Bio-safety Manual for Milk and Meat Testing Laboratory

Prepared under the
Assam Agribusiness & Rural Transformation Project (APART)
ARIAS Society, Khanapara, Guwahati-22

International Livestock Research Institute (ILRI)
and
Animal Husbandry and **Veterinary Department**, Govt. of Assam

ARIAS Society
GOVERNMENT OF ASSAM
Assam Rural Infrastructure and Agricultural Services Society

ANIMAL HUSBANDRY
& VETERINARY DEPARTMENT
Government of Assam

ILRI
INTERNATIONAL
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Bio-safety Manual

for Milk and Meat Testing Laboratory

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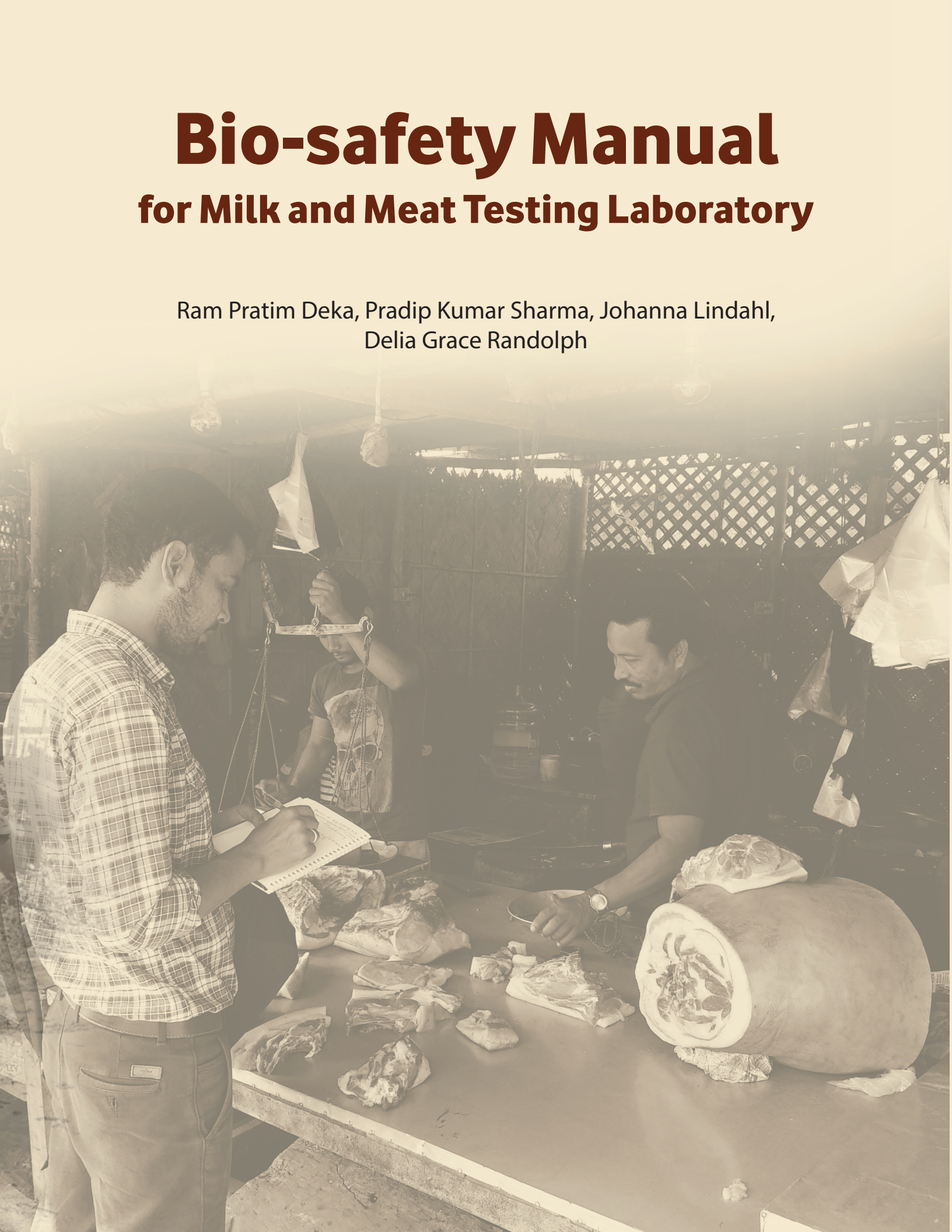

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Foreword

The Animal Husbandry and Veterinary Department (AHVD), Govt. of Assam is strengthening several laboratories of the state for conducting milk and pork safety laboratories for surveillance purpose. Operation of laboratories is a critical task and every laboratory needs to ensure that biological samples coming to the laboratory are not contaminated by any external sources, germs that may contain in biological samples are not transmitted to laboratory workers and others, chemicals and reagents are properly stored to avoid any untoward incidents, every person working in laboratories wear protective cloths for personal safety, and laboratories and biological wastes and used lab consumables are properly disposed off without causing any adverse effect on environment. As a part of the initiative, International Livestock Research Institute (ILRI), the knowledge partner under the World Bank aided Assam Agribusiness and Rural Transformation Project (APART), has come up with a Biosafety Manual for the laboratories of Animal Husbandry & Veterinary, Assam and Dairy Development, Assam.

The manual explains the simple steps that every laboratory should follow for the safety of the laboratories, persons working in the laboratories and towards the care for environment. I am confident that this manual will help in establishing a uniform process of functioning the laboratories and help the laboratory managers in managing the laboratories in a better way. I am delighted that ILRI has produced this important document in consultation with the concerned officials of AHVD and Dairy Development, Assam which will remain as an important document for the Department.

(Rajesh Prasad)

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Preface

There are some Disease Diagnostic Laboratory (DDL) under the Animal Husbandry and Veterinary Department (AHVD), Govt. of Assam for disease diagnosis and some laboratories under Dairy Development, Assam for milk testing. The department is strengthening some of these laboratories for conducing milk and pork safety test under the World Bank aided Assam Agribusiness and Rural Transformation Project (APART). As a part of the initiative and as being a knowledge partner of AHVD under APART, the International Livestock Research Institute (ILRI) has developed a Laboratory Biosafety Manual for the laboratories of both Animal Husbandry & Veterinary, Assam and Dairy Development, Assam.

Every laboratory should follow certain standard laboratory practices to avoid risk of contamination of biological samples, equipments and consumables and biological safety of the personal working in the laboratory and the environment. To achieve this every laboratory should follow certain standard biosafety practices. This requires proper guidelines and system in place which the department was lacking until now. I am delighted that ILRI has come up with this biosafety manual which will meet an important requirement of the state laboratories. I am sure this would help the Laboratory In-charge to put a system in place for biosafety in each laboratory and help in avoiding potential biosafety hazard at the laboratories.

25/08/19

Commissioner & Secretary
A.H. & Veterinary Department, Govt. of Assam

Acknowledgement

We sincerely thank and acknowledge the guidance and support that we received from the Agriculture Production Commissioner (APC) to the Govt. of Assam; Commissioner & Secretary to the Government of Assam, Animal Husbandry & Veterinary Department (AHVD); State Project Director, ARIAS Society; Director, Nodal Officer (APART) and other officials of Directorate of AHVD and Directorate of Dairy Development (DDD) and concerned officials of the ARIAS Society without which this manual compilation would not have been possible to complete.

We also express our sincere gratitude to all those mentioned under reference who documents helped us to developed this customised manual.

Team Leader and Resident Consultant, APART-ILRI
International Livestock Research Institute (ILRI)

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1. Introduction and Principles of Bio-safety

Laboratory bio-safety describes the containment principles, technologies and practices that are employed to prevent unintentional exposure to pathogens and toxins while Laboratory bio-security refers to institutional and personal security measures to prevent the loss, theft, misuse, diversion or intentional release of pathogens and toxins. Bio-safety protects the laboratory personnel, clinical specimens and the environment from the pathogens and toxins as well.

Containment refers to the safe methods, facilities and equipment for managing infectious materials in the laboratory environment being handled in or maintained. The appropriate combination of the elements of containment required in a laboratory is determined based on the risk assessment of the work to be done with a specific agent. The bio-safety measures/ guidelines proposed in this manual would be of immense use to the personnel while handling pathogens in the laboratory and the environment also to comply the recommendation of microbiological standards laid down by the FSSAI.

This Bio-safety Manual applies to bio-safety issues pertaining to personal safety, public health and environmental protection.

A biohazard can be defined as any organism, or material produced by such an organism, that is known or suspected to cause human or animal disease. Exposure to bio-hazardous agents may occur via puncture wounds or because of absorption through the respiratory tract, digestive system, skin and mucous membranes. Such exposures may result while handling samples, microbiological cultures and radioactive substances.

Bio-safety containment describes the safe methods for managing infectious agents in the laboratory environment for sample handling and aims to reduce exposure of personnel and outside environment to potentially hazardous agents.

The three elements of containment include i) laboratory practice and technique, ii) safety equipment and iii) facility design. The details are stated below

1.1 Laboratory practice and technique

- Strict adherence to standard microbiological practices and techniques
- Awareness of potential hazards
- Providing appropriate training of personnel
- Selection of safety practices in addition to standard laboratory practices, if required
- Adoption of bio-safety or operations manual which identifies the hazards

1.2 Safety equipment (primary barriers)

Safety equipment includes biological safety cabinets and a variety of enclosed containers (e.g. safety centrifuge cup). The biological safety cabinet (BSC) is the principal device used to provide containment of infectious aerosols generated by many microbiological procedures. Three types of BSCs (Class I, II, III) are used in microbiological laboratories. Safety equipment also includes items for personal protection such as gloves, coats, gowns, shoe covers, boots, respirators, face shields and safety glasses, etc.

1.3 Facility Design (Secondary barriers)

The design of the facility is important in providing a barrier to protect persons working in the facility but outside of the laboratory and those in the community from infectious agents which may be accidentally released from the laboratory.

2. Bio-safety Containment

Bio-safety concept in quality control laboratories in food industry (particularly milk and meat) ensure that adequate safety conditions are implemented to avoid potential hazards associated with the handling of food i.e. pathogenic microorganisms, antibiotics, aflatoxins and other potential contaminants. The bio-safety guidelines define the essential competencies needed by laboratory personnel to work safely in a laboratory. Competencies are measurable and include not only knowledge, skills, and abilities but also judgment and self-criticism.

A bio-safety level is a set of bio containment precautions required to isolate dangerous biological agents in an enclosed laboratory facility. The levels of containment range from the lowest bio-safety level 1 (BSL-1) to the highest at level 4 (BSL-4).

Bio-safety Level 1:

Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised.

Bio-safety Level 2:

BSL-2 is suitable for work involving agents that pose moderate risk / hazards to the personnel and environment. The personnel working in BSL-2 laboratory must have specific training in handling pathogenic agents under supervision of trained personnel. The laboratory shall be under controlled access when work is being conducted. Moreover, all procedures in which infectious aerosols or splashes may be created must be conducted in biological safety cabinets (BSCs).

Bio-safety Level 3:

Is applicable for handling clinical, diagnostic, teaching, research or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by trained scientific personnel. Also the personnel must be vaccinated for the pathogens being handled in the containment.

Bio-safety level 4:

Bio-safety level 4 (BSL-4) is the highest level of bio-safety precautions, and is appropriate for work with agents that could easily be aerosol-transmitted within the laboratory and cause severe to fatal disease in humans for which there are no available vaccines or treatments. In order to exit the BSL-4 laboratory, personnel must pass through a chemical shower for decontamination, then a room for removing the positive-pressure suit, followed by a personal shower. Entry into the BSL-4 laboratory must be restricted to trained and authorized individuals, and all persons entering and exiting the laboratory must be recorded.

