Handling Biological Specimens Safely

Introduction

Some Biological specimens used in laboratory may cause harm to humans, animals or plants.

Such activity often involves culturing large volumes of harmful micro-organisms presenting a significant risk to people and the environment.

Guidance must be effectively implemented to control the risk of harm to students, animals and plants when working with micro-organisms or with material that may contain micro-organisms.

Aseptic technique should be employed if appropriate.

There are two main goals to remember when handling Biological Specimens:

(a) To protect the people working in the lab - you, your classmates, instructors, and others, from possible infection.

(b) To prevent contamination of unwanted organisms (fungi, other bacteria) into the pure culture.

Identified Risks and Hazards

Contamination by pathogens such as viruses and bacteria through:

- Open wound
- Ingestion
- Inhalation
Pre-operational Safety

Protective gloves are always required whenever handling these biological specimens.

Laboratory gowns should be worn always inside the laboratory.

Wear additional appropriate PPE including, disposable impervious apron, sleeves, face shield, and goggles as needed.

Appropriate footwear also is recommended. Avoid using open shoes or sandals.

Wear mask and respirator if needed to protect from inhalation hazards.

All cuts and wounds should be covered with sterile dressings.

Wash hands before and after experiments.

Open-toed shoes, unstable high heals, and shorts are discouraged.

Do not wear your best clothes to lab.

Laboratory Environment

Laboratory should have adequate supplies of electricity, water, special equipments, proper storage, fume hoods, safety cupboards, refrigerator and freezers.

Operating Safely

Working with Biological Specimens

Before handling those experimental kits and delicate equipment, read the operating manuals first and be familiar with the operation procedures.
Handle all pipetting devices with special care to avoid formation of aerosols during transfer.

Mouth pipetting is prohibited. In tissue culture, plant materials are commonly used.

If animal cells are cultured, ensure that they do not harbor any pathogens.

Reagents involved in the preparation of tissue culture may be harmful and should therefore be handled with care.

Toxicity or harmfulness of reagents, such as restriction enzymes and stains, should be checked and proper precautions should be taken.

Do not eat, drink, or chew gum in the lab.

Do not place pencils, pens, labels, fingers (or any other objects) in your mouth.

Keep your hands away from your face.

Wipe off the counter with disinfectant after your lab work. A surface should be decontaminated at any other time you feel it may have become contaminated.

If you accidentally spill any bacteria, whether a drop or an entire culture, on the lab bench, yourself, or the floor, you should notify instructor.

Place paper toweling over the spill to absorb it. Without letting your hand touch the absorbed liquid, place the paper towel into the "biological waste" container.

Disinfect the area thoroughly.

Wash your hands thoroughly with disinfectant and soap. Be careful not to touch anything else, including water faucets, with your contaminated hands not spread contamination.

Label all plates, culture tubes, etc. before inoculating in order to lessen chances of error or spilling.

NEVER grab a culture tube by the cap. Many of the caps fit loosely on the tube. You won’t know until the tube crashes to the floor.

Keep cuts or open wounds covered.

Gloves are available.

Report any injuries (no matter how minor), or allergy to latex gloves, to the instructor.

Students at high risk for infection (e.g. uncontrolled diabetes, suppressed
immune system, chemotherapy, steroid treatment) or those for whom an infection could be especially devastating (e.g. impaired kidneys, lacking a spleen) should (a) have the permission of their physician and (b) preferably notify the instructor.

After lab, students should properly dispose of all cultures, glassware, plastic ware, pipettes, and other supplies that have been in contact with bacteria.

Containers marked "solid waste" and "liquid waste" are located on each bench. A "biological waste" wastebasket is available for Petri plates and other contaminated solids.

A separate waste container is provided for "sharps".

This waste is autoclaved and so it is suitable for contaminated tips, plastic pipettes, etc.

Glass pipettes are put into the cardboard "broken glass" container. If a pipette is contaminated with bacteria, it must be decontaminated first (soak in bleach).

All liquid cultures must be killed before disposal. Place cultures on a rack by the sink and add bleach solution to the tubes. Please remove tape from the tubes.

**Micro-organisms**

*Inoculation of Culture*

Always practice aseptic technique in inoculation.

