GLOBAL EDITION

Microbiology

A Laboratory Manual

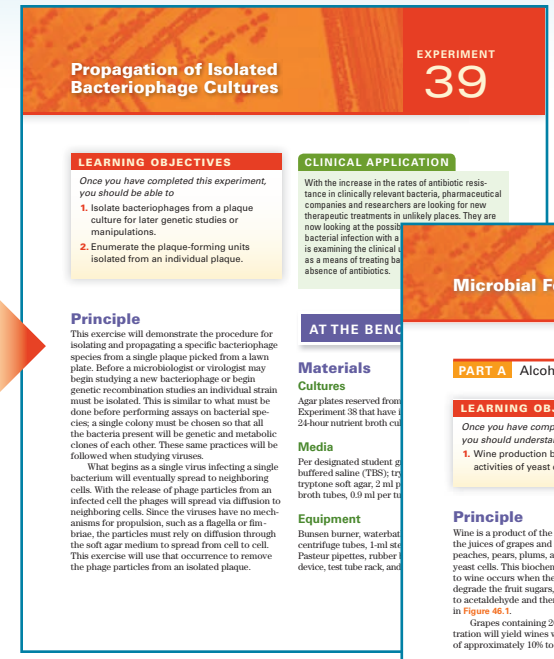
ELEVENTH EDITION

Cappuccino • Welsh

Pearson

A flexible approach to the **modern** microbiology lab

NEW! "Propagation of Isolated Bacteriophage Cultures" experiment has been added to the **Eleventh Edition**. This experiment (39) guides students to isolate bacteriophages for genetic manipulation, an important technique in current clinical research as a possible way to treat antibiotic-resistant bacterial infections.



EXPERIMENT 39

Propagation of Isolated Bacteriophage Cultures

LEARNING OBJECTIVES

Once you have completed this experiment, you should be able to

1. Isolate bacteriophages from a plaque culture for later genetic studies or manipulations.
2. Enumerate the plaque-forming units isolated from an individual plaque.

CLINICAL APPLICATION

With the increase in the rates of antibiotic resistance in clinically relevant bacteria, pharmaceutical companies and researchers are looking for new therapeutic treatments in unlikely places. They are now looking at the possibl bacterial infection with a is examining the clinical as a means of treating ba absence of antibiotics.

Principle

This exercise will demonstrate the procedure for isolating and propagating a specific bacteriophage species from a single plaque picked from a lawn plate. Before a microbiologist or virologist may begin studying a new bacteriophage or begin genetic recombination studies an individual strain must be isolated. This is similar to what must be done before performing assays on bacterial species; a single colony must be chosen so that all the bacteria present will be genetic and metabolic clones of each other. These same practices will be followed when studying viruses.

What begins as a single virus infecting a single bacterium will eventually spread to neighboring cells. With the release of phage particles from an infected cell the phages will spread via diffusion to neighboring cells. Since the viruses have no mechanisms for propulsion, such as a flagella or fimbriae, the particles must rely on diffusion through the soft agar medium to spread from cell to cell. This exercise will use that occurrence to remove the phage particles from an isolated plaque.

AT THE BENC

Materials

Cultures

Agar plates reserved from Experiment 38 that have i 24-hour nutrient broth cu

Media

Per designated student g buffered saline (TBS); try tryptone soft agar, 2 ml p broth tubes, 0.9 ml per tu

Equipment

Bunsen burner, waterbat centrifuge tubes, 1-ml ste Pasteur pipettes, rubber device, test tube rack, and

EXPERIMENT 46

Microbial Fermentation

PART A Alcohol Fermentation

LEARNING OBJECTIVE

Once you have completed this experiment, you should understand

1. Wine production by the fermentative activities of yeast cells.

Principle

Wine is a product of the natural fermentation of the juices of grapes and other fruits, including peaches, pears, plums, and apples, by the action of yeast cells. This biochemical conversion of juice to wine occurs when the yeast cells enzymatically degrade the fruit sugars, fructose and glucose, first to acetaldehyde and then to alcohol, as illustrated in Figure 46.1.

Grapes containing 20% to 30% sugar concentration will yield wines with an alcohol content of approximately 10% to 15%. Also present in grapes are acids and minerals whose concentrations are increased in the finished product and that are responsible for the characteristic tastes and bouquets of different wines. For red wine, the crushed grapes must be fermented with their skins to allow extraction of their color into the juice. White wine is produced from the juice of white grapes.