Table 1: Risks and characteristics associated with pathogens from Risk Groups 1 to 4, and recommended containment level and class of biological safety cabinet

Risk group	Risk assessment	Characteristics	Examples	Bio-safety level	Bio-safety cabinet
1	• Low individual; • low community	• Unlikely to cause disease in animals or humans	• E. coli K12	1	Not required
2	• Moderate individual; • Low community.	• Rarely cause serious human or animal disease • Limited risk of spreading • • Effective prevention and treatment available	• Salmonella typhimurium • Listeria monocytogenes • E.coli O157:H7 • Campylobacter jejuni	2	Class I or Class II
3	• High individual; • • Low community	• May cause serious disease in humans or animals • Unlikely to be spread by casual contact; • Effective prevention and treatment available	• Mycobacterium tuberculosis • Mycobacterium bovis • Ricketsia • Yersinia pestis • Brucella	3	Class II
4	• High individual; • High community.	• Likely to cause very serious disease in humans or animals; • Readily transmitted from one individual to another, or between animals and humans; • Preventative vaccines or effective treatment not available.	• Hemorrhagic fever	4	Class III

3. Laboratory Bio-safety Practices

General good microbiological lab practices

- Every individual before entering the working area in the laboratory is advised to put off shoes and wear laboratory slippers.
- Ensure that every individual working on the bench washes hands using a disinfectant soap after entry and before leaving the laboratory.
- Ensure that every individual wears protective coat, head covers and gloves while handling infectious microorganisms/ or their toxins/ or any other hazardous chemicals during working on laminar flow /biological safety cabinets/ or laboratory area.
- Ensure that the potential hazards are being handled following appropriate safety precautions as per material safety data sheet (MSDS).
- Ensure that every individual is aware about the symptoms and surveillance mechanism related to potential exposure (s) of hazardous material(s) being used in the concern laboratory.
- Ensure that work area is being kept clean using disinfectant such as 70% ethanol solution by wiping out the benches and work areas both before and after working.
- All materials like media, tubes, plates, loops, needles, pipette and other items used for culturing microbes should be sterilized by autoclaving before disposal or washing as per requirements.
- All chemicals, consumables and disinfectants should be properly labelled and in case of a hazardous nature, indicate proper hazard warning symbol/ information and store them preferably in a separate area.
- Mouth pipetting should be strictly prohibited. Pipette bulbs or mechanical pipetting devices should be used for the aspiration and dispensing of liquid microbial cultures.
- Any chemical or broken culture tube or biological fluid spills must be cleaned with 70% ethanol or 10% bleach solution, then covered with filter paper. After allowing the spill to sit with the disinfectant for a short time, carefully clean up and place the materials in bio-hazardous bags and decontaminate it by autoclaving. Wash the area again with disinfectant.
- All the hazardous materials should be segregated and collected in the respective bio-hazardous bags like green, yellow and red as per standard practices adopted in the respective laboratory.
- Do not dispose of any chemical, microbial culture or bio hazardous materials directly in the sink. The waste materials should be collected in proper waste collector, i.e., bio-hazard bags and before disposal, all microbial cultures waste must be decontaminated after autoclaving.
- The use and disposal of radioactive sources should be done as per the rules and guidelines adopted at our institute.
- Always handle inflammable liquids with great care and keep them away from naked flames. Always handle concentrated acidic and alkaline chemicals with great care.
- Ensure that any individual who is technically competent and fully trained with the working of the equipment should be allowed for its handling.
- Every individual should be familiarized with the location and working of safety devices in the lab like sinks, fire extinguisher, biological safety cabinet, first aid kit, emergency gas valve, emergency doors, etc. In case of any accident in the laboratory, concerned lab In-charge must be informed.
- Do not leave any ongoing experiment unattended. Ensure that the water and burner are turned off and electric connections are disconnected before leaving laboratory.
- Eating, drinking, gum chewing, smoking, applying cosmetics is strictly prohibited in the laboratory work area.
- In case of fire, do not throw water on a live conductor and equipment. It is dangerous. The best remedial measure is to disconnect the electric supply immediately. Fire extinguishers should not be used on electrical equipment unless it is marked as suitable for this purpose. If the fire is out of control, inform NDRI security at EPABX-1400 or call the fire brigade at 101.
- In case of electrical shock, the first attempt of the attendant should be to switch off the connection immediately. If not possible, the shocked person is either pulled touching his/her cloth with a piece of dry wood standing on a dry wooden board or thick dry paper. In no case one should touch the body of the shocked person.

4. Microbial Safety Techniques

Safety in a microbiology laboratory is important in the prevention of infection that might be caused by the microorganisms. Poor laboratory techniques, human error, and misuse of equipment cause the majority of laboratory injuries and work-related infections.

Two important objectives for ensuring the utmost safety, use of aseptic techniques and other good microbiological practices are

- Prevention of contamination of the laboratory by the organisms being handled
- Prevention of contamination of the work with organisms from the environment

Aerosols are important sources of infection that can be generated by many laboratory operations, e.g. blending, mixing, grinding, shaking, stirring, sonicating and centrifuging of infectious materials. Care should be taken to reduce the extent of their formation and dispersion. Even when safe equipment is used, it is best to carry out these operations in an approved biological safety cabinet whenever possible. The use of safety equipment is no assurance of protection unless the operator is trained and uses proper techniques. Good microbiological practice encompasses a wide range of other working methods that minimise the bi-directional cross-contamination of work and workplace.

4.1 Pipettes and pipetting aids

Pipettes are used for volumetric measurements and transfer of fluids that may contain infectious, toxic, corrosive or radioactive agents. A pipetting aid must always be used for pipetting procedures. The most common hazards associated with pipetting procedures are the result of mouth suction and hence mouth pipetting must be strictly forbidden. Oral aspiration and ingestion of hazardous materials have been responsible for many laboratory-associated infections and accidents. Pipettes with cracked or chipped suction ends should not be used as they damage the seating seals of pipetting aids and so create a hazard. Exposure to aerosols may occur when liquid from a pipette is dropped onto the work surface, when pipetting mixes cultures, or when the last drop of inoculums is blown out.

The following safe pipetting techniques are recommended to minimize the potential for exposure to hazardous materials.

- Never mouth pipette but always use a pipetting aid.
- If working with bio hazardous or toxic fluid, confine pipetting operations to a biological safety cabinet.
- Do not prepare bio hazardous materials by bubbling expiratory air through a liquid with a pipette.
- Do not forcibly expel bio hazardous material out of a pipette.
- Never mix bio hazardous or toxic material by suction and expulsion through a pipette.
- When pipetting, avoid accidental release of infectious droplets. Place a disinfectant soaked towel on the work surface and autoclave the towel after use.
- Contaminated pipettes should be completely submerged in a suitable disinfectant contained in an unbreakable container. They should be left in the disinfectant for the appropriate length of time before disposal.
- A discard container for pipettes should be placed within the biological safety cabinet, not outside it.
- Syringes fitted with hypodermic needles must not be used for pipetting.
- To avoid dispersion of infectious material dropped from a pipette, an absorbent material should be placed on the working surface; this should be disposed of as infectious waste after use.

4.2 Homogenizers, blenders, sonicators, shakers and refrigerator

Equipment designed for laboratory purpose should always be used. Domestic homogenizers are not sealed and release aerosols therefore they are not recommended to use. Homogenizers used for Risk Group 3 microorganisms should always be loaded and reopened in biological safety cabinets. Sonicators may release aerosols. They should be operated in biological safety cabinets or covered with shields during use. The shields and outsides of sonicators

should be decontaminated after use. Deep freeze, liquid nitrogen, and dry ice chests as well as refrigerators should be checked, cleaned out periodically to remove any broken ampoules, tubes, etc. containing infectious material, and decontaminated. Use rubber gloves and respiratory protection during this cleaning. All infectious or toxic material stored in refrigerators or deep freezers should be properly labelled. Shaking machines should be examined carefully for potential breakage of flasks or other containers being shaken. Screw-capped durable plastic or heavy walled glass flasks should be used. These should be securely fastened to the shaker platform.

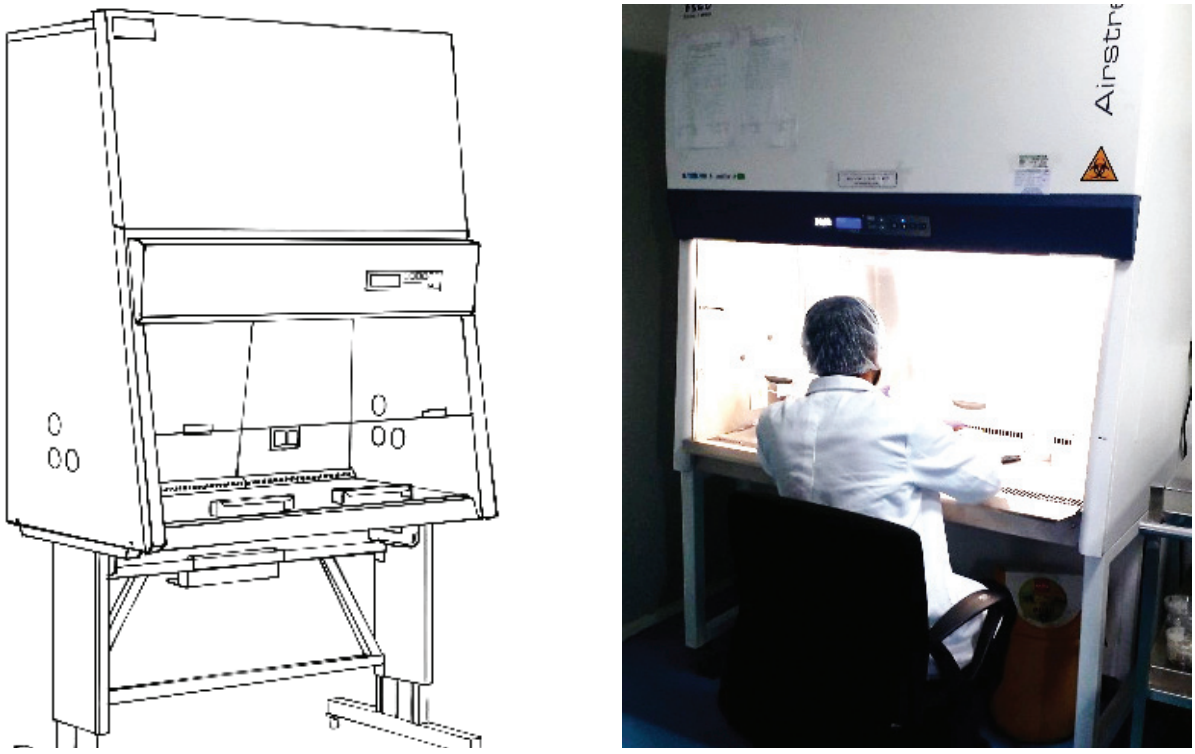
Sterilization of inoculating loops or needles in an open flame generates small-particle aerosols, which may contain viable microorganisms.

- The use of a shielded electric incinerator minimizes aerosol production during loop sterilization.
- Disposable plastic loops and needles may be used for culture work where electric incinerators or gas flames are not available. These loops should be placed in disinfectant after use and discarded as contaminated waste on contaminated (infectious) materials for disposal.
- Continuous flame gas burners should not be used in biological safety cabinets. These burners can produce turbulence, which can disturb the protective airflow patterns of the cabinet, and additionally, the heat produced by the continuous flame may damage the HEPA filter. If a gas burner must be used, one with a pilot light should be selected. Electric sterilizers should also be considered

5. Safety equipment and consumables

5.1 Biological Safety Cabinets (BSC)

Biological safety cabinets reduce the risk of airborne infection by reducing the escape of aerosolized infectious agents into the laboratory environment. In addition to protecting workers, some biological safety cabinets protect the work inside the cabinet from airborne contamination (product protection). Biological safety cabinets minimize contact between the operator and pathogens through the use of directional airflow, High Efficiency Particulate Air (HEPA) filtration of supply and/ or exhaust air, and, in some cases, a physical barrier such as a plastic or glass shield. Filters are an essential component of the biological safety cabinet, and have particle removal efficiencies of 99.97% or better for 0.3 micron diameter particles.



(a) **(b)**

Fig 1: Layout of a biosafety cabinet (a) and working condition in a biosafety containment (b)

This size particle is used as the basis for filter definition because it is considered the most difficult to remove. Thus, a filter that can trap 0.3-micron diameter particles can easily eliminate other sizes. HEPA filters consist of continuous sheets of glass fiber paper pleated over rigid corrugated separators and mounted in a wooden or metal frame.

5.2 Classes of Biological Safety Cabinets

HEPA filtered air is directed over the work surface and then discharged directly into the room. Thus, these units provide product protection, but do not protect the operator from exposure to the materials being handled; they must not be used for work with potentially infectious or toxic materials.

5.3 General principle of operation

An inward flow of room air through the work opening, away from the operator, prevents the escape of airborne pathogens into the laboratory. Negative cabinet pressure is created by a blower that exhausts the air, either into the room or to the outside, through a HEPA filter. It is this HEPA filtration of exhaust air that provides environmental protection. A disadvantage of this type of cabinet is that the product is exposed to contaminants that are pulled in from the room environment. In addition, internal air turbulence may result in cross-contamination within the cabinet.

5.4 Working safely in a Biological Safety Cabinet

Biological safety cabinets must be combined with good work practices for optimum safety and contamination control. Recommended practices when using a biological safety cabinet include the following

- Movement of arms into and out of the cabinet can disrupt airflow, adversely affecting cabinet performance.
- Whenever possible, place all materials needed for a procedure inside the cabinet before starting.
- Avoid bringing non-essential equipment and supplies into the cabinet.
- Place supplies, equipment and absorbent towels so that air intake or exhaust grilles are not obstructed.
- Keep opening and closing of lab doors and other personnel activity to a minimum.
- If a burner is deemed indispensable (remember that burners contribute to the heat load, generate convection currents that interfere with airflow and may damage the filters), use one that has a pilot flame.
- Attach a HEPA filter cartridge between the vacuum trap and the source valve.
- Work at least 4-6 inches inside the cabinet window.
- Carry out work on an absorbent pad to contain small spills.
- Clean up spills as soon as they occur; remove and disinfect the grille if contaminated.
- Designate separate areas within the cabinet for contaminated and clean materials; place contaminated material at the rear of the work area.

5.5 Personal protective equipment (PPE)

Personal protective equipment (PPE) includes clothing and equipment used to protect the laboratory person from contact with infectious, toxic, and corrosive agents, as well as excessive heat, fire etc. Proper use of protective clothing and equipment assists in reducing or minimizing exposure from hazards present in the laboratory environment (physical, biological, chemical, and radiological). The following measures should be taken in use of PPE.

- Protective clothing should be worn when working in the laboratory.
- Before leaving the laboratory, protective clothing should be removed, and hands should be washed.
- In some instances, PPE may be required to reduce the risk of exposure through direct contact, inhalation, or ingestion of an infectious agent.
- The appropriate PPE for any activity depends upon the proposed operations and the potential hazards associated with that activity or the potential routes of exposure that need to be protected to prevent exposure and infection.

