

Chapter 8

Using Human Blood, Saliva, and Dental Caries in the Biology Teaching Laboratory

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Safety in the Biology Laboratory

Since the method of transmission of Human Immunodeficiency Virus (HIV) was uncovered, questions have arisen about the safety of performing laboratory exercises using blood, saliva, and other body fluids. Science educators at all levels—from elementary schools through universities—are concerned and some have stopped using these laboratory exercises.

Laboratory exercises such as salivary amylase activity, urinalysis, and blood typing were developed because they met specific, desirable learning objectives. Moreover, because they use the student's own body, they had the advantage of high student interest. Assuming that the learning objectives are still valid, can we afford to drop these exercises from curricula? Students are told about AIDS and they hear about the disease through the news media. However, these passive activities are not usually designed to promote understanding. A hands-on laboratory (blood-typing) exercise can be used to foster an understanding of antibodies and AIDS transmission. This chapter identifies safety procedures that minimize the risk of transmitting diseases in the laboratory.

“HIV has been isolated from blood, semen, vaginal secretions, saliva, tears, breast milk, cerebrospinal fluid, amniotic fluid, and urine, and is likely to be isolated from other body fluids, secretions, and excretions. However, epidemiologic evidence has implicated only blood, semen, vaginal secretions, and possibly breast-milk in transmission.”¹ The standard practices listed here were developed to minimize the risk in handling potentially infectious urine, tears, and saliva.

Guidelines for the safe handling of blood in health-care settings have been developed to respond to the possible transmission of Hepatitis B Virus (HBV) and HIV.² Laboratory safety procedures need to be part of our instructional objectives. Safety procedures will minimize the risk of infections and accidents and teach students about safety. It is not uncommon in chemistry to require that students pass a safety test on general procedures before beginning laboratory experiments. Perhaps it is time to follow this model in biology. Standard practices can be developed for biology programs that include safe activities for all potential hazards in courses, including the use of chemicals. A sample “Student Safety Sheet” is included as Appendix A.

Safety procedures can be included in all laboratory exercises. Laboratory experiments modified to include safety precautions are described in this chapter. Each instructor (or department) can

1. Centers for Disease Control. *Morbidity and Mortality Weekly Reports*, 36(2S), August 21, 1987.

2. Centers for Disease Control. *Morbidity and Mortality Weekly Reports*, 37(S-4), April 1, 1988.

develop a list of safety procedures applicable to his or her laboratory and distribute it to each student. In this way, the issue of safety will be brought to the individual student's attention.

Many of the following guidelines, applicable to teaching facilities, are adapted from Centers for Disease Control's (CDC) *Guidelines*.³

Standard Practices for the Student

1. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the laboratory.
2. Work surfaces are disinfected at the beginning and end of every laboratory period and after any chemical spill. Appropriate disinfectants include: 1:10 dilution of household bleach (hypochlorite), phenolics, and glutaraldehyde.⁴
3. Mechanical pipetting devices are used; mouth pipetting is prohibited.
4. Wash your hands after every laboratory exercise. Bar soaps may become contaminated, therefore, liquid or powdered soaps should be used.
5. Broken glassware and other sharp objects are put in the appropriate container. Broken thermometers are handled separately.
6. Glassware and slides contaminated with microorganisms, blood, urine, and other body fluids are placed in disinfectant. They can be washed after disinfection.
7. Gloves should be worn when touching blood and body fluids.
8. Hands should be washed immediately and thoroughly if contaminated with blood or other body fluids.
9. Work only with your own saliva, blood, urine, tears, and other secretions and excretions.
10. Wear safety goggles when working with blood.
11. Don't do unauthorized experiments.
12. Don't use equipment without instruction.
13. Horseplay will not be tolerated in the laboratory.