Immerse an inoculating tool, such as inoculating loop and knife, in 70% alcohol before flaming, but not in reverse order to prevent ignition of fire.

Cool down the inoculating tool so as to avoid killing the target microorganism and to reduce the risk of generating aerosols.

Sterilize the inoculating tool immediately after use to avoid spread of the microorganisms and contamination of workplace.
Taping and Labeling

Label the base of the petri dishes rather than the lid with permanent felt pen or wax pencil to avoid mixing-up, in case the base gets separated from the lid.

Always hold a whole set of petri dish (the base and the lid together).

Seal the dishes with adhesive tape if necessary to avoid contamination or accidental separation of the base from the lid.

Sealing can be made in a manner so as to allow gaseous exchange.

Incubation of Microbial Culture

Cultures of microorganisms should be incubated in an enclosed environment, e.g. incubator.

During incubation, the covered dishes should be placed upside down.

Most microorganisms used in school microbiological experiments grow well at room temperature. Raising the temperature to 37°C favors the growth of microorganisms pathogenic to humans and thus is not encouraged.

Examination of Microbial Culture

Transfer of microbial culture by students should not be encouraged.

Examine the specimens in sealed state, e.g. in taped petri dishes or sealed transparent plastic bag.

If the petri dishes containing cultures of microorganisms must be opened for inspection, the teacher or laboratory technician should kill the microorganisms by
exposing the culture to methanol vapors (a filter paper soaked in 40% methanol solution (formalin) in petri dish for 24 hours) prior to class inspection.

**Handling Microbial Spillage**

Microbial spillage should be dealt with by teachers or laboratory technicians.

When clearing up the mess, protective gloves and laboratory gowns should be worn.

Wear masks when appropriate. It is important not to inhale aerosol cloud formed above the spill.

The spillage should be covered with a towel soaked in disinfectant (e.g. hypochlorite).

The towel should be left in place for 15 minutes and then swept into a suitable container.

The contaminated area should also be disinfected as appropriate. In case the skin is in contact with the spillage, wash with liquid soap and water immediately and thoroughly.

Seek medical help if necessary.

**Disposal of Unwanted Cultures and Contaminated Materials**

Cultures should be destroyed by steam under pressure or immersing in disinfectant for several hours before disposal.

All apparatus contaminated with microorganisms or waste materials should also be treated in the same way before disposal.

**Sterilization after Microbial Work**

After each activity, the bench surface should be wiped with disinfectant immediately.

Wash hands thoroughly with liquid soap and water after microbiological work. For drying purpose, paper towels are preferred and used ones have to be disposed of in a waste container with lid.
Using microscopes

When moving your microscope, always carry it with both hands, grasp the arm with one hand and place the other hand under the base for support.

Turn the revolving nosepiece so that the lowest power objective lens is "clicked" into position (This is also the shortest objective lens).

Your microscope slide should be prepared with a cover slip or cover glass over the specimen. This will help protect the objective lenses if they touch the slide. Place the microscope slide on the stage and fasten it with the stage clips. You can push down on the back end of the stage clip to open it.

Look at the objective lens and the stage from the side and turn the coarse focus knob so that the objective lens moves downward (or the stage, if it moves, goes upward). Move it as far as it will go without touching the slide!

Now, look through the eyepiece and adjust the illuminator (or mirror) and diaphragm for the greatest amount of light.

Slowly turn the coarse adjustment so that the objective lens goes up (away from the slide). Continue until the image comes into focus. Use the fine adjustment, if available, for fine focusing. If you have a microscope with a moving stage, then turn the coarse knob so the stage moves downward or away from the objective lens.

Move the microscope slide around so that the image is in the center of the field of view and readjust the mirror, illuminator or diaphragm for the clearest image.

Now, you should be able to change to the next objective lenses with only minimal use of the focusing adjustment. Use the fine adjustment, if available. If you cannot focus on your specimen, repeat steps 4 through 7 with the higher power objective lens in place. Do not allow the objective lens to touch the slide!