5.6 Eye and face protection

Protection of the face and eyes is of prime importance in laboratories due to the potential for foreign material, both liquid and solid; to splash on the head, face and eyes or contact lenses. The choice of equipment to protect the eyes and face from splashes and impacting objects will depend on the activity performed.

Contact lenses do not provide eye protection. It is recommended that contact lenses not be worn when working around chemicals, fumes, and other hazardous material and dust particles since these items may become trapped in the space between the contact lens and the cornea. When contact lenses are worn, eye protection, such as tight fitting goggles, must be worn.

Fig 2: Personal protective equipment for biosafety lab

5.7 Gloves

Contamination of hands may occur when laboratory procedures are performed. Hands are also vulnerable to "sharps" injuries. Disposable latex or vinyl surgical-type gloves are used widely for general laboratory work, and for handling infectious agents.

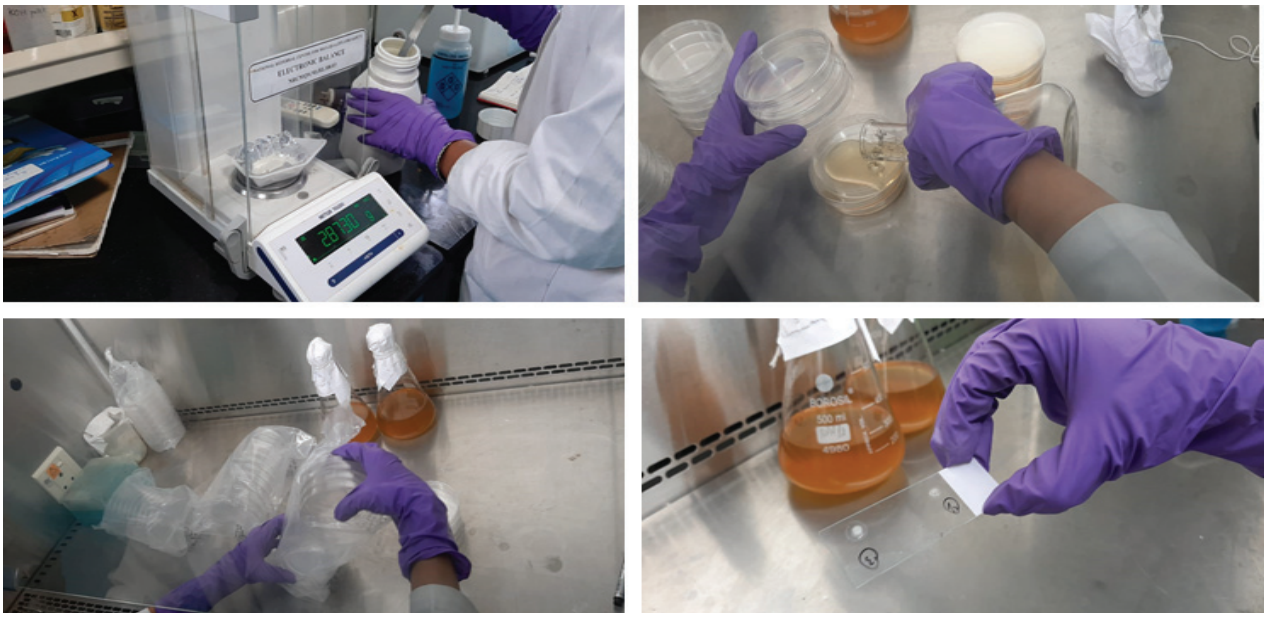


Fig 3: Application of protective gloves in different stages of handling in biosafety lab

5.7.1 Steps for safe removal of gloves

Gloves should be comfortable and of sufficient length to prevent exposure of the wrist and forearm.

- Reusable gloves may also be used but attention must be given to their correct washing, removal, cleaning and disinfection.
- Gloves should be removed and hands thoroughly washed after handling infectious materials, working in a biological safety cabinet and before leaving the laboratory.
- Used disposable gloves should be discarded with infected laboratory wastes.
- Always check the expiration date stamped on the box.

Fig 4: Safe procedure for removing hand gloves

5.8 Laboratory coats, gowns, coveralls, aprons

The lab coat is designed to protect clothing and skin from chemicals, infectious agents, and other biohazards that may be spilled or splashed.

- Laboratory coats should preferably be fully buttoned.
- Aprons should be worn over laboratory coats or gowns where necessary to give further protection against spillage of chemicals or biological materials.
- Lab coat should properly cover the whole arms and be able to be removed without pulling it over your head.
- Leave protective clothing in the lab and do not wear it to other non-lab areas.

5.9 Respirators

Protection of the respiratory system is a major concern of any biological safety program because infectious organisms can readily enter the human body through the respiratory tract. The possibility of this occurring depends on the type and infectious dose of the particular organism. For some, as few as one to ten organisms, when inhaled, may cause infection.

- Use of biological safety cabinets, should always considered as a first line of defence against respiratory infection when working with infectious organisms.
- Respirators should only be considered as a second line of defence after feasible engineering controls have been put into place and additional controls are still needed.

- Respiratory protection may be used when carrying out high-hazard procedures (e.g. cleaning up a spill of infectious material).
- The choice between mask and respirator, and type of respirator will depend on the type of hazard.
- To achieve optimal protection, respirators should be individually fitted to the operator's face and tested.
- Fully self-contained respirators with an integral air supply provide full protection.

5.10 Head covers

Hair should also be restrained because loose hair can catch fire or dip into chemical solutions. So head covers are worn to protect the hair and scalp from splatter or droplets when working with heavy contamination or when contact with the head is likely. When choosing a head cover make sure it is impervious to liquids.

Fig 5: Respirator and head cover

5.11 Closed-toe shoes

Shoes worn in the laboratory must be closed-toe.

- When working with infectious agents it is advisable to wear shoe covers, which can be decontaminated (autoclaved) before disposal, over street shoes.
- All shoes worn in the laboratory must have slip-resistant, non-absorbent soles.
- Sandals and perforated shoes are not allowed in the laboratory.
- Proper shoes reduce the potential for exposure to chemicals and injuries from broken glass and dropped items.

Fig 6: Closed-toe shoes

5.12 Sinks and hand washing facility

BSL1 and BSL2 laboratories must have a sink with running water for hand washing. In BL2 laboratories, the sink should be located near the exit door and may be manually, hands-free, or automatically operated.

- A hand washing facility must have an adequate supply of potable running water, soap, and single-use towels or hot-air drying machines.
- Hand washing sinks should be provided with a paper towel dispenser as a best management practice.

5.13 Hand-washing / hand decontamination

Whenever possible, suitable gloves should be worn when handling bio-hazardous materials. However, this does not replace the need for regular and proper hand-washing by laboratory personnel. Hands must be washed after handling bio-hazardous materials and animals, and before leaving the laboratory. In most situations, thorough washing of hands with ordinary soap and water is sufficient to decontaminate them, but the use of germicidal soaps is recommended in high-risk situations. Hands should be thoroughly lathered with soap, using friction, rinsed in clean water and dried using a clean paper or cloth towel (if available, warm-air hand-dryers may be used).

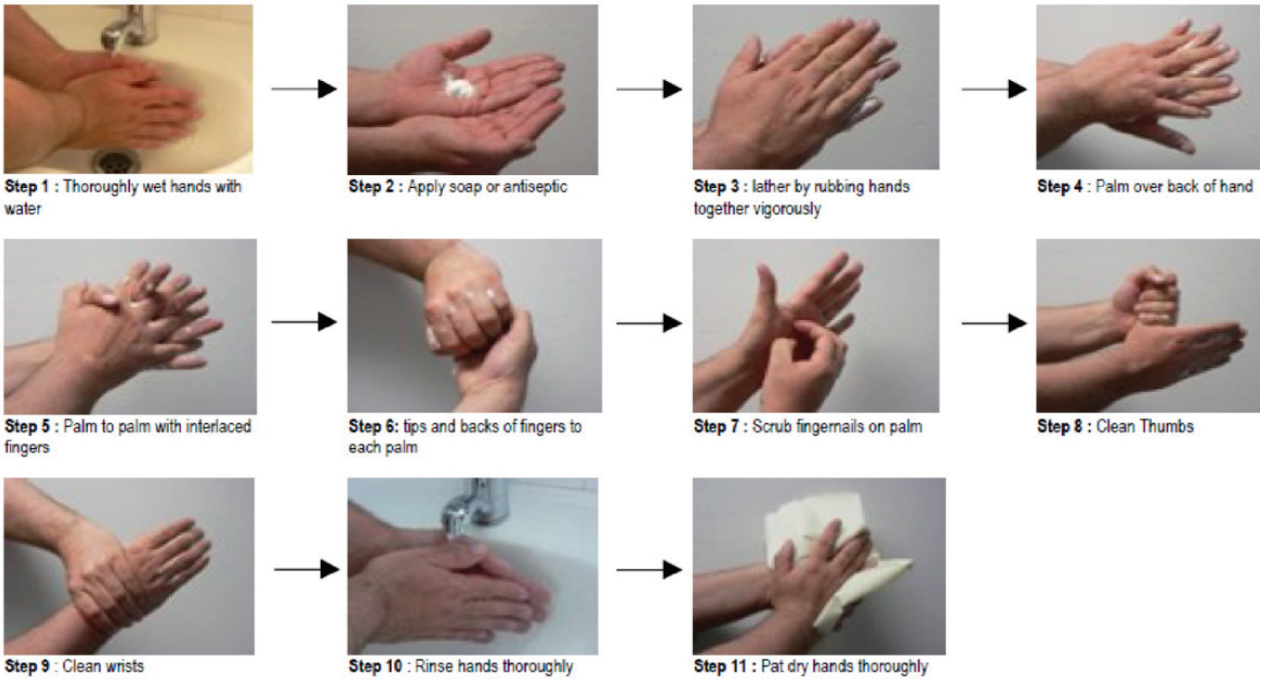


Fig 7: Proper hand washing techniques

5.14 Hygiene practices

There are generalized precautions and personal hygiene practices that have been established to protect laboratory employees from hazards associated while working with hazardous materials. These basic precautions will minimize the possibility of such exposure.

5.15 Housekeeping and maintenance

In the laboratory, keeping things clean and organized can help provide a safer environment.

- Keep drawers and cabinet doors closed and electrical cords off the floor to avoid tripping hazards.
- Keep aisles clear of obstacles such as boxes, chemical containers, and other storage items that might be put there.
- Avoid slipping hazards by cleaning up spilled liquids promptly and by keeping the floor free of loose equipment such as stirring rods, glass beads, stoppers, and other such hazards.
- Never block or even partially block the path to an exit or to safety equipment, such as fire extinguishers.
- Use the required procedure for the proper disposal of chemical wastes and solvents.
- Supplies and laboratory equipment on shelves should have sufficient clearance so that, in case of a fire, the fire sprinkler heads are able to carry out their function.
- The work area should be kept clean and uncluttered, with hazardous materials and equipment properly stored.
- Clean the work area upon completion of a task and at the end of the day.

Personnel hygiene

The liquid soap used for hand sanitization should be of good brand and certified / recommended from Indian Medical Association.

- 70% IPA (Isopropanol) solution shall be used to disinfect the hands of the personal working on biosafety cabinet/ Laminar Air Flow to carry out experimental work.
- All personal entering in the clean area shall wear lab coat, head cap and plastic/ polyethylene shoe cover.
- The personal working on hazardous material/ microbes shall wear rubber hand gloves.
- For controlling pests, suitable aerosol/ liquid pesticide shall be applied twice a month.

Cleaning and sanitizing of floorings

- Ensure that the floor is free from dust and other disposed of material.
- Ensure that the sanitizer used is of good brand containing Quaternary Aammonium Compound and certified / recommended from Indian Medical Association.
- Add half cup of sanitizer to 4 Liter of non-ionic soft water, mix it well and apply with a mop on the floor, wait until it gets dry.
- The person, performing sanitization, should be covered with protective clothing.
- The cleaning should be performed on daily basis following above practices.

6. Good Microbiological Techniques

6.1 Sterile or aseptic technique

It is a central concept in microbiology and good sterile technique is a very important part of working in a microbiology lab. The goal of sterile technique is two-fold.

- The first part is to prevent the contamination of a bacterial culture with bacteria from the environment.

- Microbiology labs both depend on a bacterial culture containing only the type or types of bacteria of interest, and no other types of bacteria which entered the culture from the environment.
- Equally important is the prevention of possible contamination of the lab worker, or others, by the potentially pathogenic bacteria he/she is working with.

Principle: Aseptic technique is a fundamental and important laboratory skill in the field of microbiology. Microbiologists use aseptic technique for a variety of procedures such as transferring cultures, inoculating media, isolation of pure cultures, and for performing microbiological tests.

- Proper aseptic technique prevents contamination of cultures from foreign bacteria inherent in the environment. For example, airborne microorganisms (including fungi), microbes picked up from the researcher's body, the lab bench-top or other surfaces, microbes found in dust, as well as microbes found on unsterilized glassware and equipment, etc. may potentially contaminate cultures, thus interfering with the lab results.
- Using proper aseptic technique can greatly minimize or even eliminate the risk of contamination.
- Aseptic technique is of at most important to maintain pure stock cultures while transferring cultures to new media.
- Aseptic technique is also essential for isolation of a single species of microorganism from a mixed culture to obtain a pure culture.
- Furthermore, proper aseptic technique prevents microbes used in the laboratory from accidentally being released into the environment and/ or infecting people working in the laboratory. This is especially relevant when pathogens are being handled.

