# Laboratory manual

## Department of Biotechnology

**St. Xavier's College, Mapusa Goa**

# FIRST AID IN LABORATORIES

## 1. Biological Laboratory:

- **Handling Spills**: Clean biological spills immediately with disinfectants like bleach, using gloves and eye protection.
- **Cuts and Punctures**: Wash with soap and water, apply an antiseptic, and cover with a sterile bandage.
- **Exposure to Biohazards**: Rinse exposed areas (skin, eyes) with water for 15 minutes; seek medical attention if necessary.
- **Needle Sticks**: Wash the area with soap and water; seek medical attention and report the incident.

## 2. Physical Laboratory (Physics/Mechanical):

- **Electric Shock**: Turn off the power, avoid touching the victim directly, use a nonconductive object to separate them from the source.
- **Burns (Heat/Cold)**: Cool burns with running water; avoid ice for severe burns. For cold burns, slowly warm the affected area.
- **Eye Injuries**: Rinse eyes immediately with water for 15-20 minutes if chemicals, dust, or particles enter.
- **Cuts and Bruises**: Clean wounds and apply pressure if bleeding, then bandage.

## 3. Chemical Laboratory:

- **Chemical Burns**: Rinse the affected area with running water for at least 15 minutes; remove contaminated clothing.
- **Inhalation of Toxic Fumes**: Move the person to fresh air, loosen clothing, and seek medical attention if symptoms persist.
- **Chemical Spills on Skin**: Wash immediately with water; for acids and bases, neutralize if advised.
- **Eye Exposure**: Flush with water for 15 minutes, holding the eyelid open, and seek medical attention.

**4. Computer Laboratory:**

- **Electric Shock**: Disconnect the power source, use non-conductive material to help the victim, and seek medical help.
- **Fire**: Use a $CO_2$ or dry chemical fire extinguisher; evacuate if necessary.
- **Minor Cuts from Equipment**: Clean and bandage minor cuts from tools or equipment.

## DOCUMENTATION: LAB NOTEBOOK, LAB REPORT, AND LOG BOOK MAINTENANCE

Documentation ensures accountability, reproducibility, and proper communication of experimental processes and findings.

**Lab Notebook:**

- **Purpose:** Records all experimental procedures, observations, and results.
- **Structure:** Include date, experiment title, objective, materials, methods, data, and conclusions.
- **Accuracy:** Write entries in real-time; use permanent ink to ensure data integrity.
- **Clarity:** Entries should be clear, well-organized, and detailed enough to allow replication.
- **Corrections:** Single-line strikeouts; no erasing or overwriting to maintain transparency.
- **Signatures:** Ensure the lab notebook is signed and dated by both the researcher and supervisor.

**Lab Report:**

- **Purpose:** A formal document summarizing an experiment's hypothesis, methods, results, and interpretation.
- **Structure:** Title, abstract, introduction, materials and methods, results, discussion, conclusion, references.
- **Content:** Include detailed explanations of procedures, data analysis, and interpretation of findings.
- **Presentation:** Data should be presented clearly with graphs, tables, and figures when necessary.
- **Conclusion:** State whether the hypothesis was supported and discuss implications of results.

**Log Book Maintenance:**

- **Purpose:** Tracks day-to-day lab activities, equipment usage, and calibration logs.
- **Organization:** Chronologically document the use of instruments and personnel involved.
- **Accuracy:** Record all events precisely, especially any anomalies or maintenance performed.

- **Accessibility:** Should be easy to retrieve and regularly updated for audit or review purposes.
- **Standardization:** Use pre-defined formats or templates to ensure uniformity across entries.

## MATH SKILLS: CONVERSION OF METRIC UNITS

Proficiency in conversions is essential for accurate measurements and calculations in scientific experiments.

➢ **Basic Metric Units:**

- **Length**: Meter (m)
- **Mass**: Gram (g)
- **Volume**: Liter (L)
- **Time**: Second (s)
- **Temperature**: Celsius (°C)

➢ **Common Metric Prefixes:**

- **Kilo- (k)**: 1,000 units (1 km = 1,000 m)
- **Centi- (c)**: 1/100 units (1 cm = 0.01 m)
- **Milli- (m)**: 1/1,000 units (1 mm = 0.001 m)
- **Micro- (μ)**: 1/1,000,000 units (1 μm = 0.000001 m)
- **Nano- (n)**: 1/1,000,000,000 units (1 nm = 0.000000001 m)

➢ **Conversion Factors:**

- To convert between units, multiply or divide by powers of 10 based on the prefixes.
- Example: To convert 5 kilometers to meters:
  - 5 km×1,000=5,000 m
- Example: To convert 200 milliliters to liters:
  - 200 mL×0.001=0.2 L

➢ **Unit Conversion Steps:**

- **Identify the unit you have** (e.g., meters).
- **Identify the unit you need** (e.g., centimeters).
- **Use the correct conversion factor** (e.g., 1 m=100 cm)
- **Multiply or divide** by the conversion factor as needed.

