DOCUMENT RESUME

ED 306 104 SE 050 502

AUTHOR Case, Christine L.
TITLE Biology Laboratory Safety Manual.
PUB DATE Mar 89
NOTE 15p.
PUB TYPE Guides - Classroom Use - Guides (For Teachers) (052)

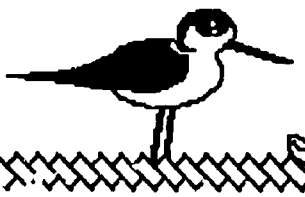
EDRS PRICE MF01/PC01 Plus Postage.
DESCRIPTORS *Biological Sciences; *College Science; *Educational Facilities Planning; Emergency Programs; *Hazardous Materials; Health; Higher Education; Instructional Materials; Laboratory Safety; Safety Education; Safety Equipment; *School Safety; Secondary Education; *Secondary School Science; Teaching Methods; Waste Disposal

ABSTRACT

The Centers for Disease Control (CDC) recommends that schools prepare or adapt a biosafety manual, and that instructors develop a list of safety procedures applicable to their own lab and distribute it to each student. In this way, safety issues will be brought to each student's attention. This document is an example of such a manual. It contains guidelines adapted from CDC guidelines for use in a school setting. Topics covered include: aerosols; chemical safety; decontamination; disinfection; waste disposal; teaching precautions; standard and special practices; universal precautions; and precautions for blood work. (CW)

BIOLOGY LABORATORY SAFETY MANUAL

CHRISTINE L. CASE
PROFESSOR, MICROBIOLOGY
SKYLINE COLLEGE
SAN BRUNO, CA 94066



Laboratory Safety

Adherence to safety procedures not only minimizes risk of infections in the laboratory, it is the students' training for minimizing transmitting nosocomial infections in their future work environments. Most laboratory-acquired infections are probably transmitted by inhaled aerosols.

The Centers for Disease Control recommends preparing or adopting a biosafety manual. It is recommended that the instructor develop a list of safety procedures applicable to his or her lab 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 CDC's Guidelines*.

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

Standard Practices

1. Work surfaces are disinfected at the beginning and end of every lab. period and after any spill of viable material.

2. Mechanical pipetting devices are used; mouth pipetting is prohibited.

3. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the laboratory.

4. Persons wash their hands after handling microbiological cultures and every time they leave the laboratory.

5. All contaminated material (including broken glassware) is decontaminated, preferably by autoclaving, before being disposed of.

6. Laboratory clothing that protects street clothing (e.g., solid-front or wrap-around gowns) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory and it is decontaminated (i.e., autoclaved) before laundering.

7. Containers for broken glassware should be provided. Contaminated glassware is deontaminated before being disposed of.

Advice to Students

1. Do not do any unauthorized experiments.

2. Do not use equipment without proper instruction in its use.

3. Be careful of laboratory burners. Long hair should be tied back.

4. No eating, drinking, or smoking in the laboratory.

Special Practices

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.

6. The instructor should be informed by students who become pregnant, are taking immunosuppressive drugs, or have any other medical condition (e.g., diabetes, 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 is present in every laboratory.

About Aerosols

Maneuver	Aerosol generated by	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; skin contamination; self-inoculation.
• Mixing and shaking	Inevitable production of stable aerosols.	Splashing.
• Transport to laboratory	Aerosol produced in airspace in tube by shaking is released on opening tube.	Leaky tubes and caps.
• Opening containers	An ever-present and inevitable danger.	Skin contamination.
• Centrifugation	Tubes leaking or breaking; aerosol in air space released on opening tubes.	Machine contamination.
• Pipetting	Dispensing last drop; dropping blood or plasma onto bench or plates, etc.	Ingestion...but mouth-pipetting is never done.
• Bacteriologic loops	Hot loops sizzle; vibrating loop; plate streaking; flaming loop.	
• Disposal and washup	Almost all activities.	

CDC's Universal Precautions

1. Gloves should be worn when touching blood and body fluid, mucous membranes, and nonintact skin and when handling items or surfaces soiled with blood or body fluids. Gloves should be changed after contact with each patient.

2. Hands and other skin surfaces should be washed immediately and thoroughly if contaminated with blood or other body fluids. Hands should be washed immediately after gloves are removed.