6.2 Precautions required for preparation of culture media

For preparation of media following precautions should be taken

- Rehydrate tablets or powder according to manufacturer's instructions.
- Before sterilization, ensure ingredients are completely dissolved, using heat if necessary.
- Avoid wastage by preparing only sufficient for either immediate use (allowing extra for mistakes) or use in the near future.
- Normally allow 15-20 cm^3 medium/ Petri dish.
- Dispense in volumes appropriate for sterilization in the autoclave/pressure cooker.
- Agar slopes are prepared in test tubes or Universal/McCartney bottles by allowing sterile molten cooled medium to solidify in a sloped position.

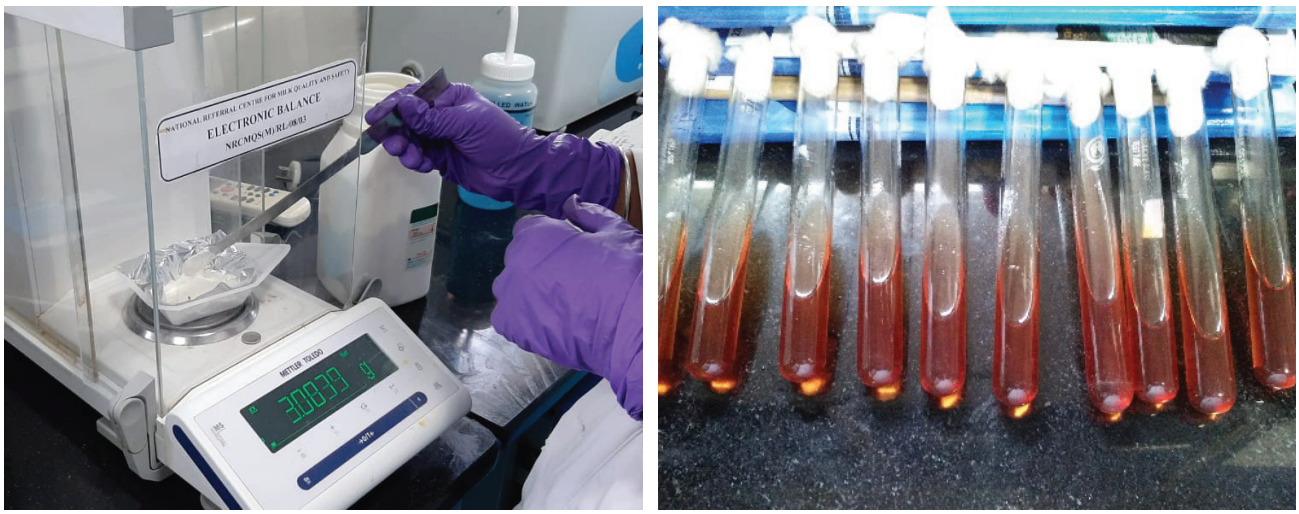


Fig 8: Preparation of culture media and slant preparation

6.3 Points to be considered in storing media

Following points are considered in storing media

- Store stocks of prepared media at room temperature away from direct sunlight.
- A cupboard is ideal but an open shelf is satisfactory.
- Media in vessels closed by cotton wool plugs that are stored for future use will be subject to evaporation at room temperature; avoid wastage by using screw cap bottles.
- Re-melt stored agar media in boiling water bath, pressure cooker or microwave oven.
- Sterile agar plates can be pre-poured and stored in well-sealed plastic bags (media-containing base uppermost to avoid heavy condensation on lid).

7. Sterilization and Disinfection

7.1 Sterilization

Any item, device or solution is considered to be sterile when it is completely free of all living microorganisms and viruses. A sterilization procedure is one that kills all micro organisms, including high numbers of bacterial end spores. Sterilization can be accomplished by heat, ethylene oxide gas, hydrogen peroxide gas, plasma, ozone, and radiation (in industry).

7.2 Disinfection

Disinfection, a procedure that reduces the level of microbial contamination, is generally a less lethal process than sterilization. It eliminates nearly all recognized pathogenic microorganisms but not necessarily all microbial forms (e.g., bacterial spores) on inanimate objects. The effectiveness of a disinfection procedure is controlled significantly by a number of factors, each one of which may have a pronounced effect on the end result. Among these are

- Nature and number of contaminating microorganisms (especially presence of bacterial spores)
- Amount of organic matter present (e.g., soil, feces, and blood)
- Type and condition of instruments, devices, and materials to be disinfected
- Temperature

7.3 Common definitions

Many different terms are used for disinfection and sterilization. The following are among the more common in bio-safety.

Antimicrobial: An agent that kills microorganisms or suppresses their growth and multiplication.

Antiseptic: A substance that inhibits the growth and development of microorganisms without necessarily killing them. Antiseptics are usually applied to body surfaces.

Biocide: A general term for any agent that kills organisms.

Chemical germicide: A chemical or a mixture of chemicals used to kill micro- organisms.

Decontamination: Any process for removing and/or killing microorganisms. The same term is also used for removing or neutralizing hazardous chemicals and radio-active materials.

Disinfectant: A chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects.

Disinfection: A physical or chemical means of killing microorganisms, but not necessarily spores.

Microbicide: A chemical or mixture of chemicals that kills microorganisms. The term is often used in place of "biocide", "chemical germicide" or "antimicrobial".

Sporocide: A chemical or mixture of chemicals used to kill microorganisms and spores.

7.4 Heat disinfection and sterilization

Heat is the most common among the physical agents used for the decontamination of pathogens. "Dry" heat, which is totally non-corrosive, is used to process many items of laboratory ware which can withstand temperatures of 160 ˚C or higher for 2–4 h. Moist heat is most effective when used in the form of autoclaving. Boiling does not necessarily kill all microorganisms and/or pathogens, but it may be used as the minimum processing for disinfection where other methods (chemical disinfection or decontamination, autoclaving) are not applicable or available. Sterilized items must be handled and stored such that they remain uncontaminated until used.

7.5 Media sterilization

When fungal spores or bacteria-laden microscopic particles make contact with plates, broths, and tubes colonies will reproduce and culture media eventually become spoiled. Most media and supplies will be sterilized in almost all laboratories. Obviously, then, all lab ware and all media must be sterilized in advance using a steam autoclave to produce moist heat. Other methods of sterilization include filtration, ethylene oxide, radiation, or ultraviolet light, may be used if components are heat-labile or materials are not heat resistant.

An autoclave is designed to deliver steam into a pressure chamber, generating high heat and pressure at the same time. Heating media to above 121°C for 4 to 20 min. destroys nearly all living cells and spores. High pressure (typically 20 lbs/sq. in) allows the temperature to exceed 100°C, which can't be accomplished with steam at one atmosphere.

(a) **(b)**

Fig 9 Autoclave- Vertical small scale(a) and double door horizontal (b)

An autoclave starts timing when the temperature reaches 121oC, and exhausts the steam slowly after the prescribed time above 121oC (to prevent exploding bottles!). The autoclave is effectively a giant pressure cooker.

To properly use an autoclave

- Know the instrument - some are fully automatic, some are fully manual
- Prepare supplies properly - the more layers or greater the volume, the longer it will take for the interior to heat up
- Check the steam pressure and ensure that the instrument is set for slow exhaust if liquids are to be sterilized
- Ensure that the door is closed properly and securely
- Check that the time and/or automatic cycle are set properly
- Ensure that the temperature is well below 100°C before attempting to open the door
- Crack the door to allow steam to vent, keeping face and hands well away from the opening

7.6 Sterilization of equipment and materials

- Wire loop: Heat to redness in Bunsen burner flame.
- Empty glassware and glass (not plastic!) pipettes and Petri dishes: At hot air oven, wrapped in either greaseproof paper or aluminium and held at 160^{0}C for 2 hrs, allowing additional time for items to come to temperature (and cool down!). Or, autoclave/pressure cooker.
- Culture media and solutions: Autoclave
- Glass spreaders and metal forceps: Flaming in alcohol (70% industrial methylated spirit).

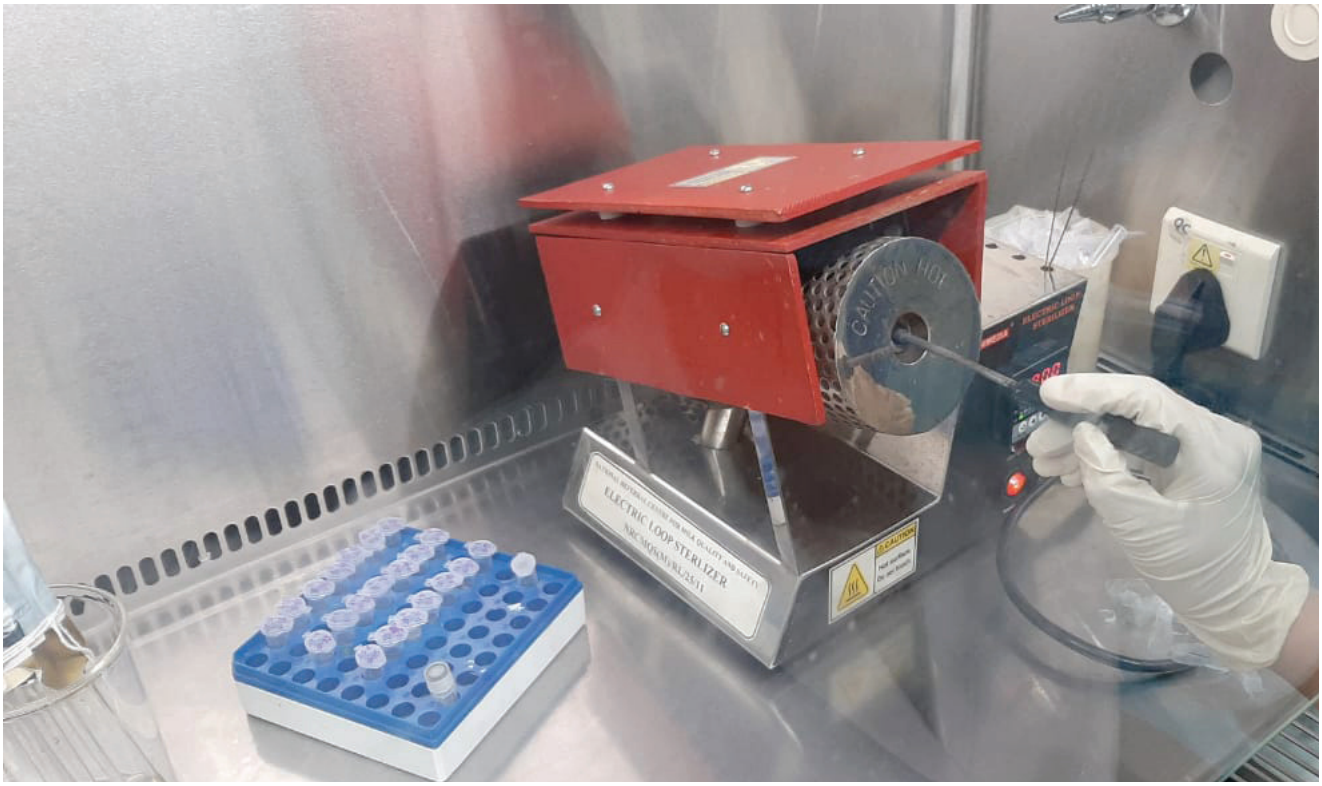


Fig 10: Electrical loop sterilizer

- **Flaming the loop:** Holding the loop in the flame of the Bunsen burner kills all contaminating organisms, thus sterilizing the loop.
  - The loop should glow red-hot for a few seconds. After flaming, make sure to slightly cool the loop before picking up organisms from the inoculums culture (the culture that is to be transferred).

- When transferring a culture from a plate, cool the loop by touching on the very edge of agar.
- When transferring from a broth, the red-hot loop will make a sizzling noise as soon as you insert it into the culture.
- The loop will automatically cool once it makes contact with the broth culture, but wait a one or two seconds before removing the loopful of inoculum from the tube.
- The hot loop may create aerosols when it touches the media containing microorganisms. It will cause some of the broth and bacteria to boil briefly, creating a bacteria-containing aerosol.
- These airborne bacteria have the chances of entering into the respiratory tract or into the body parts.
- If you hear a hissing sound when you place the heat sterilized loop into the broth culture indicates that the loop is not cooled sufficiently).

- **Flaming the mouth of the test tube:** Passing the mouth of a tube through the flame of a Bunsen burner creates a convection current which forces air out of the tube. This prevents airborne contaminants from entering the tube. The heat of the Bunsen burner also causes the air around your work area to rise, reducing the chance of airborne microorganisms contaminating your cultures.

8. Inoculation and other aseptic procedures

There are several essential precautions that must be taken during inoculation procedures to control the opportunities for the contamination of cultures, people or the environment.

- Operations must not be started until all requirements are within immediate reach and must be completed as quickly as possible.
- Vessels must be open for the minimum amount of time possible and while they are open all work must be done close to the Bunsen burner flame where air currents are drawn upwards.
- On being opened, the neck of a test tube or bottle must be immediately warmed by flaming so that any air movement is outwards and the vessel held as near as possible to the horizontal.
- During manipulations involving a petri dish, exposure of the sterile inner surfaces to contamination from the air must be limited to the absolute minimum.
- The parts of sterile pipettes that will be put into cultures or sterile vessels must not be touched or allowed to come in contact with other non-sterile surfaces, e.g. clothing, the surface of the working area, outside of test tubes/bottles.

