Acetate Differential Agar

M339

Intended Use:

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 hydrogen 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 purified/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 (6). Tatum, Ewing and Weaver (5) 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.

Sodium acetate is utilized as a sole source of carbon by some serobiotypes of *S.flexneri* such as *Shigella flexneri* 4a (1,4). Magnesium sulphate is essential ion. Sodium chloride maintains osmotic equilibrium and phosphates act as buffers.

Type of specimen

Isolated Microorganism

Specimen Collection and Handling

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Some strains of *Escherichia coli* utilize acetate slowly or not at all and therefore may produce a false negative reaction.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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
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 (00082*)	50-100	good-luxuriant	positive reaction, blue colour
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	positive reaction, blue colour
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	good-luxuriant	positive reaction, blue colour
Proteus vulgaris ATCC 13315	>=10^4	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

Key : *- Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Ewing, 1986, Edwards and Ewings Identification of *Enterobacteriaceae*, 4th Ed. Elsevier Science Publishing Co., Inc., New York.

2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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

5. 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

6. Trabulsi and Ewing, 1962, Public Health Lab., 20:137.

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