Standard Practices for the Instructor

1. Laboratory doors are kept closed when experiments are in progress.
2. The instructor controls access to the laboratory and limits access only to persons whose presence is required for program or support purposes.
3. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable, leakproof container that is closed before being removed from the laboratory.
4. An insect and rodent control program is in effect.
5. A needle should not be bent, replaced in the sheath, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before being discarded or reused.

3. Biosafety in Microbiological and Biomedical Laboratories. Available from (1) Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402, Stock #01702300167-1 or (2) National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, Stock #PB84-206879.

4. California Health and Welfare Agency, Infectious Disease Branch. California Morbidity, 37, September 23, 1988.

6. The instructor should be informed of students who become pregnant, are taking immunosuppressive drugs, or have any other medical condition (e.g., diabetes, an immunologic defect) which might necessitate special precautions in the laboratory.

Laboratory Facility

1. Interior surfaces of walls, floors, and ceiling are water resistant so that they can be easily cleaned.
2. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
3. Windows in the laboratory are closed and sealed.
4. An autoclave for decontaminating laboratory wastes is available, preferably within the laboratory.
5. An eye wash and safety shower are present in every laboratory.

Disinfection

Any powdered or liquid soaps may be used for routine handwashing in the laboratory. Bar soaps should not be used since they can become contaminated. Liquid soaps which do not contain a preservative, should be cleaned out routinely and replaced with new soap. Powdered soaps have the advantage of not becoming contaminated or allowing organisms to grow in them.

Rapid disinfection of hands may be accomplished by the use of (1) a phenolic disinfectant–detergent for 20–30 seconds and then rinse with water, or (2) alcohol (50–70%) for 20–30 seconds, followed by a soap scrub of 10–15 seconds and rinse with water.

Spills of blood, urine, or other body fluids onto bench tops may be disinfected by using a disinfectant–detergent. The spill should be covered with disinfectant for 20 minutes before cleaning up.

Potentially infectious wastes including human body secretions and fluids and objects, such as slides, syringes, lancets, and bandages, contaminated with these materials should be placed in an autoclavable container. Note that sharp objects (including broken glass) must be placed in a puncture–proof container. Contaminated glassware should be placed in a container of disinfectant. Glassware should be autoclaved before washing. Materials labelled “To Be Autoclaved” should not be stored longer than 4 days before autoclaving.

Disinfectants that disrupt the envelope of HIV and HBV are (examples in parentheses): halogens, 10% (chlorine bleach); alcohols, 70% (ethyl alcohol); phenols (Amphyl); and aldehydes (glutaraldehyde, 1%).

Autoclaving

Odd-sized supplies such as tongue depressors will require special wrapping for autoclaving. Acceptable wrapping materials for use in an autoclave include: Kraft paper, small paper bags, muslin, glassine envelopes, and autoclavable cellophane. Air in packages must be displaced by steam in an autoclave. Aluminum foil and polyethylene should not be used: aluminum foil is not permeable to steam and it is difficult to remove air from polyethylene packaging.

Aerosols

The sources of aerosols and other risks associated with common laboratory maneuvers are given in Table 8.1. Most laboratory-acquired infections are probably transmitted by inhaled aerosols.

Table 8.1. Sources of aerosols and other risks associated with common laboratory maneuvers.

Maneuver	Source of aerosol	Other risks
Blood taking	Forcing blood through needle and taking needle off syringe; squirting blood into container; vibrating needle.	Spray to eyes and mucous membranes; self-inoculation.
Mixing and shaking	Inevitable production of stable aerosols.	Mucous membranes, skin contamination.
Transport	Aerosol produced in airspace in tube by shaking is released on opening of tube.	Leaky tubes and caps.
Opening containers	An ever-present and inevitable danger.	Skin contamination.
Centrifugation	Tubes leaking or breaking; aerosol in airspace released on opening of tubes.	Machine contamination.
Pipetting	Dispensing last drop; dropping solution onto bench or plates, etc.	Ingestion – although mouth-pipetting should never be done.
Bacteriologic loops	Hot loops sizzle; vibrating loop; plate streaking; flaming loop.	
Disposal and wash-up	Almost all activities.	