9. Using a pipette

Sterile graduated or dropping (Pasteur) pipettes are used to transfer cultures, sterile media and sterile solutions.

- Remove the pipette from its container/ wrapper by the end that contains a cotton wool plug, taking care to touch no more than the amount necessary to take a firm hold.
- Fit the teat.
- Hold the pipette barrel as you would a pen but do not grasp the teat. The little finger is left free to take hold of the cotton wool plug/lid of a test tube/bottle and the thumb to control the teat.
- Depress the teat cautiously and take up an amount of fluid that is adequate for the amount required but does not reach and wet the cotton wool plug.
- Return any excess gently if a measured volume is required. The pipette tip must remain beneath the liquid surface while taking up liquid to avoid the introduction of air bubbles which may cause "spitting" and, consequently, aerosol formation when liquid is expelled.
- Immediately put the contaminated pipette into a nearby discard pot of disinfectant. The teat must not be removed until the pipette is within the discard pot otherwise drops of culture will contaminate the working surface.

9.1 Streak plate

Loop is used for preparing a streak plate. This involves the progressive dilution of an inoculum of bacteria or yeast over the surface of solidified agar medium in a Petri dish in such a way that colonies grow well separated from each other.

The aim of the procedure is to obtain single isolated pure colonies.

- Loosen the top of the bottle containing the inoculum.
- Hold the loop in the right hand.
- Flame the loop and allow cooling.
- Lift the bottle/test tube containing the inoculum with the left hand.
- Remove the lid/cotton wool plug of the bottle/test tube with the little finger of the left hand.
- Flame the neck of the bottle/test tube.
- Insert the loop into the culture broth and withdraw.

At all times, hold the loop as still as possible

- Flame neck of the bottle/test tube.
- Replace the lid/cotton wool plug on the bottle/test tube using the little finger. Place bottle/test tube on bench.
- Partially lift the lid of the Petri dish containing the solid medium.
- Hold the charged loop parallel with the surface of the agar; smear the inoculum backwards and forwards across a small area of the medium.
- Remove the loop and close the Petri dish.
- Flame the loop and allow it to cool. Turn the dish through 90° anticlockwise.
- With the cooled loop streak the plate from area across the surface of the agar in three parallel lines. Make sure that a small amount of culture is carried over.
- Remove the loop and close the Petri dish.
- Flame the loop and allow cooling. Turn the dish through 90° anticlockwise again and streak across the surface of the agar in three parallel lines.
- Remove the loop and close the Petri dish.
- Flame the loop and allow cooling. Turn the dish through 90° anticlockwise and streak loop across the surface of the agar from C into the centre of the plate.
- Remove the loop and close the Petri dish. Flame the loop 20.Seal and incubate the plate in an inverted position.

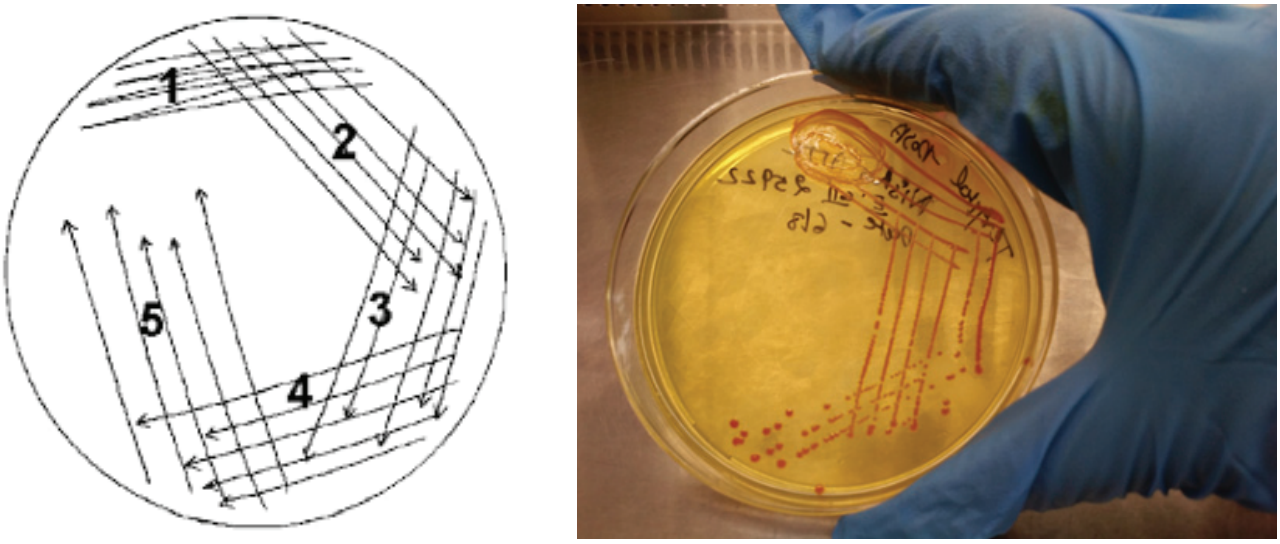


Fig 11: Proper way of streaking a culture on a plate

9.2 Pour plate

A pour plate is one in which a small amount of inoculum from broth culture is added by pipette to a molten, cooled agar medium in a test tube or bottle, distributed evenly throughout the medium, thoroughly mixed and then poured into a Petri dish to solidify. Pour plates allow micro-organisms to grow both on the surface and within the medium. Most of the colonies grow within the medium and are small in size; the few that grow on the surface are of the same size and appearance as those on a streak plate. If the dilution and volume of the inoculum, usually 1 cm^3, are known, the viable count of the sample i.e. the number of bacteria or clumps of bacteria, per cm^3 can be determined.

Fig **12: Pour plating technique**

9.3 Inoculation using a Pasteur pipette

- Loosen the top of the bottle containing the inoculum.
- Remove the sterile Pasteur pipette from its container, attach the bulb and hold in the right hand.
- Lift the bottle/test tube containing the inoculum with the left hand.
- Remove the lid/cotton wool plug with the little finger of the right hand.
- Flame the bottle/test tube neck.
- Squeeze the teat bulb of the pipette very slightly, put the pipette into the bottle/test tube and draw up a little of the culture. Do not squeeze the teat bulb of the pipette after it is in the broth as this could cause bubbles and possible aerosols.

- Remove the pipette and flame the neck of the bottle/test tube again, before replacing the lid/cotton wool plug.
- Place bottle/test tube on bench.

9.4 Pouring the pour plate

- Roll the bottle gently between the hands to mix the culture and the medium thoroughly. Avoid making air bubbles.
- Hold the bottle in the left hand; remove the lid with the little finger of the right hand.
- Flame the neck of the bottle.
- Lift the lid of the Petri dish slightly with the right hand and pour the mixture into the Petri dish and replace the lid.
- Flame the neck of the bottle and replace the lid.
- Gently rotate the dish to ensure that the medium covers the plate evenly.
- Allow the plate to solidify.
- Seal and incubate the plate in an inverted position.

9.5 Spread plate

Spread plates, also known as lawn plates, should result in a culture spread evenly over the surface of the growth medium. This means that they can be used to test the sensitivity of bacteria to many antimicrobial substances, for example mouthwashes, garlic, disinfectants and antibiotics.

Using a spreader

- Sterile glass spreaders are used to distribute inoculum over the surface of already prepared agar plates.
- Wrapped glass spreaders may be sterilized in a hot air oven.
- They can also be sterilized by flaming with alcohol.

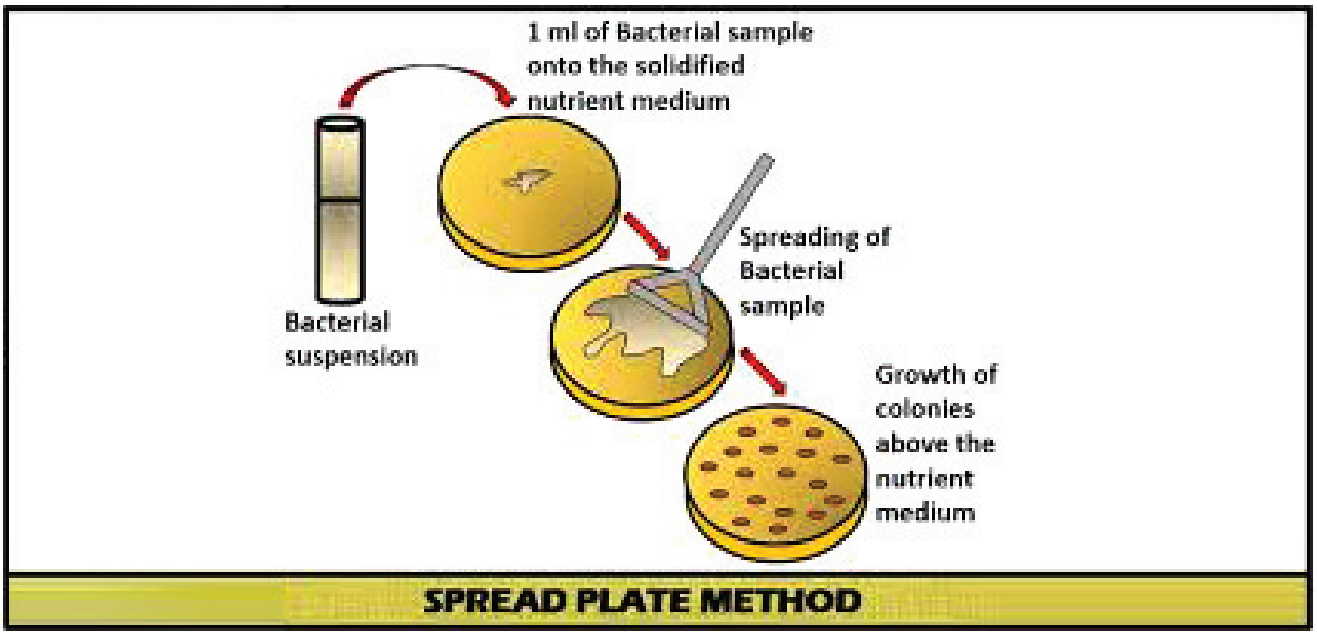


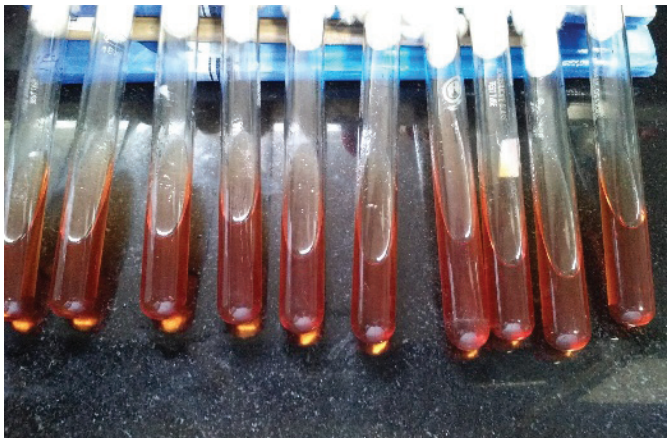
Fig 13: Spread plating technique

10. Sterilization using alcohol

- Dip the lower end of the spreader into a small volume of 70% alcohol contained in a vessel with a lid (either a screw cap or aluminium foil).
- Pass quickly through a Bunsen burner flame to ignite the alcohol; the alcohol will burn and sterilize the glass.
- Remove the spreader from the flame and allow the alcohol to burn off.
- Do not put the spreader down on the bench.
- The spread plate can be used for quantitative work (colony counts) if the inoculum is a measured volume, usually 0.1 cm^3, of each of a dilution series, delivered by pipette.
- Loosen the lid of the bottle containing the broth culture.
- Hold a sterile pipette in the right hand and the bottle/test tube containing the broth culture in the left.
- Remove the lid/plug of the bottle/test tube with the little finger of the right hand and the neck.
- With the pipette, remove a small amount of broth.
- Flame the neck of the bottle/test tube and replace the lid/plug.
- With the left hand, partially lift the lid of the Petri dish containing the solid nutrient medium.
- Place a few drops of culture onto the surface about 0.1 cm^3 (ca 5 drops, enough to cover a 5 piece).
- Replace the lid of the Petri dish.
- Place the pipette in a discard jar.
- Dip a glass spreader into alcohol, flame and allow the alcohol to burn off.
- Lift the lid of the Petri dish to allow entry of spreader.
- Place the spreader on the surface of the inoculated agar and, rotating the dish with the left hand move the spreader in a top-to-bottom or a side-to-side motion to spread the inoculum over the surface of the agar.
- Make sure the entire agar surface is covered

11. Agar Slants

Cultures are often transferred to agar slants, in addition to broth tubes and agar plates. An agar slant is a test tube containing agar, in which the solid agar forms a slant in the test tube. When inoculating an agar slant, draw the loop containing the inoculum very lightly over the surface in a zigzag formation while being careful not to break the surface. A needle can be used instead of a loop to inoculate an agar slant by stabbing the needle containing the inoculum into the agar.



