Acetate Differential Agar, Modified

M339F

Acetate Differential Agar, Modified is recommended for the differentiation of *Shigella* species from *Escherichia coli* in accordance with FDA BAM, 1998.

Composition**

Ingredients	Gms / Litre
Sodium acetate	2.000
Sodium chloride	5.000
Magnesium sulphate	0.200
Ammonium phosphate	1.000
Dipotassium phosphate	1.000
Bromothymol blue	0.080
Agar	20.000
Final pH (at 25°C)	6.70±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29.28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Distribute in tubes in sufficient amounts to give butt and slant. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool in a slanted position.

Principle And Interpretation

Shigellosis, although commonly regarded as waterborne, is also a food borne disease majorly caused to humans. It is spread among humans by food handlers with lesser personal hygiene. *Escherichia coli* is widely distributed in the intestine of humans and is an important facultative anaerobe present in the colon area of a healthy individual. Acetate Differential Agar, Modified (M339F) is recommended for the differentiation of Shigella species from *E. coli* in accordance with FDA BAM, 1998 (1).

This medium was formulated by Trabulsi and Ewing (2), by modifying Citrate Medium of Simmons(3). Most of the bacteria can utilise citrate and acetate as the carbon sources for growth in the presence of organic nitrogen, not in the absence of it. This difference in growth is helpful in differentiating *Shigella* from other closely related organisms such as *E. coli* (4). *E.coli* grows well within 24-48 hours in this media indicated by formation of blue colour (5<(>,<)>6). Magnesium sulphate is an essential ion. Sodium chloride maintains osmotic equilibrium and phosphates maintain the pH.

Aseptically weigh 25 g sample into 225 ml Shigella Broth Base (M1326) supplemented with novobiocin. Incubate jars under anaerobic conditions at 44.0°C in a water bath for 20 hrs. This can further be streaked on to a MacConkey agar plate (M081D). Incubate for 20 h at 35°C. After grams staining, the culture can be proceeded for biochemical confirmation. Inoculate the cultures into slants of Acetate Differential Agar, Modified and incubate overnight at 35°C. Acetate utilization is indicated by formation of blue colour, which is due to the utilization of sodium acetate and subsequent formation of an alkaline reaction detected by the presence of bromothymol blue indicator. *Shigella* is negative and do not show blue colouration, whereas *E.coli* is positive (1).

Quality Control

Appearance

Cream to light green homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

Emerald green coloured clear to slightly opalescent gel forms in tubes as slants

Reaction
Reaction of 2.92% w/v aqueous solution at 25°C. pH : 6.70±0.2

pH
6.50-6.90

Cultural Response
Cultural characteristics observed after an incubation at 25-30°C for upto 1-7 days.

Cultural Response

Organism	Inoculum (CFU)	Growth	Acetate utilization
Cultural Response			
Citrobacter freundii ATCC 8090	50-100	good-luxuriant	positive reaction, blue colour
Enterobacter cloacae ATCC 23355	50-100	good-luxuriant	positive reaction, blue colour
Escherichia coli ATCC 25922	50-100	good-luxuriant	positive reaction, blue colour
Klebsiella pneumoniae ATCC 13883	50-100	good-luxuriant	positive reaction, blue colour
Proteus vulgaris ATCC 13315	>=10^3	inhibited	
Salmonella Arizonae ATCC 13314	50-100	good-luxuriant	positive reaction, blue colour
Salmonella Typhi ATCC 19430	50-100	poor	negative reaction green colour
Shigella sonnei ATCC 25931	50-100	none-poor	negative reaction, no change, medium remains green

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

Reference
1.FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.
2.Trabulsi. and Ewing. 1962. Public Health Lab, 20.
3.Simmons. 1926. J. Infect. Dis, 39.
4.Cordaro, J.T. and Ball, R.J. 1966. Applied Microbiology, 14(6): 886-887.
5.Ewing. 1986. Edwards and Ewings Identification of Enterobacteriaceae. 4 ed. N.Y: Elsevier Science Pub. Co., Inc.
6.Talukder, K. A., Islam, M. A., Dutta, D.K., Hasan, F., Sada, A., Nair, G. and Bnd Sack, D. A. 2002. J. Clin. Microbiol, 40.

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