References

- American Society for Microbiology. *Laboratory Safety Principles and Practices*.
- American Society for Microbiology. *Manual of Methods for General Bacteriology*. Chapter 24, Containment and disinfection.
- California Association of Public Health Laboratory Directors. *A Laboratory Safety Guide*.
- Centers for Disease Control. *Biosafety in Microbiological and Biomedical Laboratories*. U.S. Government Printing Office, Washington, D.C. 20402, Stock #01702300167-1 or National Technical Information Service, US. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, Stock #PB84-206879.
- Centers for Disease Control. Agent summary statement for human immunodeficiency virus and Report on laboratory-acquired infection with human immunodeficiency virus. *Morbidity and Mortality Weekly Reports*, 1988; 37(S-4).
- Centers for Disease Control. Guidelines for prevention of transmission of HIV and hepatitis B virus to health-care and public-service workers. *Morbidity and Mortality Weekly Reports* 1989; 38(S-6).
- World Health Organization. *Laboratory Biosafety Manual*.

Experiment 1: Blood Typing (ABO, Rh, and MN)

Learning Objectives

1. Determine ABO, Rh, and MN blood types.
2. Determine compatible transfusions.
3. Determine your genotype for ABO, Rh, and MN.

Materials

Microscope slides (3)
Grease marking pencil
Disinfectant-soaked towel
Cotton ball
70% alcohol
Sterile lancet
Bandage
Antisera: anti-A, anti-B, anti-D, anti-M, anti-N
Toothpicks (5)

Background

Hemagglutination (blood-cell clumping) reactions are used in the typing of blood. The presence or absence of two very similar carbohydrate antigens (designated A and B) located on the surface of red blood cells is determined using specific antisera. When anti-A antiserum is mixed with type B red blood cells, no hemagglutination occurs. An individual possesses antibodies to the opposite antigen. Thus, persons of blood type A will have anti-B in their serum. Persons with type AB blood possess both A and B antigens on their red blood cells and persons with type O blood lack A and B antigens and have anti-A and anti-B in their serum.

ABO blood types are controlled by three alleles: I^A and I^B are co-dominant and both are dominant over i . The homozygous recessive condition (ii) results in type O blood. A person with blood type A may have either of the following genotypes: $I^A I^A$ or $I^A i$. What are the possible genotypes for blood type B? The genotype $I^A I^B$ results in type AB blood.

The Rh factor is a complex of over 30 different antigens on the surface of human red blood cells. The Rh factor that is routinely used in blood typing is the Rh_o antigen or D antigen. The presence of the Rh factor is determined by a hemagglutination reaction between anti-D antiserum and red blood cells. Persons possessing the Rh antigen are called Rh-positive. Although multiple alleles are responsible for the Rh antigen, it is useful to designate Rh-positive individuals as $R/_$ and Rh-negative individuals, r/r . Rh-negative individuals do not naturally have anti-Rh in their sera.

Another group of surface antigens on red blood cells is designated MN. The alleles M and N are co-dominant. Persons with type M cells possess the M antigen, persons with type N cells possess the N antigen, and MN individuals have both M and N antigens.

Isoantibodies are present in human serum. An individual possesses isoantibodies to the opposite A or B isoantigen. Thus, persons of blood type A will have serum containing isoantibodies to the B antigen. Rh-negative individuals do not normally have anti-Rh antibodies in their sera. When red blood cells with Rh antigen are introduced into Rh-negative individuals, anti-Rh antibodies will be produced. Surface antigen groups place restrictions on how blood and other tissues may be transferred from one person to another. An incompatible transfusion results when the antigens of the donor cells react with antibodies in the recipient's serum. A summary of the major characteristics of ABO, Rh, and MN human blood groups is shown in Table 8.2.

