

## CARBOHYDRATE UTILIZATION

### Purpose and Procedure Summary

Carbohydrate fermentation media, such as purple broth and phenol red broth, are used to determine whether an organism has the ability to ferment various carbohydrates. Carbohydrates typically tested include adonitol, arabinose, glucose, inositol, lactose, melibiose, rhamnose, sorbitol, and sucrose. These media are often used for distinguishing lactose-fermenting enterics, such as *Escherichia coli*, *Enterobacter aerogenes*, and *Klebsiella pneumoniae*,

from non-lactose-fermenting enterics, such as *Proteus vulgaris*, *Shigella flexneri*, and *Salmonella typhimurium*.

Purple broth contains peptone, a single carbohydrate, plus the pH indicator bromocresol purple. This indicator is purple at an alkaline pH, but yellow at an acidic pH. Phenol red broth contains peptone, a single carbohydrate, plus the pH indicator phenol red. This indicator is red at an alkaline pH, but yellow at an acidic pH. A small, inverted glass tube,

called a **durham tube**, is placed at the bottom of each tube containing carbohydrate fermentation media. The tube collects gas, which is often a product of carbohydrate fermentation.

An isolate is inoculated into a purple broth tube or a phenol red broth tube with a sterile transfer loop. The tube is incubated at 35°C for 24–48 hours before examination. The broth is observed for color change and gas production.

### Tips for Success

- When inoculating broth tubes, dip the loop into the medium and then *rub it against the tube wall* to ensure the transfer of inoculum.
- Compare results with an uninoculated control tube to determine whether changes have occurred in the medium.

### Expected Results

Bacteria that ferment a carbohydrate produce acid, or acid and gas, as end-products (Figure 5.1). Acids lower the pH of the medium, causing the bromocresol purple or phenol red pH indicator to turn yellow, while gases (if produced) collect to form bubbles in the durham tube. If bacteria do not ferment a carbohydrate, the medium will remain purple (if purple broth was used) or red (if phenol red broth was used). In addition, no bubbles will be present in the durham tube. A range of results for purple broth with glucose are shown in Figure 5.2, while results for phenol red broth with lactose are shown in Figure 5.3.

**Positive test:** carbohydrates (red, purple) → acid (pH decreases) or acid and gas (**yellow and bubbles in durham tube**)

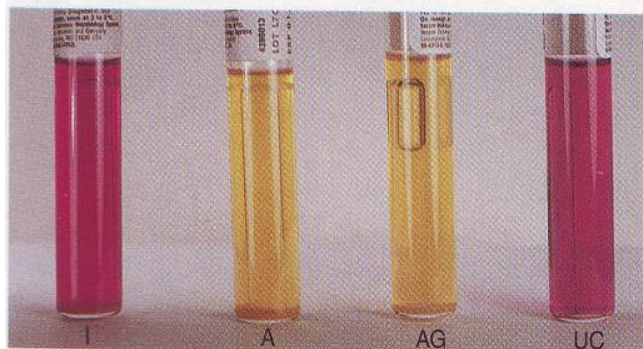
Examples: *Serratia marcescens* (acid)  
*Escherichia coli* (acid and gas)

**Negative test:** carbohydrates (red, purple) → carbohydrates (pH unchanged and no gas) (**red, purple and no bubbles in durham tube**)

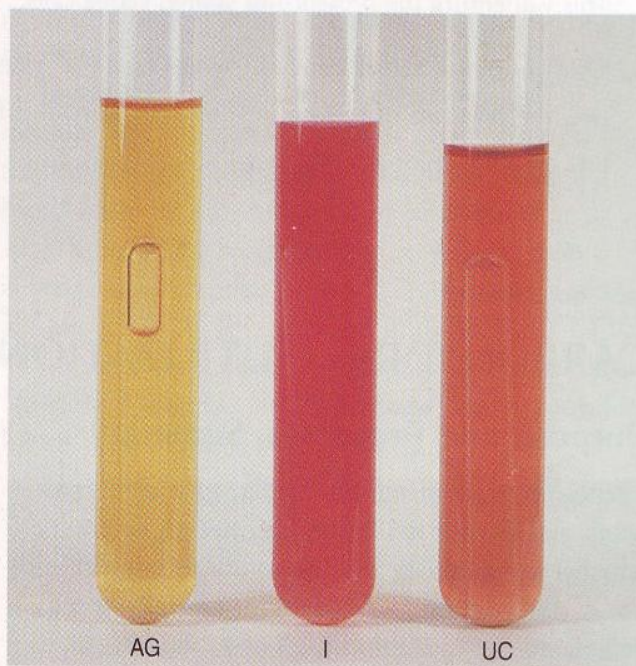
Example: *Alcaligenes faecalis* (no acid or gas)



**FIGURE 5.1** Possible reactions and results of carbohydrate utilization tests.



**FIGURE 5.2** Carbohydrate utilization test results in purple glucose broth tubes. (Left to right): *Alcaligenes faecalis*, inert = no acid or gas (I); *Serratia marcescens*, acid (A); *Escherichia coli*, acid and gas (AG); uninoculated control (UC).



**FIGURE 5.3** Carbohydrate utilization test results in phenol red lactose broth tubes. (Left to right): *Escherichia coli*, acid and gas (AG); *Alcaligenes faecalis*, inert (I); uninoculated control (UC).

