



## QUALITY CONTROL PROCEDURES

### I INTRODUCTION

Acetate Differential Agar is a medium for the differentiation of *Shigella* and *Escherichia* cultures on the basis of utilization of acetate as a sole source of carbon.

### II PERFORMANCE TEST PROCEDURE

- Inoculate representative samples with the cultures listed below.
  - Using a 0.01 mL calibrated loop, inoculate the slant surfaces with 10<sup>-1</sup> dilutions of 18- to 24-h **Trypticase™** Soy Broth cultures.
  - Incubate tubes with loosened caps at 35 ± 2°C in an aerobic atmosphere.
  - Include **Trypticase** Soy Agar slants as nonselective controls for all organisms.
- Examine tubes for up to 7 days for amount of growth, selectivity and reactions.
- Expected Results

Organisms	ATCC™	Recovery
* <i>Escherichia coli</i>	25922	Moderate to heavy growth with alkaline (blue) reaction
* <i>Shigella flexneri</i>	9199	No growth to trace growth; no change in the indicator

\*Recommended organism strain for User Quality Control.

### III ADDITIONAL QUALITY CONTROL

- Examine tubes as described under "Product Deterioration."
- Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
- Incubate uninoculated representative tubes at 20–25°C and 30–35°C in an aerobic atmosphere and examine after 7 days for microbial contamination.

## PRODUCT INFORMATION

### IV INTENDED USE

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

### V 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<sup>1</sup> and Simmons<sup>2</sup> 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 which enables the differentiation of *Shigella* species from *E. coli*.<sup>3,4</sup> Their basal medium was Simmons Citrate Agar in which sodium acetate was substituted for sodium citrate.

### VI 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 *Escherichia coli* and closely related organisms grow well within 24–48 h, 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.

### VII REAGENTS

#### Acetate Differential Agar

Approximate Formula\* Per Liter Purified Water

Sodium Acetate .....	2.0 g
Magnesium Sulfate .....	0.2 g
Sodium Chloride .....	5.0 g
Monoammonium Phosphate .....	1.0 g
Dipotassium Phosphate.....	1.0 g
Bromthymol Blue.....	0.08 g
Agar .....	14.8 g

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

#### Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

**Storage Instructions:** On receipt, store tubes in the dark at 2–25°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

**Product Deterioration:** Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

## VIII SPECIMEN COLLECTION AND HANDLING

This product is not intended for use directly with specimens or mixed cultures. The organism to be tested must first be in pure culture.

## IX PROCEDURE

**Material Provided:** Acetate Differential Agar Slants

**Materials Required But Not Provided:** Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

**Test Procedure:** Observe aseptic techniques.

Inoculate the agar slant surfaces with pure cultures of unknown organisms. Incubate all tubes for up to 7 days at  $35 \pm 2^\circ\text{C}$  in an aerobic atmosphere.

**User Quality Control:** See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

## X 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.<sup>5,6</sup>

## XI LIMITATIONS OF THE PROCEDURE

Some strains of *E. coli* utilize acetate slowly or not at all and thus may give a false-negative reaction.

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.<sup>5-10</sup>

## XII AVAILABILITY

Cat. No.	Description
221375	BBL™ Acetate Differential Agar Slants, Pkg. of 10 size K tubes

## XIII REFERENCES

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