



Acetate Differential Agar, Modified

M339F

Intended Use:

Recommended for differentiation of *Shigella* species from *Escherichia coli* in accordance with FDA BAM, 2017

Composition**

Ingredients	Gms / Litre
Sodium acetate	2.000
Sodium chloride	5.000
Magnesium sulphate	0.200
Ammonium phosphate	1.000
Dipotassium hydrogen 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 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

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, 2017 (3).

This medium was formulated by Trabulsi and Ewing (8), by modifying Citrate Medium of Simmons(6). 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* (1). *E.coli* grows well within 24-48 hours in this media indicated by formation of blue colour (2,7). Magnesium sulphate is an essential ion. Sodium chloride maintains osmotic equilibrium and phosphates maintain the pH.

Type of specimen

Isolated Microorganism

Specimen Collection and Handling

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 (3).

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 : 6.70±0.2

pH

6.50-6.90

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Acetate utilization
<i>Citrobacter freundii</i> ATCC 8090	50-100	good-luxuriant	positive reaction, blue colour
<i>Enterobacter cloacae</i> ATCC 23355 (00082*)	50-100	good-luxuriant	positive reaction, blue colour
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	good-luxuriant	positive reaction, blue colour
<i>Klebsiella pneumoniae</i> ATCC 13883 (00097*)	50-100	good-luxuriant	positive reaction, blue colour
<i>Proteus vulgaris</i> ATCC 13315	≥10 ⁴	inhibited	
<i>Salmonella</i> Arizonae ATCC 13314	50-100	good-luxuriant	positive reaction, blue colour
<i>Salmonella</i> Typhi 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

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 in order 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 (4,5).

Reference

1. Cordaro, J.T. and Ball, R.J. 1966. Applied Microbiology, 14(6): 886-887.
2. Ewing. 1986. Edwards and Ewings Identification of *Enterobacteriaceae* 4 ed. N.Y: Elsevier Science Pub. Co., Inc.
3. FDA, U.S. 2017. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. 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.
6. Simmons. 1926. J. Infect. Dis, 39.
7. 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.
8. Trabulsi. and Ewing. 1962. Public Health Lab, 20.

Revision : 02/ 2019

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