Table 8.2. Major characteristics of ABO, Rh, and MN human blood groups.

Blood Type	A	B	AB	O	Rh ⁺	RH ⁻	M	MN	N
Antigen present on the cells	A	B	A and B	Neither A nor B	D	-	M	Both M and N	N
Antigen normally present in the serum	Anti-B	Anti-A	Neither anti-A nor anti-B	Both anti-A and anti-B	-	- ¹	- ²	-	- ²
Serum causes agglutination of cells of these types	B, AB	A, AB	None	A, B, AB	-	- ¹	- ²	-	- ²
Cells agglutinated by serum of these types	B, O	A, O	A, B, O	-	- ¹	-	- ²	-	- ²
% occurrence in US population	41	10	4	45	85	15	28	50	22

1. Antibodies can be produced upon exposure to the D antigen through transplants or pregnancy.
2. Antibodies can be produced upon exposure to the M or N antigen through transplants.

Procedures

Wear gloves or work with your own blood. You should not do this laboratory experiment if you are sick.

1. With a grease pencil, draw two circles on a clean glass slide, label one A and the other B. Draw a circle on a second slide and label it D. Draw two circles on the third slide and label one M and the other N.
2. Place the slides on a disinfectant-soaked paper towel and work over this paper towel.
3. *Disinfect your finger with 70% alcohol.* Use any finger except your thumb. Unwrap a *new, sterile* lancet and pierce the disinfected finger with the sterile lancet.
4. *Place the used lancet in disinfectant.*
5. Let a drop of blood fall into each circle.
6. *Stop the bleeding with a sterile cotton ball and apply an adhesive bandage.*
7. *Dispose of the used cotton in disinfectant.*
8. To the circle A, add 1 drop of anti-A antiserum. Add 1 drop of anti-B antiserum to the B circle and 1 drop of anti-D antiserum to the D circle. Add 1 drop of anti-M antiserum to the M circle and 1 drop of anti-N to the N circle.
9. Mix each suspension with a different toothpick. Why use different toothpicks? Place the slides on a light box. The heat will improve the reaction and the light will make viewing easier.
10. Observe for agglutination and record your results in Table 8.3. Determine your blood type.
11. *Discard used slides and toothpicks in disinfectant.*
12. *Wash any spilled blood from your work area with disinfectant.*
13. *Wash your hands.*

Table 8.3. Results of blood-typing experiment.

Antiserum	Hemagglutination? (Yes or No)
Anti-A	
Anti-B	
Anti-D	
Anti-M	
Anti-N	

Conclusions and Questions

1. What is your blood type? _____
2. What is the blood type of the person on your left? _____
 Can you donate blood to this person? _____ Briefly explain.
 Can you donate a kidney to this person? _____ Briefly explain.

3. What is the blood type of the person on your right? _____
Can you receive blood from this person? _____ Briefly explain.
Can you receive a kidney from this person? _____ Briefly explain.
4. What is your ABO genotype? _____
Your Rh genotype? _____
Your MN genotype? _____

Experiment 2: Salivary Amylase Activity

Learning Objectives

1. Define enzyme.
2. Perform a chemical test to determine the presence of starch.
3. Determine the presence of salivary amylase.

Materials

Spot plate
Grease marking pencil
Lugol's iodine
Starch solution
50-ml beaker
Toothpicks (6)
Pasteur pipette

Background

Digestion is brought about by enzymes, protein molecules made by cells. An enzyme acts as a catalyst—that is, it lowers the amount of energy needed to make a reaction occur and is not used up in the reaction. Enzymes have specific reactive sites for the substrate or chemical on which they act. Most enzyme names end with the suffix *-ase*.

An enzyme and its substrate collide and briefly combine in an enzyme-substrate complex. The enzyme brings about a change in the substrate and is then free to act on a new substrate molecule. Some enzymes work outside of cells to make large molecules into smaller ones that can then be acted on by intracellular enzymes. Most extracellular digestion in humans takes place in the small intestine.

