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# ACETATE DIFFERENTIAL AGAR

## (Sodium Acetate)

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### INTENDED USE

Remel Acetate Differential Agar is a solid medium recommended for use in qualitative procedures to differentiate species of *Shigella* from *Escherichia coli* and nonfermentative gram-negative bacilli.

### SUMMARY AND EXPLANATION

Organic acids have been widely used in media containing organic nitrogen sources as a means of differentiating *Enterobacteriaceae*. Most bacteria can use citrate and acetate in the presence of organic nitrogen. Simmons Citrate Agar was developed by Simmons to measure citrate utilization without organic nitrogen present.<sup>1</sup> Trabulsi and Ewing replaced sodium citrate with sodium acetate in their formulation of Acetate Differential Agar.<sup>2</sup> They demonstrated a high percentage of *E. coli* strains, primarily nonmotile, anaerogenic biotype A-D, grew on Acetate Differential Agar within 48 hours, whereas none of the *Shigella* strains tested grew. *E. coli* grow by utilizing acetate as a sole source of carbon. Other *Enterobacteriaceae*, *Salmonella*, *Citrobacter*, *Klebsiella*, *Enterobacter*, and *Serratia*, usually exhibit growth within 1 to 7 days but *Proteus* and *Providencia* do not.

### PRINCIPLE

This medium contains a mixture of salts and sodium acetate as the carbon source. *Shigella* spp. fail to utilize sodium acetate, therefore, no growth or color change is observed on the medium. Most strains of *E. coli* grow well within 24 to 48 hours, but some strains grow more slowly. Growth results in the production of an alkaline by-product which increases the pH causing the brom thymol blue indicator to change from green to blue.

### REAGENTS (CLASSICAL FORMULA)\*

Sodium Chloride.....	5.0 g	Magnesium Sulfate.....	0.1 g
Sodium Acetate.....	2.0 g	Brom Thymol Blue.....	0.08 g
Dipotassium Phosphate .....	1.0 g	Agar.....	20.0 g
Monoammonium Phosphate .....	1.0 g	Demineralized Water .....	1000.0 ml

pH 6.7 ± 0.1 @ 25°C

\*Adjusted as required to meet performance standards.

### PROCEDURE

1. Emulsify a portion of a colony from a pure, 18-24 hour culture in 1.0 ml of sterile physiological saline (0.85%). A broth suspension should not be used due to carryover of peptones (a carbon source).
2. Using a sterile inoculating loop, transfer a loopful of the suspension to the surface of the Acetate Differential Agar slant.
3. Incubate aerobically at 33-37°C for at least 7 days with intermittent observation.
4. Observe for a color change from green to blue along the slant.

### INTERPRETATION OF THE TEST

Positive Test - Blue color development along the slant

Negative Test - No color change

### QUALITY CONTROL

All lot numbers of Acetate Differential Agar have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

### CONTROL

*Escherichia coli* ATCC® 9339

*Shigella sonnei* ATCC® 9290

### INCUBATION

Aerobic, up to 7 days @ 33-37°C

Aerobic, up to 7 days @ 33-37°C

### RESULTS

Positive

Negative

### LIMITATIONS

1. This test is usually performed when other biochemical reactions fail to differentiate *E. coli* from *Shigella*.<sup>3</sup>
2. Some strains of *E. coli* do not use acetate as a carbon source.<sup>4</sup>
3. A heavy inoculum may cause false-positive results.<sup>3</sup>

### BIBLIOGRAPHY

1. Simmons, J.S. 1926. J. Infect. Dis. 39:209.
2. Trabulsi, L.R. and W.H. Ewing. 1962. Public Health Lab. 20:137.
3. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.
4. Edwards, P.R. and W.H. Ewing. 1972. Identification of *Enterobacteriaceae*. 3<sup>rd</sup> ed. Burgess Publ. Co., Minneapolis, MN.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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