➢ **Examples of Common Conversions:**

- **Length**:
  - 1 meter (m) = 100 centimeters (cm) = 1,000 millimeters (mm)
  - 1 kilometer (km) = 1,000 meters (m)
- **Mass**:
  - 1 kilogram (kg) = 1,000 grams (g)
  - 1 gram (g) = 1,000 milligrams (mg)
- **Volume**:
  - 1 liter (L) = 1,000 milliliters (mL)
  - 1 cubic meter ($m^3$) = 1,000 liters (L)

## STERILIZATION USING PHYSICAL AGENTS

Each physical sterilization method has its own ideal applications depending on the material being sterilized and the nature of contamination.

**1. Dry Heat Sterilization:**

- **Method**: Uses hot air without moisture to kill microorganisms.
- **Mechanism**: Causes oxidative damage and protein denaturation in microbes.
- **Common Techniques**:
    - **Hot Air Oven**: Heated to 160°C–180°C for 1–2 hours.
    - **Incineration**: Used for complete destruction of contaminated materials (e.g., medical waste).
- **Applications**: Sterilizing glassware, metal instruments, powders, and oils.

**2. Moist Heat Sterilization:**

- **Method**: Uses steam under pressure to achieve higher temperatures.
- **Mechanism**: Causes coagulation and denaturation of proteins in microbes.
- **Common Techniques**:
    - **Autoclaving**: Steam at 121°C under 15 psi pressure for 15–20 minutes.
    - **Boiling**: Can disinfect, but not completely sterilize, due to lower temperature (100°C).
- **Applications**: Sterilizing surgical instruments, culture media, and liquids.

**3. UV Sterilization (Ultraviolet Radiation):**

- **Method**: Uses UV light (usually 254 nm wavelength) to sterilize surfaces.
- **Mechanism**: UV radiation damages microbial DNA, preventing replication and causing cell death.
- **Common Techniques**:
    - **UV Lamps**: Used in biological safety cabinets, operating rooms, and water purification systems.
- **Limitations**: Effective only on exposed surfaces; cannot penetrate solid or opaque objects.
- **Applications**: Disinfecting air, water, surfaces, and equipment.

## STERILIZATION USING CHEMICAL AGENTS

Each chemical sterilizing agent has its specific use cases, advantages, and limitations, requiring proper handling and safety protocols.

**1. Alcohol:**

- **Common Types**: Ethanol (70%) or Isopropanol (60–90%).
- **Mechanism**: Denatures proteins and dissolves lipids in microbial cell membranes.
- **Effectiveness**: Effective against bacteria, fungi, and viruses; not sporicidal.
- **Applications**: Disinfection of skin, surfaces, and medical instruments.

**2. Formaldehyde:**

- **Form**: Gas or liquid (formalin).
- **Mechanism**: Alkylates proteins, DNA, and RNA, leading to microbial death.
- **Effectiveness**: Effective against bacteria, viruses, fungi, and spores.
- **Applications**: Sterilization of equipment, rooms (fumigation), and vaccines.
- **Caution**: Toxic, irritating, and requires proper ventilation.

**3. Sodium Hypochlorite (Bleach):**

- **Concentration**: Commonly used at 0.1–0.5%.
- **Mechanism**: Releases chlorine, which oxidizes and destroys proteins and nucleic acids in microbes.
- **Effectiveness**: Broad-spectrum disinfectant; effective against bacteria, viruses, fungi, and spores.
- **Applications**: Surface disinfection (e.g., hospitals, households), water treatment, and cleaning spills.
- **Limitations**: Corrosive and can be inactivated by organic matter.

**4. Chromic Acid:**

- **Form**: Solution of chromium trioxide in water.
- **Mechanism**: Strong oxidizing agent that damages microbial proteins and DNA.
- **Effectiveness**: Broad-spectrum, but primarily used for sterilizing glassware.
- **Applications**: Cleaning and sterilizing glass laboratory equipment.
- **Caution**: Highly toxic and carcinogenic; requires careful handling and disposal.

## GUIDELINES FOR DISPOSING HARMFUL CHEMICALS

By adhering to these guidelines, one can ensure the safe and environmentally responsible disposal of harmful chemicals while complying with legal and safety standards.

**1. Segregation of Waste:**

- **Separate chemicals by category**: Acids, bases, solvents, heavy metals, and oxidizers should be stored and disposed of separately to avoid dangerous reactions.
- **Label containers**: Ensure that all chemical waste is clearly labeled with the content, concentration, and hazard classification.

**2. Consult Safety Data Sheets (SDS):**

- **Review SDS for each chemical**: The Safety Data Sheet provides specific disposal guidelines, hazards, and regulations for handling the chemical.

**3. Neutralization (If Applicable):**

- **Neutralize certain chemicals**: Acids and bases can sometimes be neutralized (acid with base or vice versa) before disposal if permitted by local regulations.
- **Follow protocols for neutralization**: Ensure safety measures are in place (e.g., use of fume hoods, protective gear).

**4. Use Designated Chemical Waste Disposal Services:**

- **Certified waste disposal companies**: Hire licensed hazardous waste disposal services for chemicals that require professional handling (e.g., heavy metals, radioactive materials, highly toxic substances).
- **Collection services**: Regularly schedule pickups for chemical waste from laboratories or industrial sites.