Enzymes are widely used in industry. For example, amylases are used in producing syrups from corn starch, in making coating for papers, and in the production of glucose (also called dextrose) used in food manufacturing.

We will test salivary amylase. This is an enzyme that is secreted from cells in the salivary gland and hydrolyzes starch molecules. Polysaccharides such as starch and cellulose consist of simple sugar subunits. Both starch and cellulose are made of glucose molecules attached together. When these carbohydrates are hydrolyzed or broken down, sugar is released. The glucose subunits are bonded differently in starch and cellulose. The enzyme amylase breaks the bonds in the starch molecule only to produce maltose and glucose. The enzyme cellulase which is not found in animals, breaks the bonds in cellulose.

To test whether a particular enzyme is active, we can test for the presence of substrate or product. In this determination of amylase activity we will test for starch using iodine. Iodine turns blue in the presence of starch.

Procedures

Work with your own saliva.

1. Collect about 5 ml of saliva in a small beaker.
2. Number eight depressions in a spot plate 1 through 8. Place 5 drops of cornstarch solution in each of depressions 2 through 8.
3. Add 5 drops of water to depressions 1 and 2. Add 5 drops of saliva to depressions 3 through 8. What is the purpose of adding water to 1 and 2?
4. Add 2 drops of Lugol's iodine to depressions 1 and 2. Record the color in Table 8.4.
5. Add 2 drops of iodine to depression 3, mix, and record any color change.
6. After 1 minute, add 2 drops of iodine to depression 4, mix with a toothpick, and record the color.
7. To test the remaining depressions, add 2 drops of iodine to depression 5 after 5 minutes; 6 after 10 minutes; 7 after 20 minutes; and 8 after 30 minutes.
8. Be sure to record your results. What is the longest time starch and saliva are mixed together?
9. *Discard used toothpicks in disinfectant. Wash the beaker and spot plate with soap and water. Rinse with disinfectant, then rinse with water.*

Table 8.4. Results of salivary amylase activity.

Depression #	Color	Presence of starch
1		
2		
3		
4		
5		
6		
7		
8		

Conclusions and Questions

1. Do you have amylase in your saliva? How can you tell?
2. Is amylase an intracellular or extracellular enzyme? How can you tell?
3. What would you see if you dropped iodine on a potato or slice of bread?
4. Identify the substrate and products for amylase.

Experiment 3: Determining Susceptibility to Dental Caries

Learning Objectives

1. Describe the formation of dental caries.
2. Explain the relationship between sucrose and caries.

Materials

Rodac plate containing Mitis-Salivarius-Bacitracin (MSB) agar
 Paraffin
 Sterile tongue blade
 Candle jar
 Glucose broth tube
 Fructose broth tube
 Sucrose broth tube
 Sterile popsicle sticks (3)

Background

The mouth contains millions of bacteria in each milliliter of saliva. Some of these bacteria are transient flora carried on food. Some species of *Streptococcus* are part of the normal flora of the mouth.

Streptococcus mutans, *S. salivarius*, and *S. sanguis* produce sticky polysaccharides from sucrose. Bacterial exoenzymes hydrolyze sucrose into its component monosaccharides, glucose and fructose. *S. mutans* and *S. sanguis* polymerize the glucose moiety and release fructose. Energy released in the hydrolysis is used to polymerize the glucose to form dextran. Fructose released from the sucrose is fermented to produce lactic acid. Enzymes of *S. salivarius* have similar specificity for sucrose but the fructose is polymerized into levan (a fructose polymer) and the liberated glucose is fermented.

The dextran capsule enables the bacteria to adhere to surfaces in the mouth. *S. salivarius* colonizes the surface of the tongue. *S. sanguis* and *S. mutans* colonize teeth. Masses of bacterial cells, dextran, and debris adhering to the teeth constitute dental plaque. Production of lactic acid by bacteria in the plaque initiates dental caries by eroding tooth enamel. Streptococci and other bacteria, such as *Lactobacillus*, are able to grow on the exposed dentin and tooth pulp.