The commercial production of wine is a long and exacting process. First, the grapes are crushed or pressed to express the juice, which is called **must**. Potassium metabisulfite is added to the must to retard the growth of acetic acid bacteria, molds, and wild yeast that are endogenous to grapes in the vineyard. A wine-producing strain of yeast, *Saccharomyces cerevisiae* var. *ellipsoideus*, is used to inoculate the must, which is then incubated for 3 to 5 days under aerobic conditions at 21°C to 32°C. This is followed by an anaerobic incubation period. The wine is then aged for 1 year to 5 years in aging tanks or wooden barrels. During this time, the wine is clarified of any turbidity, thereby producing volatile esters that are responsible for characteristic flavors. The clarified product is then filtered, pasteurized at 60°C for 30 minutes, and bottled.

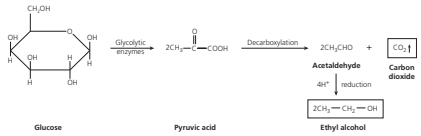


Figure 46.1 Biochemical pathway for alcohol production

REVISED EXPERIMENTS include options for alternate media, making the experiments affordable and accessible to all sizes of lab programs. Experiment 46 now includes both wine and lactic acid fermentation, looking at the production of wine and yogurt.

AT THE BENCH

Materials

Cultures

48- to 72-hour nutrient broth cultures (50 ml per 250-ml Erlenmeyer flask) of *Staphylococcus aureus* BSL-2 *and Bacillus cereus*; 72- to 96-hour Sabouraud broth cultures (50 ml per 250-ml Erlenmeyer flask) of *Aspergillus niger* and *Saccharomyces cerevisiae*.

Media

Per designated student group (pairs or groups of four): five nutrient agar plates, five Sabouraud agar plates, and one 10-ml tube of nutrient broth.

Equipment

Microincinerator or Bunsen burner, 800-ml beaker (waterbath), tripod and wire gauze screen with heat-resistant pad, thermometer, sterile test tubes, glassware marking pencil, and inoculating loop.

Procedure Lab One

1. Label the covers of each of the nutrient agar and Sabouraud agar plates, indicating the experimental heat temperatures to be used: 25°C (control), 40°C, 60°C, 80°C, and 100°C.
2. Score the underside of all plates with a glassware marking pencil into two sections. On the nutrient agar plates, label one section *S. aureus* BSL-2 and the other *B. cereus*. On the Sabouraud agar plates, label one section *A. niger* and the second *S. cerevisiae*.
3. Using aseptic technique, inoculate the nutrient agar and Sabouraud agar plates labeled 25°C by making a single-line loop inoculation of each test organism in its respective section of the plate.
4. Using a sterile pipette and mechanical pipetter, transfer 10 ml of each culture to four sterile test tubes labeled with the name of the organism and the temperature (40°C, 60°C, 80°C, and 100°C).
5. Set up the waterbath as illustrated in Figure 40.2, inserting the thermometer in an uncapped tube of nutrient broth.

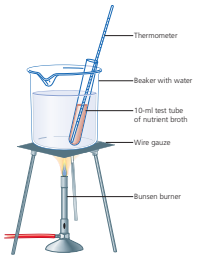


Figure 40.2 Waterbath for moist heat experiment

6. Slowly heat the water to 40°C; check the thermometer frequently to ensure that it does not exceed the desired temperature. Place the four cultures of the experimental organisms into the beaker and maintain the temperature at 40°C for 10 minutes. Remove the cultures and aseptically inoculate each organism in its appropri- ate section on the
7. Raise the waterbat repeat Step 6 for th plates labeled 60°C
8. Raise the waterbat repeat Step 6 for th plates labeled 80°C
9. Raise the waterbat repeat Step 6 for th plates labeled 100°
10. Incubate the nutrie inverted position fo and the Sabouraud 5 days at 25°C in a

Procedure L

1. Observe all plates f the test organisms
2. Record your result the Lab Report.

NEW! BioSafety Levels (BSLs) alert students to appropriate safety techniques. The organisms within this manual are mostly BSL-1 organisms, with any BSL-2 organisms now marked within the text. The Eleventh Edition also reflects the most up to date safety protocols from governing bodies such as the EPA, ASM, and AOAC, better preparing students for professional lab work.

