Dehydrated Culture Media
Bases / Media Supplements

Technical Information

Acetate Differential Agar

Product Code: DM 1339

Application: - 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

Principle & Interpretation

Trabulsi and Ewing [1] formulated acetate differential agar by which was modified by replacing sodium citrate by sodium acetate, for the differentiation of *Shigella* species from *Escherichia coli* [2]. Organic acids have been used widely as an aid in the differentiation of *Enterobacteriaceae,* Most bacteria, 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. For this purpose this medium contains sodium acetate as the sole source of nitrogen. 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 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 serobiotypes 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.

Methodology

Suspend 29.18 grams of powder media in 1000 ml distilled water. Shake well & 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 (12 1°C) for 15 minutes. Allow the tubes to cool in a slanted position.

Quality Control

Physical 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 Range:- 6.50-6.90

Cultural Response/Characteristics

DM 1339: Cultural characteristics observed after an incubation at 25-30°C for upto 1-7 days.

Organism	Inoculum (CFU)	Growth	Acetate utilization
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	50100	None-poor	Negative Reaction no change, medium remain green

Storage and Shelf Life

Dried media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

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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