Other carbohydrates such as glucose or starch may be fermented by bacteria, but they are not converted to dextran and hence do not promote plaque formation. “Sugarless” candies contain mannitol or sorbitol, which cannot be converted to dextran although they may be fermented by normal flora such as *S. mutans*. Acid production increases the size of dental caries.

The number of *S. mutans* present in paraffin-stimulated saliva has been correlated with the potential for caries formation. In this exercise, you will observe and count *S. mutans* and other polysaccharide producing streptococci in a paraffin-stimulated saliva sample. The media used contain sucrose to promote capsule formation and Mitis-Salivarius-Bacitracin (MSB) agar inhibits the growth of most oral bacteria, except *S. mutans*.

Procedure

The Rodac plate and glucose, fructose, and sucrose broths are sterile. Do not open the plate or tubes until you are ready to inoculate them. Replace the tops immediately.

Part A

1. Label an MSB plate with your name and date.
2. Allow a small piece of paraffin to soften under your tongue and then chew it for 1 minute. Do not swallow your saliva or the paraffin.
3. Rotate a sterile tongue blade in your mouth 10 times so both sides of the blade are thoroughly inoculated with saliva. Remove the tongue blade through closed lips to remove excess saliva. Don't swallow yet, you'll need the saliva in Part B.
4. Press one side of the tongue blade onto one half of the surface of the MSB agar. Then, press the other side of the blade onto the other side of the agar. *Discard the tongue depressor in the disinfectant jar.*
5. Incubate the plate inverted in a candle jar for 72–96 hours at 35°C.
6. Examine the MSB plate with a dissecting microscope for the presence of *S. mutans*. *S. mutans* colonies are light blue to black, raised, and rough—their surface resembles etched glass or “burnt” sugar. Other bacteria occasionally grow on MSB, therefore careful observation of colony morphology is necessary to accurately estimate the number of *S. mutans* present.
7. *Discard your cultures in the “To Be Autoclaved” area.*

Part B

1. Write your name and date on the glucose, fructose, and sucrose tubes.
2. Rotate a sterile popsicle stick in your mouth 10 times so both sides of the stick are thoroughly inoculated with saliva. Remove the stick through closed lips to remove excess saliva.
3. Place this stick in the glucose broth tube. Replace the cap on the tube.
4. Inoculate another stick using the procedure in step 2. Place this stick in the fructose broth tube.
5. Moisten a third stick and place this stick in the sucrose broth tube. It's okay to swallow now. *Discard the paraffin in the disinfectant jar.*
6. Incubate the three tubes for 72–96 hr at 35°C.
7. Examine the sticks for the presence of bacterial colonies and record results in Table 8.5. The sticky capsules allow the bacteria to adhere to the stick just as they would to teeth.
8. *Discard your tubes in the “To Be Autoclaved” area.*

Table 8.5. Results of dental-carries susceptibility tests.

Part A: MSB agar		
Species	Number	Description

Part B: Broth tubes		
Sugar	Number of colonies on stick	Average diameter of colonies
Glucose		
Fructose		
Sucrose		

Conclusions and Questions

1. Look at the plates of your classmates and compare your oral flora.
2. Studies have shown that both sucrose and bacteria are necessary for tooth decay. Why should this be true?
3. Even if a large number of *S. mutans* is in your saliva, how might you avoid tooth decay?