TIPS FOR SUCCESS

- Gram stain your unknown culture first and then determine which tests would be useful in identifying your bacteria. For example, the oxidase test and the citrate test would be of no use in identifying a Gram positive cocci bacteria.
- Since many of the tests utilize agars that are similar in appearance, be sure to label all tubes and plates to ensure that results are collected for the correct test.

NEW! Tips for Success appear throughout the experiments and draw attention to common mistakes and stumbling blocks in the lab. Each tip explains why specific techniques are necessary to yield accurate results and helps guide students on how to perform crucial procedural steps correctly.

Procedure Lab One

1. Prepare the starch agar, tributyrin agar, and milk agar plates for inoculation as follows:
   a. Short procedure: Using two plates per medium, divide the bottom of each Petri dish into two sections. Label the sections as *E. coli*, *B. cereus*, *P. aeruginosa*, and *S. aureus* BSL-2, respectively.
   b. Long procedure: Repeat Step 1a, dividing three plate bottoms into three sections and one plate bottom into four sections for each of the required media, to accommodate the 13 test organisms.
2. Using aseptic technique, make a single-line streak inoculation of each test organism on the agar surface of its appropriately labeled section on the agar plates.
3. Using aseptic technique, inoculate each experimental organism in its appropriately labeled gelatin deep tube by means of a stab inoculation.
4. Incubate all plates in an inverted position for 24 to 48 hours at 37°C. Incubate the gelatin deep tube cultures for 48 hours. Re-incubate all negative cultures for an additional 5 days.

Procedure Lab Two

Starch Hydrolysis

1. Flood the starch agar plate cultures with Gram's iodine solution, allow the iodine to remain in contact with the medium for 30 seconds, and pour off the excess.
2. Examine the cultures for the presence or absence of a blue-black color surrounding the growth of each test organism. Record your results in the chart provided in the Lab Report.
3. Based on your observations, determine and record the organisms that were capable of hydrolyzing the starch.

Lipid Hydrolysis

1. Examine the tributyrin agar plate cultures for the presence or absence of a clear area, or zone of lipolysis, surrounding the growth of each of the organisms. Record your results in the chart provided in the Lab Report.
2. Based on your observations, determine and record which organisms were capable of hydrolyzing the lipid.

Casein Hydrolysis

1. Examine the milk agar plate cultures for the presence or absence of a clear area, or zone of proteolysis, surrounding the growth of each of the bacterial test organisms. Record your results in the chart provided in the Lab Report.
2. Based on your observations, determine and record which of the organisms were capable of hydrolyzing the milk protein casein.

Gelatin Hydrolysis

1. Place all gelatin deep tube cultures into a refrigerator at 4°C for 30 minutes.
2. Examine all the cultures to determine whether the medium is solid or liquid. Record your results in the chart provided in the Lab Report.
3. Based on your observations following the 2-day and 7-day incubation periods, determine and record in the Lab Report (a) which organisms were capable of hydrolyzing gelatin and (b) the rate of hydrolysis.

EXPERIMENT
20
Lab Report

Name: _____

Date: _____ Section: _____

Observations and Results

Starch and Lipid Hydrolysis

	STARCH HYDROLYSIS		LIPID HYDROLYSIS	
Bacterial Species	**Appearance of Medium**	**Result (+) or (−)**	**Appearance of Medium**	**Result (+) or (−)**
E. coli				
B. cereus				
P. aeruginosa				
S. aureus				
S. typhimurium				
P. vulgaris				
K. pneumonia				
A. faecalis				
M. luteus				
L. lactis				
S. dysenteriae				
E. aerogenes				
C. xerosis				
Alternate organism				

Casein and Gelatin Hydrolysis

	CASEIN HYDROLYSIS		GELATIN HYDROLYSIS		
			Liquefaction (+) or (−)		
Bacterial Species	**Appearance of Medium**	**Result (+) or (−)**	**2 days**	**7 days**	**Rate of Hydrolysis (Slow or Rapid)**
E. coli					
B. cereus					
P. aeruginosa					
S. aureus					
S. typhimurium					
P. vulgaris					
K. pneumonia					
A. faecalis					
M. luteus					
L. lactis					
S. dysenteriae					
E. aerogenes					
C. xerosis					
Alternate organism					

Review Questions

1. Why is the catalytic activity of enzymes essential to ensure and regulate cellular metabolism?

2. Why are microorganisms able to cause dairy products, such as milk, to sour or curdle?

3. Give a reason why it is necessary for polysaccharides, such as starch or cellulose, to be digested outside of the cell even though disaccharides, such as lactose or sucrose, are digestible inside the cell.

