



A

**Principles of
Biosafety**

When working with human tissue samples, an acute awareness of possible risks and a clear concept of biological safety are essential to prevent occupational acquired infections as well as the release of pathogens into the environment.

The laboratory facilities should have restricted access to persons whose presence is required to perform cell culture work and who have been instructed in biosafety principles.

A.1 Categories of Biosafety

Biosafety Level 1 (low risk)

- Laboratories appropriate for undergraduate training and teaching
- Work is done with defined and characterised strains of viable microorganisms not known to cause any disease in healthy adult humans

Safety Requirements: sink for handwashing

Biosafety Level 2 (moderate risk)

- Laboratories appropriate for diagnostics and teaching (graduate and postgraduate level)
- Work is done with agents that are associated with human diseases (microorganisms like: Hep. B, HIV, most bacteria) as well as human body fluids, tissues and primary human cell lines

Safety Requirements:

1. Primary Barriers: face protection, gowns, gloves, Biosafety Class II cabinet
2. Secondary Barriers: sinks for handwashing, waste decontamination facilities

Biosafety Level 3 (moderate-high risk)

- Laboratories appropriate for diagnostics, teaching, research or production facilities
- Work is done with exotic agents with a potential of respiratory transmission which may cause serious and potentially lethal infections (*Myc. Tub. Cox. Burnetti*)

Safety Requirements:

1. Primary Barriers: Aerosol-tight chamber for work
2. Secondary Barriers: Controlled access to the laboratory

Biosafety Level 4 (high risk)

- Laboratory appropriate for research

- Work is done with dangerous and exotic agents that poses a high individual risk of life-threatening disease, which is transmitted via the aerosol route and for which there is no vaccine or therapy available (Marburg virus, Ebola virus)

Safety Requirements:

1. Primary Barriers: complete full-body air-supplied, positive pressure personal suit (Biosafety Class III cabinets)
2. Secondary Barriers: complete isolated zone in a separate building

A.2 Biohazard Materials

1. Human pathogens (bacteria, fungi, viruses, parasites, prions)
2. All human blood products, tissues and body fluids
3. Cultured cells
4. Toxins
5. Infected tissues

A.3 Recommended Work Practices

1. Practice aseptic culture techniques
2. Keep good record of tissue specimens — source, date etc.
3. Maintain proper containment — where and how to handle specimens in the laboratory



Fig. A.1 Biohazard bag and container.

4. Use appropriate lab wear — gloves (if necessary double gloving), lab coat, proper footwear, safety goggles (if needed) and do not wear protective clothing outside the laboratory
5. Maintain a proper working environment — clean up and disinfect equipment after use! (10% household bleach or Chlorox is an effective disinfectant)
6. Keep laboratory doors closed when experiments are in progress

A.4 General Hygiene

1. Maintain personal hygiene — wash your hands, tie back long hair
2. Do not touch your face/hair with gloves and do not use mobile phones with gloves
3. Do not eat, drink, smoke in the laboratory
4. Avoid talking when doing sterile work



Fig. A.2 Proper disposal of biological wastes.

A.4.1 Pipettes

- No mouth pipetting
- Always use cotton plugged pipettes
- Avoid creating bubbles
- Do not mix fluids in the pipette
- Place contaminated reusable pipettes in a container with disinfectant
- Autoclave contaminated disposable pipettes in an appropriate bag/container before disposal

A.4.2 Syringes and Scalpels

- Use disposable needles
- Never put the cap back on the needle
- Do not use excessive force when fitting a needle or a scalpel
- Dispose in container meant for sharp objects after use

A.4.3 Biosafety Cabinets

- Use at least a class II biosafety cabinet for human cell culture work
- Plan your work in advance



Fig. A.3 Disposal of sharp objects.

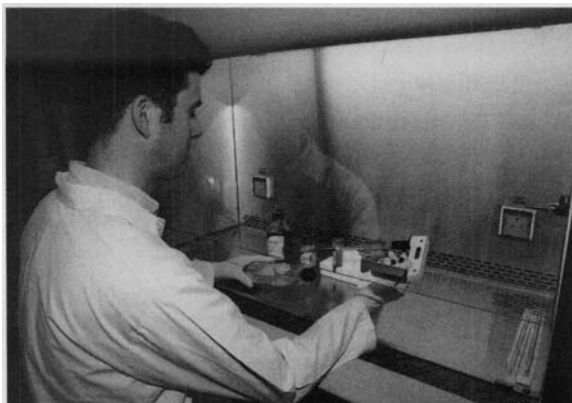


Fig. A.4 Working at the biosafety cabinet.