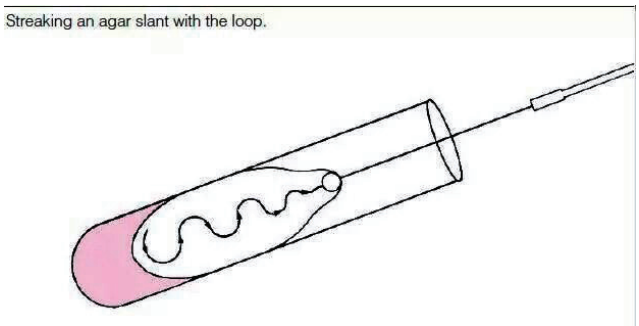


Fig 14: Preparation and inoculation on a slant

12. Incubation

The lid and base of an agar plate should be taped together with 2-4 short strips of para-film as a protection from accidental (or unauthorized!) opening during incubation. Although tape is the preferred method Para film could be used as an alternative for sealing the plates.

- Agar plates must be incubated with the medium-containing half (base) of the Petri dish uppermost otherwise condensation will occur on the lid and drip onto the culture. This might cause colonies to spread into each other and risk the spillage of the contaminated liquid.
- The advantages of incubators are that they may be set at a range of temperatures and reduce the possibility of cultures being interfered with or accidentally discarded. The temperature of an incubator varies from the set temperature, oscillating by several degrees in the course of use.
- Water baths are used when accurately controlled temperatures are required, e.g. for enzyme reactions and growth temperature relationships, when temperature control of incubators is not sufficiently precise. They should be used with distilled or deionised water to prevent corrosion and emptied and dried for storage.

Fig 15: Heating of samples in a water bath

13. Decontamination

In order to implement a laboratory bio-safety program, it is important to understand the principles of decontamination, cleaning, sterilization, and disinfection. Definitions of sterilization, disinfection, antisepsis, decontamination, and sanitization are discussed here to avoid misuse and confusion regarding the terms.

13.1 Cleaning laboratory materials

Cleaning is the removal of dirt, organic matter and stains.

- Cleaning includes brushing, vacuuming, dry dusting, washing or damp mopping with water containing a soap or detergent.
- Dirt, soil and organic matter can shield microorganisms and can interfere with the killing action of decontaminants (antiseptics, chemical germicides and disinfectants).
- Pre-cleaning is essential to achieve proper disinfection or sterilization. Many germicidal products claim activity only on pre-cleaned items.
- Pre-cleaning must be carried out with care to avoid exposure to infectious agents.
- Materials chemically compatible with the germicides to be applied later must be used.
- It is quite common to use the same chemical germicide for pre-cleaning and disinfection.

13.2 Chemical germicides

Many types of chemicals can be used as disinfectants and/or antiseptics. As there is an ever increasing number and variety of commercial products, formulations must be carefully selected for specific needs.

- The germicidal activity of many chemicals is faster and better at higher temperatures. At the same time, higher temperatures can accelerate their evaporation and also degrade them. Particular care is needed in the use and storage of such chemicals.
- Many germicides can be harmful to humans or the environment. They should be selected, stored, handled, used and disposed of with care, following manufacturers' instructions.
- For personal safety, gloves, aprons and eye protection are recommended when preparing dilutions of chemical germicides.
- Chemical germicides are generally not required for regular cleaning of floors, walls, equipment and furniture. However, their use may be appropriate in certain cases of outbreak control.

Commonly used classes of chemical germicides are described below, with generic information on their applications and safety profiles. Unless otherwise indicated, the germicide concentrations are given in weight/volume (w/v).

13.3 Chlorine (sodium hypochlorite)

Chlorine, a fast-acting oxidant, is a widely available and broad-spectrum chemical germicide. It is normally sold as bleach, an aqueous solution of sodium hypochlorite (NaOCl), which can be diluted with water to provide various concentrations of available chlorine.

- A general all-purpose laboratory disinfectant should have a concentration of 1 g/l available chlorine.
- A stronger solution, containing 5 g/l available chlorine, is recommended for dealing with bio hazardous spillage and in the presence of large amounts of organic matter.
- Sodium hypochlorite solutions, as domestic bleach, contain 50 g/l available chlorine and should therefore be diluted 1:50 or 1:10 to obtain final concentrations of 1 g/l and 5 g/l, respectively.
- Industrial solutions of bleach have a sodium hypochlorite concentration of nearly 120 g/l and must be diluted accordingly to obtain the levels indicated above.
- Granules or tablets of calcium hypochlorite Ca $(ClO)_2$ generally contain about 70% available chlorine.
- Solutions prepared with granules or tablets, containing 1.4 g/l and 7.0 g/l, will then contain 1.0 g/l and 5.0 g/l available chlorine, respectively.

Bleach is not recommended as an antiseptic, but may be used as a general-purpose disinfectant and for soaking contaminated metal-free materials. In emergencies, bleach can also be used to disinfect water for drinking, with a final concentration of 1–2 mg/l available chlorine. Many by-products of chlorine can be harmful to humans and the environment, so that indiscriminate use of chlorine-based disinfectants, in particular bleach, should be avoided.

13.4 Formaldehyde

Formaldehyde (HCHO) is a gas that kills all microorganisms and spores at temperatures above 200 ⁰C. However, it is not active against prions. Formaldehyde is relatively slow-acting and needs a relative humidity level of about 70%. It is marketed as the solid polymer, par formaldehyde, in flakes or tablets, or as formalin, a solution of the gas in water of about 370 g/l (37%), containing methanol (100 ml/l) as a stabilizer. Both formulations are heated to liberate the gas, which is used for decontamination and disinfection of enclosed volumes such as safety cabinets and rooms. Formaldehyde (5% formalin in water) may be used as a liquid disinfectant. Formaldehydes are suspected carcinogen, dangerous, irritant gas that has a pungent smell and its fumes can irritate eyes and mucous membranes. It must therefore be stored and used in a fume-hood or well-ventilated area. National chemical safety regulations must be followed.

13.5 Glutaraldehyde

Like formaldehyde, glutaraldehyde (OHC $(CH_2)_3$CHO) is also active against vegetative bacteria, spores, fungi and lipid- and non-lipid-containing viruses. It is non-corrosive and faster acting than formaldehyde. However, it takes several hours to

kill bacterial spores. Glutaraldehyde is generally supplied as a solution with a concentration of about 20 g/l (2%) and some products may need to be "activated" (made alkaline) before use by the addition of a bicarbonate compound supplied with the product. The activated solution can be reused for 1–4 weeks depending on the formulation and type and frequency of its use. Dipsticks supplied with some products give only a rough indication of the levels of active glutaraldehyde available in solutions under use. Glutaraldehyde solutions should be discarded if they become turbid. Glutaraldehyde is toxic and an irritant to skin and mucous membranes, and contact with it must be avoided. It must be used in a fume-hood or in well ventilated areas. It is not recommended as a spray or solution for the decontamination of environmental surfaces. National chemical safety regulations must be followed.

13.6 Alcohols

Ethanol (ethyl alcohol, C_2H_5OH) and 2-propanol (isopropyl alcohol, $(CH_3)_2CHOH$) have similar disinfectant properties. They are active against vegetative bacteria, fungi and lipid-containing viruses but not against spores. Their action on non-lipid viruses is variable. For highest effectiveness they should be used at concentrations of approximately 70% (v/v) in water: higher or lower concentrations may not be as germicidal. A major advantage of aqueous solutions of alcohols is that they do not leave any residue on treated items.

Mixtures with other agents are more effective than alcohol alone, e.g. 70% (v/v) alcohol with 100 g/l formaldehyde, and alcohol containing 2 g/l available chlorine. A70% (v/v) aqueous solution of ethanol can be used on skin, work surfaces of laboratory benches and bio-safety cabinets, and to soak small pieces of surgical instruments. Since ethanol can dry the skin, it is often mixed with emollients. Alcohol-based hand-rubs are recommended for the decontamination of lightly soiled hands in situations where proper hand-washing is inconvenient or not possible. However, it must be remembered that ethanol is ineffective against spores and may not kill all types of non-lipid viruses. Alcohols are volatile and flammable and must not be used near open flames. Working solutions should be stored in proper containers to avoid the evaporation of alcohols. Alcohols may harden rubber and dissolve certain types of glue. Proper inventory and storage of ethanol in the laboratory is very important to avoid its use for purposes other than disinfection. Bottles with alcohol-containing solutions must be clearly labelled to avoid autoclaving.

13.7 Hydrogen peroxide

Like chlorine, hydrogen peroxide (H_2O_2) and per acids are strong oxidants and can be potent broad spectrum germicides. They are also safer than chlorine to humans and the environment. Hydrogen peroxide is supplied either as a ready-to-use 3% solution or as a 30%aqueous solution to be diluted to 5–10 times its volume with sterilized water. However, such 3–6% solutions of hydrogen peroxide alone are relatively slow and limited as germicides. Products now available have other ingredients to stabilize the hydrogen peroxide content, to accelerate its germicidal action and to make it less corrosive. Hydrogen peroxide can be used for the decontamination of work surfaces of laboratory benches and bio-safety cabinets, and stronger solutions may be suitable for disinfecting heat-sensitive medical/dental devices. The use of vaporized hydrogen peroxide or per acetic acid (CH_3COOOH) for the decontamination of heat-sensitive medical/surgical devices requires specialized equipment.

13.8 Local environmental decontamination

Decontamination of the laboratory space, its furniture and its equipment requires a combination of liquid and gaseous disinfectants. Surfaces can be decontaminated using a solution of sodium hypochlorite (NaOCl); a solution containing 1 g/l available chlorine may be suitable for general environmental sanitation, but stronger solutions (5 g/l) are recommended when dealing with high risk situations. For environmental decontamination, formulated solutions containing 3% hydrogen peroxide (H2O2) make suitable substitutes for bleach solutions. Rooms and equipment can be decontaminated by fumigation with formaldehyde gas generated by heating par formaldehyde or boiling formalin. This is a highly dangerous process that requires specially trained personnel. All openings in the room (i.e. windows, doors, etc.) should be sealed with masking tape or similar before the gas is generated. Fumigation should be conducted at an ambient temperature of at least 21 °C and a relative humidity of 70%.

After fumigation the area must be ventilated thoroughly before personnel are allowed to enter. Appropriate respirators must be worn by anyone entering the room before it has been ventilated. Gaseous ammonium bicarbonate can be used to neutralize the formaldehyde. Fumigation of smaller spaces with hydrogen peroxide vapours is also effective but requires specialized equipment to generate the vapours.

13.9 Decontamination of biological safety cabinets

To decontaminate Class I and Class II cabinets, equipment that independently generates, circulates and neutralizes formaldehyde gas is available. Alternatively, the appropriate amount of par formaldehyde (final concentration of 0.8% par formaldehyde in air) should be placed in a frying pan on an electric hot plate. Another frying pan, containing 10% more ammonium bicarbonate than par formaldehyde, on a second hot plate is also placed inside the cabinet. The hot plate leads are plugged in outside the cabinet, so that operation of the pans can be controlled from the outside by plugging and unplugging the hot plates as necessary. If the relative humidity is below 70%, an open container of hot water should also be placed inside the cabinet before the front closure is sealed in place with strong tape (e.g. duct tape). Heavy gauge plastic sheeting is taped over the front opening and exhaust port to make sure that the gas cannot seep into the room. Penetration of the electric leads passing through the front closure must also be sealed with duct tape.

The plate for the par formaldehyde pan is plugged in. It is unplugged when all the par formaldehyde has vaporized. The cabinet is left undisturbed for at least 6 hour. The plate for the second pan is then plugged in and the ammonium bicarbonate is allowed to vaporize. This plate is then unplugged and the cabinet blower is switched on for two intervals of approximately 2 each to allow the ammonium bicarbonate gas to circulate. The cabinet should be left undisturbed for 30 min before the front closure (or plastic sheeting) and the exhaust port sheeting are removed. The cabinet surfaces should be wiped down to remove residues before use.

14. Bio-medical waste and its disposal

The disposal of laboratory and medical waste is subject to various regional, national and international regulations, and the latest versions of such relevant documents should be consulted before designing and implementing a programme for handling, transportation and disposal of bio-hazardous waste. In general, ash from incinerators may be handled as normal domestic waste and removed by local authorities. Autoclaved waste may be disposed off by off-site incineration or in licensed landfill sites.

Medical waste is any kind of waste that contains infectious material (or material that's potentially infectious). This definition includes waste generated by healthcare facilities like physician's offices, hospitals, dental practices, laboratories, medical research facilities, and veterinary clinics. Medical waste can contain bodily fluids like blood or other contaminants. The 1988 Medical Waste Tracking Act defines is as waste generated during medical research, testing, diagnosis, immunization, or treatment of either human beings or animals. Some examples are culture dishes, glassware, bandages, gloves, discarded sharps like needles or scalpels, swabs, and tissue. Following figures reveal about the segregation of bio0-medical waste.

Fig 16: Biomedical waste management rules

Medical Waste Types

The term "medical waste" can cover a wide variety of different by-products of the healthcare industry. The broadest definition can include office paper and hospital sweeping waste. The list below displays the most common waste categories as identified by the WHO.

Sharps. This kind of waste includes anything that can pierce the skin, including needles, scalpels, lancets, broken glass, razors, ampoules, staples, wires, and trocars.

Infectious Waste. Anything infectious or potentially infectious goes in this category, including swabs, tissues, excreta, equipment, and lab cultures.

R**adioactive.** This kind of waste generally means unused radiotherapy liquid or lab research liquid. It can also consist of any glassware or other supplies contaminated with this liquid.

Pathological. Human fluids, tissue, blood, body parts, bodily fluids, and contaminated animal carcasses come under this waste category.

Pharmaceuticals. This grouping includes all unused, expired, and/or contaminated vaccines and drugs. It also encompasses antibiotics, injectables, and pills.