EXPERIMENT 21

Carbohydrate Fermentation

LEARNING OBJECTIVES

Once you have completed this experiment, you should

1. Understand the difference between cellular respiration and fermentation.
2. Be able to determine the ability of microorganisms to degrade and ferment carbohydrates with the production of acid and gas.

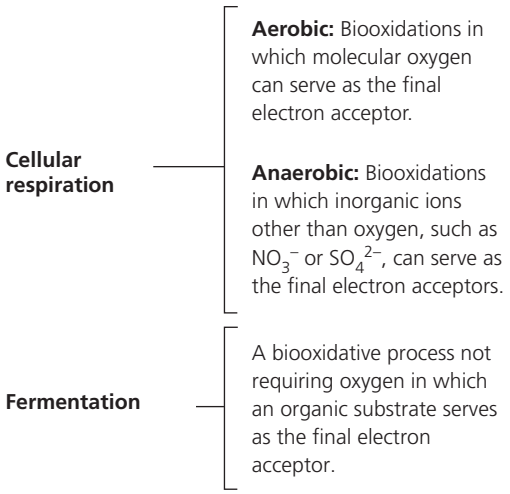


Figure 21.1 **Biooxidative pathways**

Principle

Most microorganisms obtain their energy through a series of orderly and integrated enzymatic reactions leading to the biooxidation of a substrate, frequently a carbohydrate. The major pathways by which this is accomplished are shown in Figure 21.1.

Organisms use carbohydrates differently depending on their enzyme complement. Some organisms are capable of fermenting sugars such as glucose anaerobically, while others use the aerobic pathway. Still others, facultative anaerobes, are enzymatically competent to use both aerobic and anaerobic pathways, and some organisms lack the ability to oxidize glucose by either. In this exercise, the fermentative pathways are of prime concern.

In fermentation, substrates such as carbohydrates and alcohols undergo anaerobic dissimilation and produce an organic acid (for example, lactic, formic, or acetic acid) that may be accompanied by gases such as hydrogen or carbon dioxide. Facultative anaerobes are usually the so-called fermenters of carbohydrates. Fermentation is best described by considering the degradation of glucose by way of the **Embden-Meyerhof pathway**, also known as the **glycolytic pathway**, illustrated in Figure 21.2.

As the diagram shows, one mole of glucose is converted into two moles of pyruvic acid, which is the major intermediate compound produced by glucose degradation. Subsequent metabolism of pyruvate is not the same for all organisms, and a variety of end products result that define their different fermentative capabilities. This can be seen in Figure 21.3.

Fermentative degradation under anaerobic conditions is carried out in a fermentation broth tube containing a Durham tube, an inverted inner vial for the detection of gas production as illustrated in Figure 21.4. A typical carbohydrate fermentation medium contains

1. Nutrient broth ingredients for the support of the growth of all organisms.
2. A specific carbohydrate that serves as the substrate for determining the organism's fermentative capabilities.
3. The pH indicator phenol red, which is red at a neutral pH (7) and changes to yellow at a slightly acidic pH of 6.8, indicating that slight amounts of acid will cause a color change.

The critical nature of the fermentation reaction and the activity of the indicator make it imperative that all cultures should be observed within 48 hours. Extended incubation may mask acid-producing reactions by production of alkali because of enzymatic action on substrates other than the carbohydrate.

