



Acetate Differential Agar

M339

Acetate Differential Agar is recommended for the differentiation of *Shigella* species from *Escherichia coli*.

Composition**

Ingredients	Gms / Litre
Sodium acetate	2.000
Magnesium sulphate	0.100
Sodium chloride	5.000
Monoammonium phosphate	1.000
Dipotassium phosphate	1.000
Bromothymol blue	0.080
Agar	20.000
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29.18 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

Acetate Differential Agar was formulated by Trabulsi and Ewing (1). Tatum, Ewing and Weaver (2) modified the medium by replacing sodium citrate by sodium acetate, which enables the differentiation of *Shigella* species from *Escherichia coli*. Organic acids have been used widely as an aid in the differentiation of *Enterobacteriaceae*, usually in formulae that contained organic nitrogen sources. Most bacteria, however, can use citrate and acetate in the presence of organic nitrogen. The differentiation of groups is based on the ability or failure of the test culture to utilize acetate in a medium devoid of trace organic nitrogen. This medium contains sodium acetate as the sole source of carbon. Trabulsi and Ewing demonstrated that *Shigella* and *Proteus* species are unable to utilize acetate and therefore fails to grow. Majority of *Escherichia coli* and closely related organisms grow well within 24-48 hours but some strains grow very slowly and a few strains are unable to utilize acetate as a sole carbon source. 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. Some strains of *Escherichia coli* utilize acetate slowly or not at all and therefore may produce a false negative reaction. Sodium acetate is utilized as a sole source of carbon by some serotypes of *S. flexneri* such as *Shigella flexneri 4a* (3, 4). Magnesium sulphate is essential ion. Sodium chloride maintains osmotic equilibrium and phosphates act as buffers.

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 : 6.7±0.2

pH

6.50-6.90

Cultural Response

M339: Cultural characteristics observed after an incubation at 25-30°C for upto 1-7 days.

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

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. Trabulsi and Ewing, 1962, Public Health Lab., 20:137.
2. Tatum H. W., Ewing W. H., and Weaver R. E., 1974, Manual of Clinical Microbiology, , 2nd Ed., American Society for Microbiology, Washington D.C. Pg.-270
3. Ewing, 1986, Edwards and Ewings Identification of Enterobacteriaceae , 4th Ed. Elsevier Science Publishing Co., Inc., New York.
4. Talukder K. A, Islam M. A., Dutta D.K., Hasan F., Sada A., Nair G. B . and Sack D. A., 2002, J. Clin. Microbiol., 40:2490

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