APPENDIX A
“Student Safety Sheet” for Use in the Biology Laboratory

Biosafety

1. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the laboratory.
2. Work surfaces are disinfected at the beginning and end of every laboratory period and after every spill. The disinfectant used in this laboratory is _____.
3. Mechanical pipetting devices are used; mouth pipetting is prohibited.
4. Place a disinfectant-soaked paper towel on desk while pipetting.
5. Wash your hands after every laboratory exercise. Bar soaps may become contaminated, therefore, liquid or powdered soaps should be used.
6. Cover spilled microbial cultures with paper towels and squirt disinfectant on towel. Leave for 20 minutes then clean up the spill. Do not touch broken glassware with your hands, use the broom and dustpan. Broken glassware contaminated with microbial cultures or body fluids are placed in the “To Be Autoclaved” container. (See the Glassware section on broken glassware that is not contaminated.)
7. Glassware and slides contaminated with microorganisms, blood, urine, and other body fluids are placed in disinfectant.
8. Gloves should be worn when touching blood and body fluids from other people.
9. Hands should be washed immediately and thoroughly if contaminated with blood or other body fluids.
10. Work only with your own saliva, blood, urine, tears, and other secretions and excretions.
11. Wear safety goggles when working with blood.
12. Long hair should be tied back.
13. Don't do unauthorized experiments.
14. Don't use equipment without instruction.
15. Horseplay will not be tolerated in the laboratory.

Specific Hazards

Alcohol

Keep containers of alcohol away from open flames. When it is necessary to heat alcohol, pour alcohol into a heat-resistant container and heat on a hot plate.

Glassware

If you break a glass object, sweep up the pieces using a broom. Do not pick up pieces of broken glass with your bare hands. Broken glass is to be placed in one of the containers marked for this purpose. The one exception to this rule concerns broken thermometers; consult your instructor if you break a thermometer.

Electrical equipment

The basic rule to follow is electricity and water don't mix. Do not allow water or any water-based solution to come into contact with electrical cords or electrical conductors. Your hands should be dry when you handle electrical connectors. If your electrical equipment crackles, snaps, or begins to give off smoke, do not attempt to disconnect it. Call your instructor immediately.

Fire

If *gas burns* from a leak in the burner or tubing, turn off the gas.

your hand). Smother the fire quickly.

laboratory to put it out. Your instructor will demonstrate the use of the fire extinguishers.

according to the following procedure:

1. Turn off all gas burners and unplug electrical equipment.
2. Leave the room and proceed _____.
3. It is imperative that you assemble in front of the building so that your instructor can take roll to determine if anyone is still in the building. Do not wander off.

First aid

1. Report all accidents immediately. Your instructor will administer first aid as required.
2. For spills in or near the eyes, use the eyewash.
3. For large spills on your person, use the safety shower.
4. For heat burns, the affected part should be chilled with ice as soon as possible.

Power outage

If the electricity goes off, be sure to turn your gas jet off. When the power is restored, the gas will come on.

Earthquake

Turn off your gas jet and get under your laboratory desk during the temblor. Your instructor will give any necessary evacuation instructions.

Orientation walkabout

Locate the following items in the laboratory:

- | | |
|-------------------|-------------------------|
| Broom and dustpan | Reference books |
| Eyewash | Safety shower |
| Fire blanket | “To Be Autoclaved” Area |
| Fire extinguisher | |
| First aid cabinet | |
| Fume hood | |
| Instructor's desk | |

APPENDIX B
Materials for Experiment 1

Notes

1. This exercise can be used for immunology or genetics.
2. This exercise can be done using synthetic blood which is available from biological supply houses or blood from a blood bank. Blood obtained from blood banks is tested for HBV and HIV. No test method can offer complete assurance that laboratory specimens do not contain these viruses.
3. Used slides can be placed in disinfectant. The slides can then be autoclaved and washed or discarded. If slides are not placed in disinfectant for mass decontamination, students may wash slides with abrasive cleanser, rinse in disinfectant, and rinse with water.