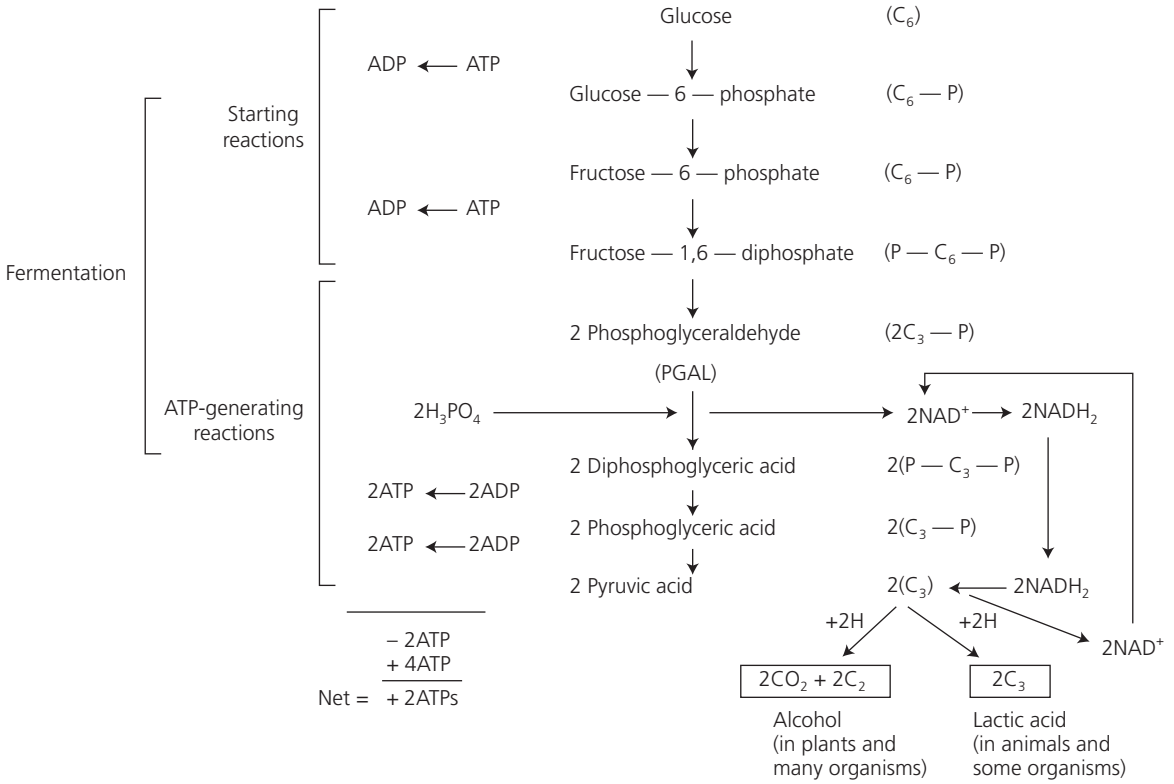


Figure 21.2 **The Embden-Meyerhof pathway**

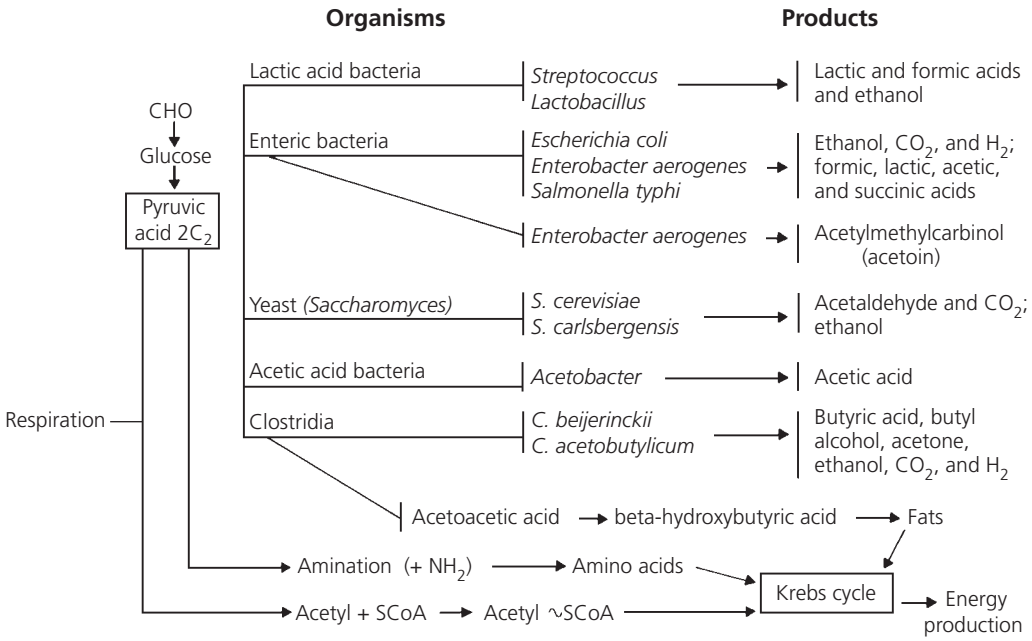


Figure 21.3 **Variations in the use of pyruvic acid**

Following incubation, carbohydrates that have been fermented with the production of acidic wastes will cause the phenol red (Figure 21.5a) to turn yellow, thereby indicating a positive reaction (Figures 21.5b and c). In some cases, acid production is accompanied by the evolution of a gas (CO_2) that will be visible as a bubble in the inverted tube (Figure 21.5b). Cultures that are not capable of fermenting a carbohydrate substrate will not change the indicator, and the tubes will appear