3. Masks and protective eyewear or face shields should be worn during procedures that are likely to generate droplets of blood or other body fluids.

4. Gowns or aprons should be worn during procedures that are likely to generate splashes of blood or other body fluids.

5. To prevent needlestick injuries, needles should not be recapped, purposely bent or broken, or otherwise manipulated by hand. After disposable syringes and needles, scalpel blades, and other sharp items are used, they should be placed in puncture-resistant containers for disposal.

6. Although saliva has not been implicated in HIV transmission, mouthpieces, resuscitation bags, or other ventilation devices should be available for use in areas in which the need for resuscitation is predictable. Emergency mouth-to-mouth resuscitation should be minimized.

7. Health care workers who have exudative lesions or weeping dermatitis should refrain from all direct patient care and handling patient-care equipment.

8. Pregnant health care workers are not known to be at greater risk of contracting HIV infection than health care workers who are not pregnant; however, if a health care worker develops HIV infection during pregnancy, the infant is at risk of infection. Because of this risk, pregnant health care workers should be especially familiar with and strictly adhere to precautions to minimize the risk of HIV transmission.

Precautions in the Teaching Laboratory

1. Gloves should be worn when touching blood and body fluid, mucous membranes, and nonintact skin and when handling items or surfaces soiled with blood or body fluids. Gloves should be changed after contact with each student. Gloves should be worn when performing lancet-sticks.

2. Hands and other skin surfaces should be washed immediately and thoroughly if contaminated with blood or other body fluids. Hands should be washed immediately after gloves are removed.

3. Masks and protective eyewear or face shields should be worn during procedures that are likely to generate droplets of blood or other body fluids.

4. Lab coats should be worn at all times in the laboratory.

5. To prevent needlestick injuries, needles should not be recapped, purposely bent or broken, or otherwise manipulated by hand. After disposable syringes and needles, scalpel blades, and other sharp items are used, they should be placed in puncture-resistant containers for autoclaving.

6. Although saliva has not been implicated in HIV transmission, mouthpieces, resuscitation bags, or other ventilation devices should be used in respiratory therapy and EMT training. Emergency mouth-to-mouth resuscitation should be minimized.

7. Students who have exudative lesions or weeping dermatitis should refrain from all direct student contact and handling patient-care equipment.

8. Pregnant students are not known to be at greater risk of contracting HIV infection than students who are not pregnant; however, if a woman develops HIV infection during pregnancy, the infant is at risk of infection. Because of this risk, pregnant students should be especially familiar with and strictly adhere to precautions to minimize the risk of HIV transmission.

Disinfection

For routine handwashing when not working with infectious agents, liquid or powdered soaps may be used. Bar soaps should **not** be used. Liquid soaps which lack 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 wescodyne-detergent preparation and scrub for 30 to 60 sec. Rinse with water.

✓ Use of 4% chlorhexidine-detergent and scrub for 30 to 60 seconds. Rinse with water.

✓ Use of a phenolic disinfectant-detergent for 20 to 30 sec and then rinse with water.

✓ Use of alcohol (50-70%) for 20 to 30 sec, followed by a soap scrub of 10 to 15 sec and rinse with water.

Bench tops may be disinfected by using a disinfectant-detergent according to the manufacturers directions. If a 1:10 hypochlorite solution (household bleach) is used, remember that it is easily neutralized by organic matter, may discolor surfaces, is corrosive to metals, and has an unpleasant odor.

For laboratories where infectious material is used, floors should be disinfected daily with a suitable phenolic-deteregent.

When pipetting infectious agents place a disinfectant soaked towel on the work surface.

Contaminated glassware should be placed in disinfectant; pipettes may be soaked in a vertical or horizontal container. All contaminated glassware should be autoclaved before washing.

Disinfectants that disrupt the envelope of HIV and HBV	Examples
Halogens	10% chlorine bleach
Alcohols	70% ethyl alcohol
Phenols	Vestal Staphene
Glutaraldehyde	1%

Blood Group Determination – ABO + Rh typing

Wear **gloves** or work with your **own** blood*. You should not do this lab. 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.
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.
9. Mix each suspension with a different toothpick.
10. Observe for agglutination and 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.*****

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*Blood obtained from blood banks is tested for hepatitis B virus and HIV. No test method can offer complete assurance that laboratory specimens do not contain these viruses.