Materials

Per student

- Sterile cotton ball (1)
- Sterile lancet (1)
- Glass microscope slides (3)
- Toothpicks (5)
- Grease marking pencil (1)
- Band-Aid (1)

Per pair

- Disinfectant in wash bottle (1)

Per class

- 70% ethyl alcohol (250 ml)
- Anti-A antiserum (2)
- Anti-B antiserum (2)
- Anti-M antiserum (2)
- Anti-N antiserum (2)
- Anti-D antiserum (2)
- Rh view box (Carolina Biological Supply Company #70-0636) (1)

Disposal area

Separate counter or cart with:

- (1) 1-gallon jar of disinfectant for discarding lancets, cotton, tongue depressors, toothpicks, etc.;
- (2) disinfectant-container labeled "Slide Disposal"; and
- (3) disinfectant-container for used pipettes.

APPENDIX C
Materials for Experiment 2

Materials

Per student

Spot plate (1)
Toothpicks (6)
Grease marking pencil (1)
Pasteur pipette with bulb (1)
50-ml beaker (1)

Per pair

Disinfectant in wash bottle (1)

Per class

Lugol's iodine in dropper bottle (4)
(Dissolve 10 g KI in 100 ml distilled water; then add 5 g iodine. Dispense in dark dropper bottles.)
0.1% cornstarch (boil to dissolve) (4)
(Dispense in dropper bottle.)

Disposal area

Separate counter or cart with:

- (1) 1-gallon jar of disinfectant for discarding lancets, cotton, tongue depressors, toothpicks, etc.;
- (2) disinfectant-container labeled "Slide Disposal"; and
- (3) disinfectant-container for used pipettes.

APPENDIX D
Materials for Experiment 3

Notes

To use MSB results to quantify susceptibility to tooth decay see Newbrun et al. (Comparison of two screening tests for *Streptococcus mutans* evaluation of their suitability for mass screening and private practice. *Community Dentistry and Oral Epidemiology*, 12:325–331; 1984).

Materials

Per student

Rodac plate containing MSB agar (1)

MSB agar

Mitis-Salivarius (MS) agar

Sucrose

Bacitracin (3 mg/ml), filter-sterilized

Chapman Tellurite Solution

Dissolve, with heat 90 g MS agar and add 150 g sucrose with 1 litre water. Autoclave and cool to 50°C. Aseptically add 1 ml of bacitracin and 1 ml Chapman Tellurite Solution. Mix well, pour plates, air-dry overnight at room temperature. Refrigerate until use. Plates should be used within 2 weeks of preparation.

Rodac plates are made by Falcon plastics. They allow the agar surface to extend above the sides of the plate bottom. Instructions for pouring come with the plates. Plates are available from Fisher Scientific #08-7570152.

Glucose broth tube (1)

To 2% yeast extract (aqueous) add 20% glucose. Mix thoroughly and dispense 10 ml into 15-mm × 125-mm tubes. Cap tubes. Sterilize by autoclaving at 118°C for 15 minutes.

Fructose broth tube (1)

To 2% yeast extract (aqueous) add 20% fructose. Mix thoroughly and dispense 10 ml into 15-mm × 125-mm tubes. Cap tubes. Sterilize by autoclaving at 118°C for 15 minutes.

Sucrose broth tube (1)

To 2% yeast extract (aqueous) add 20% sucrose. Mix thoroughly and dispense 10 ml into 15-mm × 125-mm tubes. Cap tubes. Sterilize by autoclaving at 118°C for 15 minutes.

Popsicle sticks, sterile (3)

Tongue blade, sterile (1)

Wax marking pencil (1)

Small piece of paraffin (1)

Per class

Candle jar *or* GasPak CO₂ Pouch (BBL) and jar (1)

Disposal area

Separate counter or cart with:

- (1) 1-gallon jar of disinfectant for discarding lancets, cotton, tongue depressors, toothpicks, etc.;
- (2) disinfectant-container labeled “Slide Disposal”;
- (3) disinfectant-container for used pipettes;
- (4) rack for discarded tubes; and
- (5) bucket for discarded plates.