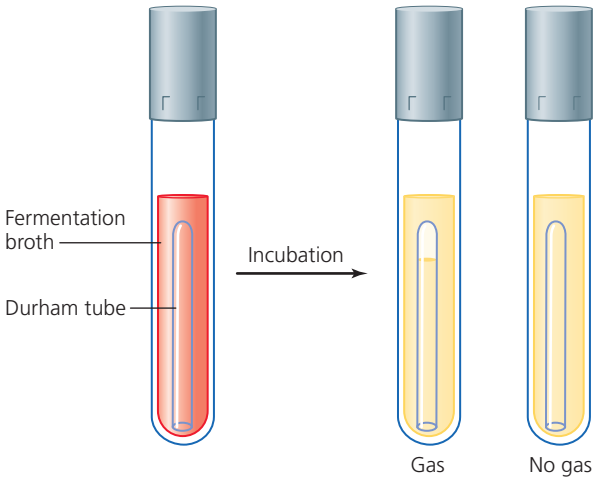


Figure 21.4 Detection of gas production

red; there will not be a concomitant evolution of gas. This is a negative reaction (Figure 21.5d).

The lack of carbohydrate fermentation by some organisms should not be construed as absence of growth. The organisms use other nutrients in the medium as energy sources. Among these nutrients are peptones present in nutrient broth. Peptones can be degraded by microbial enzymes to amino acids that are in turn enzymatically converted by oxidative deamination to ketoamino acids. These are then metabolized through the Krebs cycle for energy production. These reactions liberate ammonia, which accumulates in the medium, forming ammonium hydroxide (NH_4OH) and producing an alkaline environment. When this occurs, the phenol red turns to a deep red in the now basic medium. This alternative pathway of aerobic respiration is illustrated in Figure 21.6.

CLINICAL APPLICATION

Using Fermentation Products to Identify Bacteria

The fermentation of carbohydrates assists in the identification of some bacteria by determining what nutrients they are using and what products they produce. The pattern of sugars fermented may be unique to a particular genus, species, or strain. Lactose fermentation is one test that distinguishes between enteric and non-enteric bacteria. Dextrose fermentation allows for the differentiation between the oxidase (+) *Vibrio* and *Pseudomonads* species in patients suffering from septicemia after eating contaminated fish.

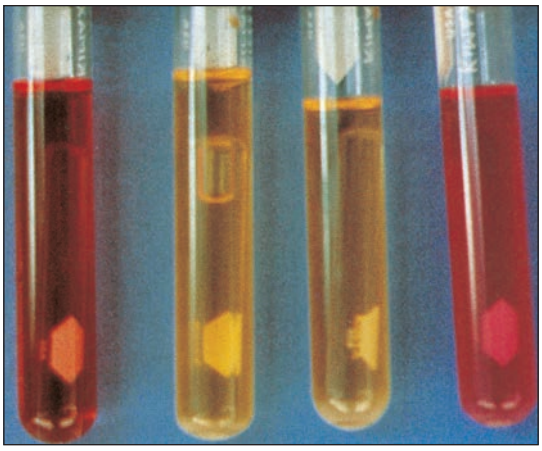


Figure 21.5 Carbohydrate fermentation test. **(a)** Uninoculated, **(b)** acid and gas, **(c)** acid, and **(d)** negative.

AT THE BENCH

Materials

Cultures

24- to 48-hour Trypticase soy broth cultures of *Escherichia coli*, *Alcaligenes faecalis*, *Salmonella typhimurium* BSL-2, and *Staphylococcus aureus* BSL-2 for the short version. 24- to 48-hour Trypticase soy broth cultures of the 13 organisms listed on page 164 for the long version.

Media

Per designated student group: phenol red lactose, dextrose (glucose), and sucrose broths: 5 of each for the short version, 14 of each for the long version.

Equipment

Microincinerator or Bunsen burner, inoculating loop, and glassware marking pencil.

Controls

	REACTION	
Sugar	***Acid***	***Acid w/Gas***
Dextrose	*S. aureus*	*E. coli*
Sucrose	*S. aureus*	*K. pneumoniae*
Lactose	*S. aureus*	*E. coli*