**10% chlorine (household) bleach.

*** Bar soaps should not be used. Use a liquid or powdered soap.

Decontamination of CPR Training Mannequins

1. Become thoroughly familiar with the manufacturer's recommendations and provisions for sanitary practicies.

2. Do not have students participate who have current weeping dermatologic lesions on hands or in oral or circumoral areas or who have upper respiratory disease.

3. Routinely inspect mannequins for signs of physical deterioration, such as cracks or tears in plastic surfaces.

4. Use protective face shields.

5. Clean and decontaminate the mannequin consistently after <u>each</u> class. Completely disassemble the mannequin head, thoroughly wash all external and internal surfaces with warm soapy water and brushes. Rinse all surfaces with fresh water. Wet all surfaces with 10% chlorine (household) bleach for 10 min, rinse again with fresh water, and dry with clean absorbent towel. The disinfectant should be made fresh at each class and discarded after use.

6. Establish a routine of vigorous wiping of the mannequin's face and inside mouth with clean absorbent towel that has been wetted with either 10% bleach or 70% isopropyl alcohol. The surface should remain wet for at least 30 sec before it is wiped dry with a second piece of clean aborbent towel.

Decontamination of Respiratory Equipment

Use disposable mouthpieces. Dispose of used items in a suitable container. Used, disposable items should not be kept longer than 4 days before autoclaving.

Use only sterile water in humidification equipment and change the water after each class.

Equipment and oxygen cylinders should be dusted and wiped regularly with a cloth moistened in disinfectant.

Decontamination of nondisposable equipment:

1. Place articles to be decontaminated in glutaraldehyde for 15 min.

2. Wash articles in Alconox®.

3. Immerse articles in tap water containing 0.2% acetic acid for 15 min. Acetic acid removes any residual soap and the taste and smell of glutaraldehyde.

Culture equipment in use to determine the adequacy of decontamination procedures. More than 10 gram-negative rods in the tubing is unacceptable. To culture tubing: rinse tube with nutrient broth, membrane filter broth, and place filter on nutrient medium.

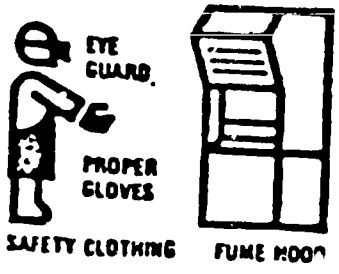
Disposal of Infectious Wastes

Infectious wastes including bacterial and fungal cultures, human body secretions and fluids and objects, such as slides, syringes, lancets,, and broken glassware contaminated with these materials should be placed in an autoclavable container. Note that "sharps" must be placed in a puncture-proof container. These "to be autoclaved" materials should be not be stored longer than 4 days before autoclaving.

Chemical Safety

Students should be given appropriate instructions for handling hazardous chemicals.

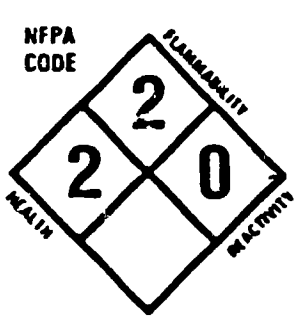
Personal protection.



Degree of hazard. This symbol, developed by the National Fire Protection Association shows a red segment (flammability), blue segment (toxicity), and yellow one (reactivity). Printed over each is a bold black number showing degree of hazard involved. The fourth segment is left blank and is reserved for specific warnings, such as radioactivity.

The numerical ratings are

4 = extreme hazard
3 = severe hazard
2 = moderate hazard
1 = slight hazard
0 = according to current information, no hazard



Chemical storage information for laboratory personnel.

RED

Store in area for flammable reagents.

BLUE

Toxic if inhaled, ingested, or absorbed through skin. Store in secure area.

YELLOW

May react violently with air, water, or other chemicals. Store away from flammable and combustible materials.

WHITE

Corrosive. May harm skin, eyes, mucous membranes, Store away from red-, yellow-, and blue-coded reagents.

GRAY

General chemical storage.

EXCEPTION

Reagent incompatible with other of same color code. Store separately.

References

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