultural identification and differentiation of enteropathogenic *Escherichia coli* , which do not ferment sorbitol.

Composition**

Ingredients	Gms / Litre
Beef extract	3.000
Proteose peptone	15.000
D-Sorbitol	2.000
Sodium chloride	5.000
Ferric ammonium citrate	0.500
Sodium thiosulphate	0.500
Phenol red	0.030
Agar	20.000
Final pH (at 25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 46.03 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in test tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool in a slanted position.

Principle And Interpretation

Escherichia coli is the most common bacterium isolated in clinical samples, the most prevalent facultative gram-negative rods in faeces, the most common cause of urinary tract infection and a common cause of both intestinal and extra-intestinal infections (1). Strains of *E. coli* that are primary intestinal pathogens of man are described in four groups namely Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Verocytotoxin-producing *E. coli* (VTEC) and Enteropathogenic *E. coli* (EPEC) (2). EPEC causes infantile diarrhea (1).

Sorbitol Iron Agar is a differential tube medium described by Rappaport and Henig (1). It is a modification of Kligler Iron Agar where dextrose and lactose is substituted with D-sorbitol. The pathogenic strain of *E. coli* is identified on the basis of inability to ferment sorbitol and hydrogen sulfide production.

Proteose peptone and beef extract in the medium provide carbon, nitrogen, vitamins and minerals required for the growth of organisms. D-Sorbitol is the fermentable carbohydrate source. Sodium chloride provides essential ions. The combination of ferric ammonium citrate and sodium thiosulphate enables the detection of hydrogen sulphide production, which is evidenced by a black colour formation. Phenol red is the pH indicator, detecting the fermentation of sorbitol and subsequent formation of acidic conditions.

Colourless colonies from Sorbitol Agar (M298) are inoculated into Sorbitol Iron Agar by stabbing the butts and streaking the slants. After 18-24 hours, freshly isolated pathogenic strains of *E. coli* show neither acid nor blackening of the medium. *Proteus* species may or may not blacken the medium, may produce acid in the butt; and on transfer to urease test medium, will give a positive urease test. Ordinary strains of *E. coli* produce acid and gas on Sorbitol Iron Agar, some pathogenic strains after laboratory cultivation may develop the capacity to ferment sorbitol and produce acid. Subsequently transfer of such cultures on Kligler Iron Agar (M078) or Triple Sugar Iron Agar (M021), Urease Test Medium will help in identification.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium
Red coloured clear to slightly opalescent gel forms in tubes as slants

Reaction
Reaction of 4.6% w/v aqueous solution at 25°C. pH : 7.6±0.2

pH
7.40-7.80

Cultural Response
M299: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Sorbitol	H2S
Growth Promoting Cultural Response				
Escherichia coli ATCC 25922	50-100	luxuriant	positive reaction, yellow colour with gas formation	negative reaction
Enterobacter aerogenes ATCC 13048	50-100	luxuriant	positive reaction, yellow colour	negative reaction
Enterococcus faecalis ATCC 29212	50-100	luxuriant	positive reaction, yellow colour	negative reaction
Klebsiella pneumoniae ATCC 13883	50-100	luxuriant	positive reaction, yellow colour	negative reaction
Proteus vulgaris ATCC 13315	50-100	luxuriant	negative reaction	positive reaction, blackening of medium
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	positive reaction, yellow colour	positive reaction, blackening of medium
Shigella flexneri ATCC 12022	50-100	luxuriant	negative reaction	negative reaction
Escherichia coli serotype 011 and 055	50-100	luxuriant	negative reaction	negative reaction
Escherichia coli O157:H7 NCTC12900	50-100	luxuriant	negative reaction	negative reaction

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
1. Rappaport F. and Henig E., 1952, J. Clin. Pathol., 5:361.
2. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone.

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