The proper way to use a monocular microscope is to look through the eyepiece with one eye and keep the other eye open (this helps avoid eye strain). If you have to close one eye when looking into the microscope, it's ok. Remember,
everything is upside down and backwards. When you move the slide to the right, the image goes to the left!

Do not touch the glass part of the lenses with your fingers. Use only special lens paper to clean the lenses.

When finished, raise the tube (or lower the stage), click the low power lens into position and remove the slide.

Always keep your microscope covered when not in use. Dust is the number 1 enemy!

**Maintenance**

All used glassware and plastic ware that have been in contact with DNA, bimolecular, bacterial cells and tissue culture should be considered as contaminated.

They should be soaked in disinfectant (e.g. hypochlorite) for at least one hour or sterilized using steam under pressure before cleaning or disposal.

After each practical on biotechnology, the bench surface should be wiped with disinfectant immediately.

Wash hands thoroughly with liquid soap and water.

**Operating Procedures**

Cultures of microorganisms should be treated as potentially hazardous due to the possibility of being contaminated by pathogens or becoming virulent as a result of mutation.

Pathogenic microorganisms may gain access into the human body if the body surface with wounds or cuts is in direct contact with the culture, if aerosols above the culture are inhaled, or if contaminated food/drink is ingested. All microorganisms and their cultures should therefore be handled with caution.

Hygienic measures should be emphasized when working with microorganisms.

Protective gloves and laboratory gowns should be worn if necessary. All wounds and cuts on body surface should be covered with sterile dressings before starting microbiological experiments.

Always employ aseptic technique when working with microbial cultures. Before and after work, clean the bench surface with disinfectant as well as wash hands. All unwanted cultures should be disposed of properly after experiment.

Pipette fillers should be used to help transfer liquid cultures. Mouth pipetting should be strictly forbidden during microbiological experiments.
Do not eat, drink, smoke, and apply cosmetics or lip balm, or store food in the laboratory. Bring any necessary equipment, laboratory instructions and your lab notebook to the work bench. A flow chart and/or notes may help you carry out the lab work with a minimum of page shuffling.

Wear a protective laboratory coat or apron when you are working with cultures, and avoid wearing long, full sleeves if possible.

Tie long hair back or put it up. If hair hangs loose, it becomes a contamination hazard and also may catch on fire in the Bunsen burner flame.

Carry and store cultures of microorganisms in racks or baskets.

Do not leave cultures on the table or in unmarked areas when the laboratory session is completed.

Place cultures to be discarded in racks or trays designated for contaminated material; these racks should be clearly labeled. All such materials should be autoclaved before further handling, discarding, or washing.

Decontaminate work surface after spills and at the beginning and end of each laboratory period with the disinfectant provided in the laboratory.

Cover small spills with paper towels and soak the towels well with disinfectant. Let the towels stand for one-half an hour. Place these materials in a container with disinfectant or in a plastic bag which can be sealed.

Autoclave container before discarding.

Mix liquid cultures gently to avoid foaming and splashing which may produce an aerosol. Do not mix cultures by bubbling expiratory air through the liquid with a pipette.

Never pipette cultures by mouth; mechanical pipetting devices are to be used. Place contaminated pipettes in a pan which contains an appropriate disinfectant.

Wash hands carefully with soap after any possible contamination and before leaving laboratory.

Dry hands thoroughly after washing.

Open cuts should be covered with bandages; if they are on the hands, wear disposable gloves.

Individuals who have special health problems such as diabetes or suppressed immunity from disease or therapy should be encouraged to discuss them privately with the instructor.
Develop the habit of keeping your hands away for your mouth, nose, eyes, and face to prevent self-inoculation.
Before centrifuging, inspect tubes for cracks, whenever possible; use autoclavable plastic centrifuge tubes with screw caps.
Avoid filling the tube to the point that the rim becomes wet with the culture. Avoid the use of hypodermic syringes. Use a pipette whenever possible. If a syringe is needed, use a needle-locking hypodermic syringe.
Before and after injecting an animal, swab the site of injection with disinfectant. Shake broth cultures in a manner that avoids wetting the plug or cap.
Periodically clean out deep freeze and dry-ice chests in which cultures are stored to remove broken ampoules and tubes.
Use rubber gloves and respiratory protection during the cleaning.