Chemical. These are disinfectants, solvents used for laboratory purposes, batteries, and heavy metals from medical equipment such as mercury from broken thermometers.

Genotoxic Waste. This is a highly hazardous form of medical waste that's either carcinogenic, teratogenic, or mutagenic. It can include cytotoxic drugs intended for use in cancer treatment.

General Non-Regulated Medical Waste. Also called non-hazardous waste, this type doesn't pose any particular chemical, biological, physical, or radioactive danger.

Disposal of medical waste

On-Site Treatment

The on-site treatment of medical waste is generally limited to large, well-monied hospitals and facilities. On-site treatment is extremely cost-prohibitive. That's because the required equipment is expensive to buy, expensive to maintain, and expensive to manage and run. The regulatory maze around such equipment (and its use) presents yet another barrier to entry.

Off-Site Treatment

Off-site medical waste treatment is a far more cost-effective option for most small and mid-sized medical practices and facilities. Third-party vendors whose main business is healthcare waste collection and disposal have the equipment and training needed to handle the process. Vendors can collect the waste either by truck or by mail.

No matter where medical waste is processed, it's ultimately treated by incineration, autoclaving, microwave, biological, or chemical treatment. Incineration, once by far the most popular method, has decreased in usage since the 1990's, as regulation has forced other methods to come online.

Incineration. Before 1997, over 90% of all infectious medical waste was disposed of by incineration. Changes to EPA regulations has led providers to seek other disposal means. This is still the only method used on pathological waste, for example body parts and recognizable tissues.

Autoclaving. Steam sterilization renders biohazardous waste non-infectious. After it's been sterilized, the waste can be disposed of normally in solid waste landfills, or it can be incinerated under less-stringent regulation.

Fig17 :Packing of biological waste in specialized autoclave bags

Microwaving: Another way to render hazardous healthcare waste non-hazardous is to microwave it with high-powered equipment. As with autoclaving, this method opens up the waste to normal landfill disposal or incineration afterward.

Chemical: Some kinds of chemical waste may be neutralized by applying reactive chemicals that render it inert. This is generally reserved for waste that's chemical in nature.

Biological: This experimental method of treating biomedical waste uses enzymes to neutralize hazardous, infectious organisms. It's still under development and rarely used in practice.

Personnel safety devices for handling of Bio-medical waste

The use of protective gears should be made mandatory for all the personnel handling waste.

Gloves: Heavy-duty rubber gloves should be used for waste handling by the waste retrievers. This should be bright yellow in colour. After handling the waste, the gloves should be washed twice. The gloves should be washed after every use with carbolic soap and a disinfectant. The size should fit the operator.

Aprons, gowns, suits or other apparels: Apparel is worn to prevent contamination of clothing and protect skin. It could be made of cloth or impermeable material such as plastic. People working in incinerator chambers should have gowns or suits made of non-inflammable material.

Masks: Various types of masks, goggles, and face shields are worn alone or in combination, to provide a protective barrier. It is mandatory for personnel working in the incinerator chamber to wear a mask covering both nose and mouth, preferably a gas mask with filters.

Boots: Leg coverings, boots or shoe-covers provide greater protection to the skin when splashes or large quantities of infected waste have to be handled. The boots should be rubber-soled and anti-skid type. They should cover the leg up to the ankle.

15. Handling of spillage

15.1 Spills of biohazards

Each laboratory working with potentially hazardous biological material must be prepared and trained to handle its own biological spills. Performing all work on plastic-backed liners to absorb spills can minimize the consequences of a spill of a bio hazardous or select agent. The quantities of these materials should be limited so they can be easily contained, cleaned, or destroyed. A simple spill kit should be on hand including

- Chlorine bleach or some other concentrated disinfectant
- A package or roll of paper towels
- Autoclavable bags
- Rubber gloves
- Forceps for picking up broken glass

15.2 Broken containers and spilled infectious substances

- Broken containers contaminated with infectious substances and spilled infectious substances should be covered with a cloth or paper towels.
- Disinfectant should then be poured over these and left for the appropriate amount of time.
- The cloth or paper towels and the broken material can then be cleared away; glass fragments should be handled with forceps.
- The contaminated area should then be swabbed with disinfectant.
- If dustpans are used to clear away the broken material, they should be autoclaved or placed in an effective disinfectant.
- Cloths, paper towels and swabs used for cleaning up should be placed in a contaminated waste container.
- Gloves should be worn for all these procedures.
- If laboratory forms or other printed or written matter are contaminated, the information should be copied onto another form and the original discarded into the contaminated-waste container.

Fig 18: Handling of large spill

15.3 Small spill of material in a Biological Safety Cabinet (BSC)

- Alert the other laboratory employees.
- Leave the cabinet turned on.
- While wearing gloves, spray or wipe cabinet walls, work surfaces and equipment with disinfectant equivalent to 1:10 bleach solution. If necessary, flood the work surface, as well as drain-pans and catch basins below the work surface, with disinfectant for a contact time of at least 20 minutes.
- Soak up disinfectant and spill with paper towels. Drain catch basin into a container. Lift front exhaust grill and tray and wipe all surfaces. Ensure that no paper towels or solid debris are blown into the area beneath the grill. Report the spill to the laboratory's PI.
- Autoclave all clean-up materials before disposal in the biohazard waste container.
- Wash hands and any exposed surfaces thoroughly after the clean-up procedure.

15.4 Large spill of material (>500 ml) in a Biological Safety Cabinet (BSC)

BSC must run during cleanup to contain aerosols and HEPA-filter exhaust air.

- Ware an appropriate personal protective gear before initiating cleanup
- Initiate clean up as soon as possible using a germicidal disinfectant (phenolic or iodophor). Alcohol should be used.
- If the spill is contained on a bench pad, remove the contaminated bench pad discard as infectious waste.
- If the spill is on the work area surface, cover spilled material with disinfectant-soaked towels. Allow 20 minutes contact time then remove the contaminated towels and discard as infectious waste.
- Wipe down the interior of the cabinet and any splatter on items within the cabinet with a disinfectant-soaked towel.
- Wipe down non-autoclavable materials with disinfectant. Allow 20 minutes of contact time with disinfectant before any items are removed from cabinet.
- Place items designated as contaminated used sharps in an appropriate infectious waste sharps container using tongs/forceps. Place other contaminated disposable materials used in the cleanup process in an autoclave bag. Process as infectious waste.
- Place contaminated re-usable items in biohazard bags, autoclavable pans with lids or wrap them in newspaper. Sterilize, preferably by autoclaving, and then clean for re-use.
- If the cabinet has a catch basin beneath the work surface and the spill resulted in liquids flowing into this area, more extensive decontamination is required as follows
    - Ensure the drain valve under the cabinet is closed.
    - Pour disinfectant onto the work surface and through the front and rear grilles into the drain pan. Allow 20-30 minutes contact time.
    - Absorb spilled fluid-disinfectant from work surface with paper towels and discard in biohazard bag.
    - Prepare to empty drain pan. Place disinfectant solution in a collection vessel. Attach flexible tubing to the drain valve. The tube should be of sufficient length to allow the open end to be submerged in the collection vessel to minimize aerosol generation.
    - Open the drain valve and empty the drain pan into the collection vessel containing disinfectant. Flush the drain pan with water and remove the flexible tubing. Manage contaminated materials as if they are infectious.
    - Remove protective clothing used during cleanup and place in a biohazard bag for autoclaving. Wash hands when gloves are removed.
    - Notify Principal Investigator, supervisor, and EH&S. Consult with EH&S to determine whether formaldehyde decontamination of the cabinet and filters is necessary, especially if a high-risk agent or a major spill of a moderate-risk agent occurred.
    - Run BSC at least 10 minutes after cleanup, before resuming activity in the cabinet.

15.5 Small spill of material outside of a BSC

- Alert laboratory employees.
- Wearing gloves, safety glasses, and a lab coat, cover the spill with paper towels and gently apply disinfectant, proceeding from the outer edge of the spill to its centre. Leave in place for 20 minutes.
- Report the spill to the laboratory's PI
- Pick up the towels and discard into a biohazard container. Pick up any pieces of broken glass with forceps and place in sharps container.
- Re-wipe the spill area with disinfectant and thoroughly wash hands after glove removal.

Illustration needed.

15.6 Large spill of material (>500 ml) outside of a BSC

- Hold your breath and leave the room immediately.
- Warn others to stay out of the spill area to prevent spread of contamination.
- Post a sign stating: "DO NOT ENTER, BIOHAZARD SPILL, contact (name and phone #) for information."
- Remove any contaminated clothing, ensuring that clothing is not pulled over the face, and put into a biohazard bag for later autoclaving.
- Wash hands and exposed skin.
- Notify the supervisor and in-charge of the laboratory
- Put on protective clothing (lab coat, gloves and, if indicated, respirator, eye protection, shoe covers) and assemble clean-up materials.
- Wait 30 minutes before re-entering the contaminated area to allow for dissipation of aerosols.
- Cover the spill with paper towels and gently apply disinfectant, proceeding from the outer edge of the spill to its centre. Leave in place for 20 minutes.
- Collect and autoclave all treated material and discard in a biohazard container. Pick up any broken glass with forceps and place them into a sharps container.
- Re-wipe the spill area with disinfectant and wash hands thoroughly at completion of clean-up.

15.7 Potentially infectious aerosol release (outside a biological safety cabinet)

- All persons should immediately vacate the affected area and any exposed persons should be referred for medical advice.
- The laboratory supervisor and the bio safety officer should be informed at once.
- No one should enter the room for an appropriate amount of time (e.g. 1 h), to allow aerosols to be carried away and heavier particles to settle.
- If the laboratory does not have a central air exhaust system, entrance should be delayed (e.g. for 24 hour).
- Signs should be posted indicating that entry is forbidden.
- After the appropriate time, decontamination should proceed, supervised by the bio-safety officer.
- Appropriate protective clothing and respiratory protection should be worn.

15.8 Breakage of tubes containing potentially infectious material in centrifuges not having sealable buckets

- If a breakage occurs or is suspected while the machine is running, the motor should be switched off and the machine left closed (e.g. for 30 min) to allow settling.
- If a breakage is discovered after the machine has stopped, the lid should be replaced immediately and left closed (e.g. for 30 min).
- In both instances, the bio-safety officer should be informed.
- Strong (e.g. thick rubber) gloves, covered if necessary with suitable disposable gloves should be worn for all subsequent operations.
- Forceps, or cotton held in the forceps, should be used to retrieve glass debris.
- All broken tubes, glass fragments, buckets, trunnions and the rotor should be placed in a noncorrosive disinfectant known to be active against the organisms concerned.
- Unbroken, capped tubes may be placed in disinfectant in a separate container and recovered.
- The centrifuge bowl should be swabbed with the same disinfectant, at the appropriate dilution, and then swabbed again, washed with water and dried.
- All materials used in the clean-up should be treated as infectious waste.

15.9 Breakage of tubes inside sealable buckets (safety cups)

- All sealed centrifuge buckets should be loaded and unloaded in a biological safety cabinet.
- If breakage is suspected within the safety cup, the safety cup should be loosened and the bucket autoclaved.
- Alternatively, the safety cup may be chemically disinfected.

16. Emergency Procedures

An emergency situation is declared if a release or spill of a hazardous substance occurs that poses a significant threat to the health and safety of the person in the vicinity of the release. Methods of handling emergency situations and specific response procedures to handle injuries, emergencies, and spills that occur in a safe, orderly and efficient manner when research involves biological materials are delineated hereunder. Each bio-safety laboratory should have safety plan contains procedures for spills, contact numbers, and the location of emergency equipment. The PI or designee must review the guide with new personnel and on an annual basis. Emergency plans will be tested, to ensure they are capable of effectively responding to the emergency in a timely manner, before they are adopted. The Following emergency procedures should be followed in accidental spills or releases of bio hazardous material in microbiological laboratories.

16.1 Puncture wounds, cuts and abrasions

- The affected individual should remove protective clothing
- Wash the hands and any affected area(s)
- Apply an appropriate skin disinfectant, and seek medical attention as necessary.
- The cause of the wound and the organisms involved should be reported, and appropriate and complete medical records kept.

16.2 Intact Skin

- Remove contaminated clothing. Clothing should not be pulled over the face as contact with eyes, nose, and mouth may occur. Shirts should be cut off.
- Vigorously wash contaminated skin for 1 minute with soap and water.
- Call medical attention at the Campus Health Services, if necessary.
- Inform the laboratory's PI

16.3 Eye

- Immediately flush eyes for at least 15 minutes with water, using eyewash. Hold eyelids away from your eyeball and rotate your eyes so that all surfaces may be washed thoroughly.
- Remove contaminated clothing. Clothing should not be pulled over the face as contact with eyes, nose, and mouth may occur. Shirts should be cut off.
- Call medical attention at the Campus Health Services, if necessary.
- Inform the laboratory's in-charge immediately.

16.4 Ingestion of potentially infectious material

- Protective clothing should be removed and medical attention sought.
- Move to fresh air immediately
- Do not induce vomiting unless advised to do so by a health care provider.
- Call medical attention at the Campus Health Services, if necessary
- Identification of the material ingested and circumstances of the incident should be reported, appropriate and complete medical records kept.
- Inform the laboratory's in-charge immediately and also refer to the MSDS of the related chemical.