**5. Do Not Dispose of Chemicals in Drains or Trash:**

- **No disposal down sinks**: Harmful chemicals should never be poured down drains, as they can contaminate water supplies and harm the environment.
- **No disposal in regular trash**: Never dispose of hazardous chemicals in regular waste bins to prevent accidental exposure.

**6. Proper Storage Before Disposal:**

- **Use compatible containers**: Store chemical waste in containers that are compatible with the chemical (e.g., no plastic for strong solvents).
- **Seal containers tightly**: Ensure chemical waste containers are properly sealed to prevent leaks or evaporation.

**7. Personal Protective Equipment (PPE):**

- **Wear appropriate PPE**: Gloves, goggles, lab coats, and respirators should be used when handling hazardous chemicals.
- **Training**: Ensure all personnel handling chemical waste are trained in proper disposal procedures and emergency protocols.

**8. Documentation and Tracking:**

- **Keep disposal records**: Maintain a log of all hazardous chemical waste, including the type of chemical, amount, disposal method, and date.
- **Waste manifests**: Obtain and retain manifests or certifications from waste disposal services to confirm proper disposal.

## MICROBIAL DECONTAMINATION

Microbial decontamination refers to the process of removing or killing microorganisms (bacteria, viruses, fungi, etc.) from surfaces, air, water, or materials to prevent infection and contamination. Key methods and practices used for microbial decontamination are as below. Each method is tailored to the specific environment, type of microorganism, and level of decontamination required, ensuring safety and preventing contamination.

**PHYSICAL METHODS:**

**Heat (Sterilization)**:

- **Dry Heat**: Use of hot air (160-180°C) for 1-2 hours in a hot air oven to kill microorganisms.
- **Moist Heat (Autoclaving)**: Steam under pressure (121°C at 15 psi) for 15-20 minutes to sterilize medical instruments, liquids, and other materials.
- **Pasteurization**: Heating liquids (like milk) to 60-70°C for a short time to reduce microbial load without sterilizing.

**Radiation**:

- **Ultraviolet (UV) Light**: Destroys microbial DNA, preventing replication; used for surface and air decontamination in labs and hospitals.
- **Ionizing Radiation (Gamma Rays)**: Used for sterilizing medical equipment and food products by penetrating deeper into materials.

**Filtration**:

- **HEPA Filters**: Trap airborne microorganisms in clean rooms, biological safety cabinets, and ventilation systems.
- **Membrane Filters**: Remove microorganisms from liquids (e.g., water, culture media) by physically filtering them out.

**CHEMICAL METHODS: Disinfectants and Antiseptics**:

- **Alcohol (Ethanol, Isopropanol)**: Commonly used at 70% concentration to disinfect skin, surfaces, and small instruments.
- **Chlorine Compounds (Sodium Hypochlorite)**: Used for surface disinfection in hospitals, laboratories, and water treatment (bleach solutions).
- **Hydrogen Peroxide**: Used as a surface and wound disinfectant; vaporized hydrogen peroxide is used to sterilize rooms and equipment.

- **Quaternary Ammonium Compounds**: Effective against bacteria and some viruses; used for disinfecting surfaces, floors, and medical instruments.
- **Formaldehyde/Glutaraldehyde**: High-level disinfectants and sterilizing agents for medical instruments and surfaces (especially in fumigation).

**MECHANICAL METHODS:**

- **Scrubbing and Cleaning**: Physically removing dirt and organic matter from surfaces to reduce microbial load.
- **Vacuuming (HEPA Filters)**: Removes airborne particles, including microorganisms, in controlled environments such as cleanrooms or surgical theatres.

**BIOLOGICAL METHODS:**

- **Biocontrol Agents**: Use of natural or genetically engineered microorganisms (e.g., bacteriophages, probiotics) to inhibit harmful bacteria.
- **Enzymatic Cleaners**: Enzymes are used to break down biofilms and organic matter where microbes thrive, aiding in microbial decontamination.

**AIR DECONTAMINATION:**

- **UV Air Sterilizers**: Installed in ventilation systems or as standalone units to destroy airborne microorganisms.
- **HEPA Filtration Systems**: Remove bacteria, fungi, and viral particles from the air in hospitals, laboratories, and cleanrooms.
- **Ozone Generators**: Used in some industrial applications to decontaminate air by oxidizing microbial structures.

**WATER DECONTAMINATION:**

- **Chlorination**: Adding chlorine or chloramine to water supplies to kill pathogens.
- **UV Sterilization**: Exposing water to UV light to destroy microbial DNA, commonly used in drinking water purification.
- **Boiling**: Simple method to kill bacteria and other pathogens in contaminated water.

**DECONTAMINATION OF SURFACES AND EQUIPMENT:**

- **Surface Disinfectants**: Using chemical agents like alcohol, bleach, or hydrogen peroxide to wipe down surfaces.
- **Autoclaving**: Sterilizing reusable equipment like surgical tools, lab glassware, and media with steam.
- **Fumigation**: Using gaseous agents like formaldehyde or ethylene oxide to disinfect entire rooms or large areas.