- Switch on the UV lamp 10 mins before use with front panel closed to maximise sterility
- Switch off the UV lamp before starting work; it is harmful for the eyes and the cultures
- Turn the blower on and leave it running at least 5 mins prior to use
- Wipe bench top with 70% ethanol before use
- Spray items outside the cabinet with 70% ethanol before bringing them in
- Minimise storage of things in the cabinet
- Do not place objects on the front air intake grill
- Clean up spills immediately
- Clean up the cabinet with disinfectant after use
- Close front panel and switch on the UV lamp

A.4.4 Chemicals

A number of chemicals used in the laboratory are hazardous. All manufacturers of hazardous materials are required by law to supply the user with pertinent information on any hazards associated with their chemicals. This information is supplied in the form of Material Safety Data Sheets or MSDS. This information contains the chemical name, CAS#, health hazard data including first aid treatment, physical data, fire and explosion hazard data, reactivity data, spill or leak procedures, and any special precautions needed when handling this chemical.

MSDS information can be accessed on World Wide Web (www.msdssearch.com, www.msds.com). Researchers are strongly urged to make use of this information prior to using a new chemical, and certainly in the case of any accidental exposure or spill. The principal investigator or laboratory head must be notified immediately in the case of an accident involving any potentially hazardous reagents.

The following chemicals are particularly noteworthy:

- Phenol — can cause severe burns
- Acrylamide — potential neurotoxin
- Ethidium bromide — carcinogen

These chemicals are not harmful if used properly: always wear gloves when using potentially hazardous chemicals and never mouth-pipette them. If you accidentally splash any of these chemicals on your skin, rinse the area thoroughly with water immediately, and inform the laboratory safety officer. Discard chemical wastes in appropriate containers.

Important

Do not discard chemical waste down the sink!

A.4.5 Ultraviolet Light

Exposure to ultraviolet (UV) light can cause acute eye irritation. Since the retina cannot detect UV light, you may not realize that you have serious eye damage until 30 mins to 24 hours after exposure. Therefore, always turn off the UV light or wear appropriate eye protection before entering the laboratory or starting to work at the biosafety cabinet.

A.4.6 General Housekeeping

All common areas should be kept free of clutter and all dirty dishes. Since you have only a limited amount of space to call your own, it is to your advantage to keep your own area clean. As you will be using common facilities, all solutions and everything stored in an incubator, refrigerator, etc. must be

labelled. In order to limit confusion, each person should use his initials or some other unique designation for labelling plates, etc. Unlabelled material found in the biosafety cabinets, incubators or freezers may be destroyed. Always mark culture vessels with your initials, the date and relevant experimental data, e.g. strain numbers. Each person should be assigned general lab duties that may include keeping track of inventory, making sure a given area is kept clean, or maintaining equipment.

Note

“Think about what you are doing. The best defense is common sense.”

A.5 Handling Biological Specimens

Researchers working with primary human tissue samples should know about the existing risks of disease transmission while working with the specimen. The most important and fundamental precaution would be to follow proper work practice in an appropriate laboratory environment designed for primary cell culture work.

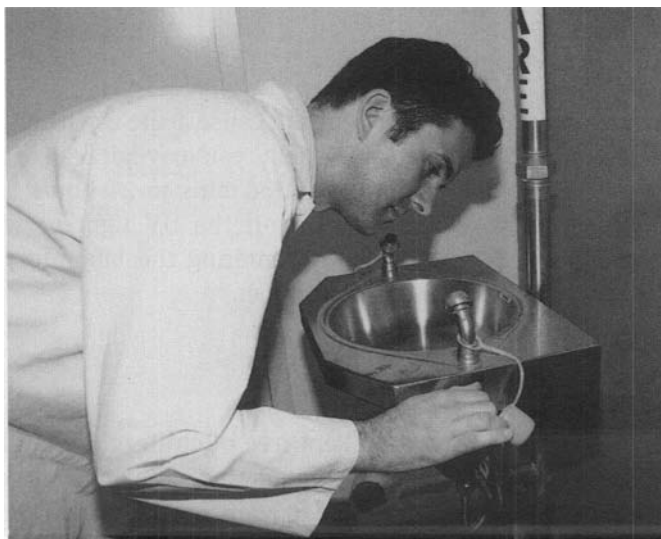


Fig. A.5 Irrigation of the eyes using an eye shower.

In addition, it is mandatory that new researchers joining the group receive proper training in specimen handling and that they are informed about the biological specimens involved. **Remember: All primary human tissue samples are potentially contagious!** We also recommend vaccinations against **Hepatitis A/B, Tuberculosis and Tetanus.**

In the event of an “occupational exposure” — contact of potentially infectious material with the body, either via contact with the skin or any mucus membrane, ingestion (swallowing of material) or any other parenteral means (e.g. accidental needle puncture), the following steps should be taken immediately:

1. Stop working
2. Inform your colleagues/lab safety officer immediately
3. Irrigate the skin/mucus/eye area with water
4. Consult a medical doctor immediately
5. Keep a sample of the material for further diagnostic tests



Fig. A.6 Safety kit and fire extinguisher.

A.5.1 Checklist (Update the Equipment Every 2 Months!)

- Equipped first aid kit (disinfectant solution, absorbent gauzes, disposable gloves, adhesive bandage, crepe bandage, antiseptic cream, triangular bandage, plasters, general guidance booklet)
- Working eye shower
- Working fire extinguisher
- Accessible emergency exits
- Available emergency telephone numbers
- Lab staff trained in first aid procedures
- Biosafety manual