16.5 Fire and natural disasters

- Fire and other services should be involved in the development of emergency preparedness plans.
- There should a fire exit plan for the laboratory and directions toward the exit must be pasted throughout the laboratory
- They should be told in advance which rooms contain potentially infectious materials.
- It is beneficial to arrange for these services to visit the laboratory to become acquainted with its layout and contents.
- After a natural disaster, local or national emergency services should be warned of the potential hazards within and/or near laboratory buildings.
- They should enter only when accompanied by a trained laboratory worker. Infectious materials should be collected in leak proof boxes or strong disposable bags.
- Salvage or final disposal should be determined by bio-safety staff on the basis of local ordinances.

16.6 Emergency equipment

The following emergency equipment must be available:

- First-aid kit, including universal and special antidotes
- Appropriate fire extinguishers, fire blankets

The following are also suggested but may be varied according to local circumstances

- Full protective clothing
- Full-face respirators with appropriate chemical and particulate filter canisters
- Room disinfection apparatus, e.g. sprays and formaldehyde vaporizers
- Tools, e.g. hammers, axes, spanners, screwdrivers, ladders, ropes
- Hazard area demarcation equipment and notices.

16.7 Emergency services: whom to contact

The telephone numbers and addresses of the following should be prominently displayed in the facility

- The institution or laboratory itself (the address and location may not be known in detail by the caller or the services called)
- Director of the institution or laboratory
- Laboratory supervisor
- Bio safety officer
- Fire services
- Hospitals/ambulance services/ medical staff (names of individual clinics, departments, and/ or medical staff, if possible)
- Police
- Medical officer
- Responsible technician
- Water, gas and electricity services.

17. Chemical Hazards and Safety

Workers in microbiological laboratories are exposed to chemical hazards as well as to pathogenic microorganisms. It is therefore vital that they have proper knowledge of the toxic effects of these chemicals, the routes of exposure, and the hazards that may be associated with their handling and storage. Material safety data sheets (MSDS), which describe the hazards associated with the use of a given chemical, are available from the manufacturer, and should be made available in laboratories where these chemicals are used, e.g. as part of a safety or operations manual.

17.1 Definitions and classifications

Hazardous chemicals are often defined and classified according to regulations written for the transport of dangerous goods or by the hazards and degrees of danger they present. They may be listed by their degree of reactivity, instability, fire or health hazard or by toxic effects.

17.2 Routes of exposure

Exposure to hazardous chemicals may occur in several ways

- Inhalation: Chemicals may cause irritation, sensitization, allergic reactions, respiratory disease or cancer.
- Contact: Contact with skin may cause chemical burns, conjunctivitis of the eyes, or systemic poisoning.
- Ingestion: Hazardous chemicals may be accidentally swallowed via mouth pipetting, or contamination of food or drinks.
- Through broken skin: Hazardous chemicals may enter the body via cuts, abrasions or needlesticks.

17.3 Storage of chemicals

Only minimum amounts of the chemicals listed below should be stored in the laboratory for daily use. Bulk stocks should be kept in specially designated rooms or buildings, which should have concrete floors with sills at doorways to retain spills. Flammable substances should be stored separately in buildings that are some distance from any others. To avoid ignition of flammable and explosive vapours by the sparking of electrical contacts, light switches for these stores should be on the outside of the building and the lights themselves should be in bulkheads.

Fig 19: Arrangement of chemicals in a biosafety laboratory

Chemicals should not be stored in alphabetical order otherwise incompatible chemicals may be in close proximity and some hazardous chemicals may be on high shelves. All large bottles and all bottles containing strong acids and alkalis should be at floor level and in drip trays. Bottle carriers and siphoning devices for filling bottles from bulk containers should be provided.

17.4 Incompatible chemicals

Many common laboratory chemicals react in a dangerous manner if they come into contact with one another. Some such incompatible chemicals are listed below.

- **Acetic acid:** with chromic acid, nitric acid, hydroxyl compounds, ethylene glycol, perchloric acid, peroxides and permanganates.
- **Acetone:** with concentrated sulphuric and nitric acid mixtures.

- **Acetylene:** with copper (tubing), halogens, silver, mercury and their compounds.
- **Alkali metals:** with water, carbon dioxide, carbon tetrachloride and other chlorinated hydrocarbons.
- **Ammonia, anhydrous**: with mercury, halogens, calcium hypochlorite and hydrogen fluoride.
- **Ammonium nitrate:** with acids, metallic powders, flammable liquids, chlorates, nitrites, sulphur and finely divided organic or combustible compounds.
- **Aniline:** with nitric acid and hydrogen peroxide.
- **Bromine:** with ammonia, acetylene, butadiene, butane, hydrogen, sodium carbide, turpentine and finely divided metals
- **Carbon, activated:** with calcium hypochlorite and all oxidizing agents.
- **Chlorates:** with ammonium salts, acids, metal powders, sulphur and finely divided organic or combustible compounds.
- **Chlorine:** with ammonia, acetylene, butadiene, benzene and other petroleum fractions, hydrogen, sodium carbide, turpentine and finely divided metals.
- **Chlorine dioxide:** with ammonia, methane, phosphine and hydrogen sulfide.
- **Chromic acid:** with acetic acid, naphthalene, camphor, alcohol, glycerol, turpentine and other flammable liquids.
- **Copper:** with acetylene, azides and hydrogen peroxide.
- **Cyanide:** with all acids
- **Flammable liquids:** with ammonium nitrate, chromic acid, hydrogen peroxide, nitric acid, sodium peroxide and halogens.
- **Hydrocarbons:** with fluorine, chlorine, bromine, chromic acid and sodium peroxide.
- **Hydrogen peroxide:** with chromium, copper, iron, most other metals or their salts, flammable liquids and other combustible products, aniline and nitro methane.
- **Hydrogen sulphide:** with fuming nitric acid and oxidizing gases.
- **Iodine:** with acetylene and ammonia.
- **Mercury:** with acetylene, fulminic acid and ammonia.
- **Nitric acid:** with acetic acid, chromic acid, hydrocyanic acid, aniline, carbon, hydrogen sulphide, fluids, gases and other substances that are readily nitrated.
- **Oxygen:** with oils, greases, hydrogen and flammable liquids, solids and gases.
- **Oxalic acid:** with silver and mercury.
- **Perchloric acid:** with acetic anhydride, bismuth and its alloys, alcohol, paper, wood and other organic materials.
- **Phosphorus pent oxide:** with water.
- **Potassium permanganate:** with glycerol, ethylene glycol, benzaldehyde and sulphuric acid
- **Silver :** with acetylene, oxalic acid, tartaric acid and ammonium compounds.
- **Sodium:** with carbon tetrachloride, carbon dioxide and water.
- **Sodium azide:** with lead, copper and other metals. This compound is commonly used as a preservative but forms unstable, explosive compounds with metals. If it is flushed down sinks, the metal traps and pipes may explode when worked on by a plumber.
- **Sodium peroxide:** with any oxidizable substance, e.g. methanol, glacial acetic acid, acetic anhydride, benzaldehyde, carbon disulfide, glycerol, ethyl acetate and furfural
- **Sulphuric acid:** with chlorates, per chlorates, permanganates and water.

17.5 Chemical spills

Most manufacturers of laboratory chemicals issue charts describing methods for dealing with spills. Spillage charts and spillage kits are also available commercially. Appropriate charts should be displayed in a prominent position in the laboratory. The following equipment should also be provided

- Chemical spill kits

- Protective clothing, e.g. heavy-duty rubber gloves, overshoes or rubber boots, respirators
- Scoops and dustpans
- Forceps for picking up broken glass
- Mops, cloths and paper towels
- Bucket
- Soda ash (sodium carbonate, Na_2CO_3) or sodium bicarbonate ($NaHCO_3$) for neutralizing acids and corrosive chemicals
- Sand (to cover alkali spills
- Non-flammable detergent.

The following actions should be taken in the event of a significant chemical spill.

- Notify the appropriate safety officer.
- Evacuate non-essential personnel from the area.
- Attend to persons who may have been contaminated
- If the spilled material is flammable, extinguish all open flames, turn off gas in the room and adjacent areas, open windows (if possible), and switch off electrical equipment that may spark.
- Avoid breathing vapours from spilled material.
- Establish exhaust ventilation if it is safe to do so.
- Secure the necessary items (see above) to clean up the spill.

18. Bio-safety Safety Checklist

This checklist is intended to assist in assessments of microbiological laboratory safety and security status of biomedical laboratories.

- Have guidelines for commissioning and certification been considered for facility construction or post-construction evaluations?
- Is the working space adequate for safe operation?
- Are the circulation spaces and corridors adequate for the movement of people and large equipment?
- Are the benches, furniture and fittings in good condition?
- Is there a hand-washing sink in each laboratory room?
- Are the premises constructed and maintained to prevent entry and harbourage of rodents and arthropods?
- Can access to laboratory areas be restricted to authorized personnel?

Application of protective gloves

Protective laboratory coats / gowns

Face protective mask

18.1 Heating and ventilation air conditioning (HVAC)

- Is there a comfortable working temperature?
- Are blinds fitted to windows that are exposed to full sunlight?
- Are there HEPA filters in the ventilation system?

18.2 Fire prevention and fire protection

- Is there a fire alarm system?
- Are the fire doors in good order?
- Is the fire detection system in good working order and regularly tested?
- Do all exits lead to an open space?
- Are corridors, aisles and circulation areas clear and unobstructed for movement of staff and fire-fighting equipment?
- Is all fire-fighting equipment and apparatus easily identified by an appropriate colour code?
- Are portable fire extinguishers maintained fully charged and in working order, and kept in designated places at all times?

18.3 Electrical hazards

- Are all new electrical installations and all replacements, modifications or repairs made and maintained in accordance with some certificate?
- Does the interior wiring have an earthed/grounded conductor (i.e. a three-wire system)?
- Are circuit-breakers and earth-fault interrupters fitted to all laboratory circuits?
- Do all electrical appliances have testing laboratory approval?
- Are the flexible connecting cables of all equipment as short as practicable, in good condition, and not frayed, damaged or spliced?
- Is each electric socket outlet used for only one appliance (no adapters to be used)?

18.4 Personal protection

- Is protective clothing of approved design and fabric provided for all staff for normal work, e.g. gowns, coveralls, aprons, gloves?
- Are safety glasses, goggles and shields (visors) provided?
- Are appropriate filters provided for the correct types of respirators, e.g. HEPA filters for microorganisms, appropriate filters for gases or particulates?
- Are respirators fit-tested?
- Are persons washing their hands after working with potentially hazardous materials and before leaving the laboratory?
- Is Hand washing protocols rigorously followed?

18.5 Instruction for visitors/lab persons

- Is sign/label for entry of authorized personnel displayed in clean room and bio-safety lab (Level –II) area?
- Are street foot ware removed and separate lab foot ware has been worn by lab persons?
- Are shoe covers, masks, head caps and aprons provided to the visitors at entry point?
- Are all laboratory doors closed after entering or exiting the facility?
- Are Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption not in practice in laboratory areas?
- Are gloves worn to protect hands to prevent injury or direct skin contact with biological materials?
- Are visitors following the complete instructions laid by the safety officer/in charge of the laboratory?

		
Entry of authorized personnel only	Removal of street foot ware is recommended	Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food not allowed

18.6 Health and safety of staff

- Is there an occupational health service?
- Are first-aid boxes provided at strategic locations?
- Are notices prominently posted giving clear information about the location of first-aiders, telephone numbers of emergency services, etc.?
- Are warning and accident prevention signs used to minimize work hazards?
- Are personnel trained to follow appropriate bio-safety practices?
- Are laboratory staffs encouraged to report potential exposures?

18.7 Laboratory equipment

- Are all equipments certified safe for use?
- Are procedures available for decontaminating equipment prior to maintenance?
- Are biological safety cabinets regularly tested and serviced?
- Are autoclaves and other pressure vessels regularly inspected?
- Are centrifuge buckets and rotors regularly inspected?
- Are HEPA filters regularly changed?
- Are pipettes used instead of hypodermic needles?
- Is cracked and chipped glassware always discarded and not reused?
- Are there safe receptacles for broken glass?
- Are plastics used instead of glass where feasible?
- Are sharps disposal containers available and being used?

18.8 Infectious materials

- Are specimens / samples received in a safe condition?
- Are records kept of incoming materials?
- Are specimens unpacked in biological safety cabinets with care and attention to possible breakage and leakage?
- Are gloves and other protective clothing worn for unpacking specimens?
- Are personnel trained to ship infectious substances according to current national and/or international regulations?
- Are work benches kept clean and tidy?
- Are discarded infectious materials removed daily or more often and disposed of safely?
- Are all members of the staff aware of procedures for dealing with breakage and spillage of cultures and infectious materials?
- Is the performance of sterilizers checked by the appropriate chemical, physical and biological indicators?
- Are appropriate disinfectants being used? Are they used correctly?
- Are all laboratory work surface and floor cleaned with disinfectant (70% alcohol) before and after day's work?
- Are all laboratory waste properly decontaminated before discarding i.e. after autoclaving?

18.9 Chemicals and radioactive substances

- Are incompatible chemicals effectively separated when stored or handled?
- Are all chemicals correctly labelled with names and warnings?
- Are chemical hazard warning charts prominently displayed?
- Is mouth pipetting is strictly prohibited?
- Is mechanical pipetting devices are being used?
- Are flammable substances correctly and safely stored in minimal amounts in approved cabinets?
- Are staffs appropriately trained to safely work with radioactive materials?
- Are proper records of stocks and use of radioactive substances maintained?
- Are radioactivity screens provided?

		
Mouth pipetting is strictly prohibited	Radioactive Hazard	No food storage

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