Acetate Differential Agar

Intended Use
Acetate Differential Agar is used for the differentiation of *Shigella* species from *Escherichia coli*.

Summary and Explanation
Organic acids have been used widely as an aid to 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 citrate media of Koser[1] and Simmons[2] were free of organic nitrogen and, therefore, were a true measure of citrate utilization. In a further extension of this approach, Trabulsi and Ewing developed Acetate Differential Agar, a chemically defined medium utilizing sodium acetate that enables the differentiation of *Shigella* spp. from *E. coli*, particularly anaerogenic, nonmotile biotypes.[3,4] Their basal medium was Simmons Citrate Agar in which sodium acetate was substituted for sodium citrate.

Principles of the Procedure
Acetate Differential Agar consists of a mixture of salts and sodium acetate, as a sole source of carbon, in a chemically defined medium devoid of organic nitrogen.

Typical cultures of *Shigella* are unable to utilize acetate and fail to grow; therefore, the medium remains unchanged. Most cultures of *E. coli* and closely related organisms grow well within 24-48 hours, but some strains grow more slowly and a few cannot use the acetate as a source of carbon. The blue color of the bromthymol blue is due to the production of alkaline products from the utilization of the sodium acetate.

Formula
Difco™ Acetate Differential Agar

Approximate Formula* Per Liter		
Sodium Acetate	2.0	g
Magnesium Sulfate	0.1	g
Sodium Chloride	5.0	g
Monoammonium Phosphate	1.0	g
Dipotassium Phosphate	1.0	g
Bromthymol Blue	0.08	g
Agar	20.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product
1. Suspend 29.2 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense into tubes to allow a 10 mm butt and a 30 mm slant.
4. Autoclave at 121°C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure
Inoculate the agar slant surfaces with pure cultures of unknown organisms. Incubate all tubes for up to 7 days at 35 ± 2°C in an aerobic atmosphere.

Expected Results
Bacteria capable of utilizing acetate as the sole carbon source will grow on the medium and produce an alkaline reaction (blue color). For a listing of organisms capable of utilizing acetate, consult appropriate texts.[4-6]

Limitations of the Procedure
Some strains of *E. coli* utilize acetate slowly or not at all and may give a false-negative reaction. Sodium acetate is utilized as a sole source of carbon by some biotypes of *S. flexneri* 4a.[4]

User Quality Control
Identity Specifications
Difco™ Acetate Differential Agar

Dehydrated Appearance:	Medium yellowish-tan to light green, free-flowing, homogeneous.
Solution:	2.92% solution, soluble in purified water upon boiling. Solution is emerald green, slightly opalescent.
Prepared Appearance:	Emerald green to green, slightly opalescent.
Reaction of 2.92% Solution at 25°C:	pH 6.7 ± 0.1

Cultural Response
Difco™ Acetate Differential Agar

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 2-7 days. Acetate utilization is indicated by a color change of the slant from green to blue.

ORGANISM	ATCC™	RECOVERY	ACETATE UTILIZATION
Escherichia coli	25922	Good	Positive (blue)
Shigella sonnei	25931	Poor to good	Negative (green)

Uninoculated Tube

Escherichia coli ATCC™ 25922

References

1. Koser. 1923. J. Bacteriol. *8*:493.
2. Simmons. 1926. J. Infect. Dis. *39*:209.
3. Trabulsi and Ewing. 1962. Public Health Lab. *20*:137.
4. Ewing. 1986. Edwards and Ewing's identification of Enterobacteriaceae, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
5. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
6. Farmer. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Acetate Differential Agar

BAM COMPF SMD

Cat. No. 274210 Dehydrated – 500 g

BBL™ Acetate Differential Agar

BAM COMPF SMD

Cat. No. 221375 Prepared Slants – Pkg. of 10