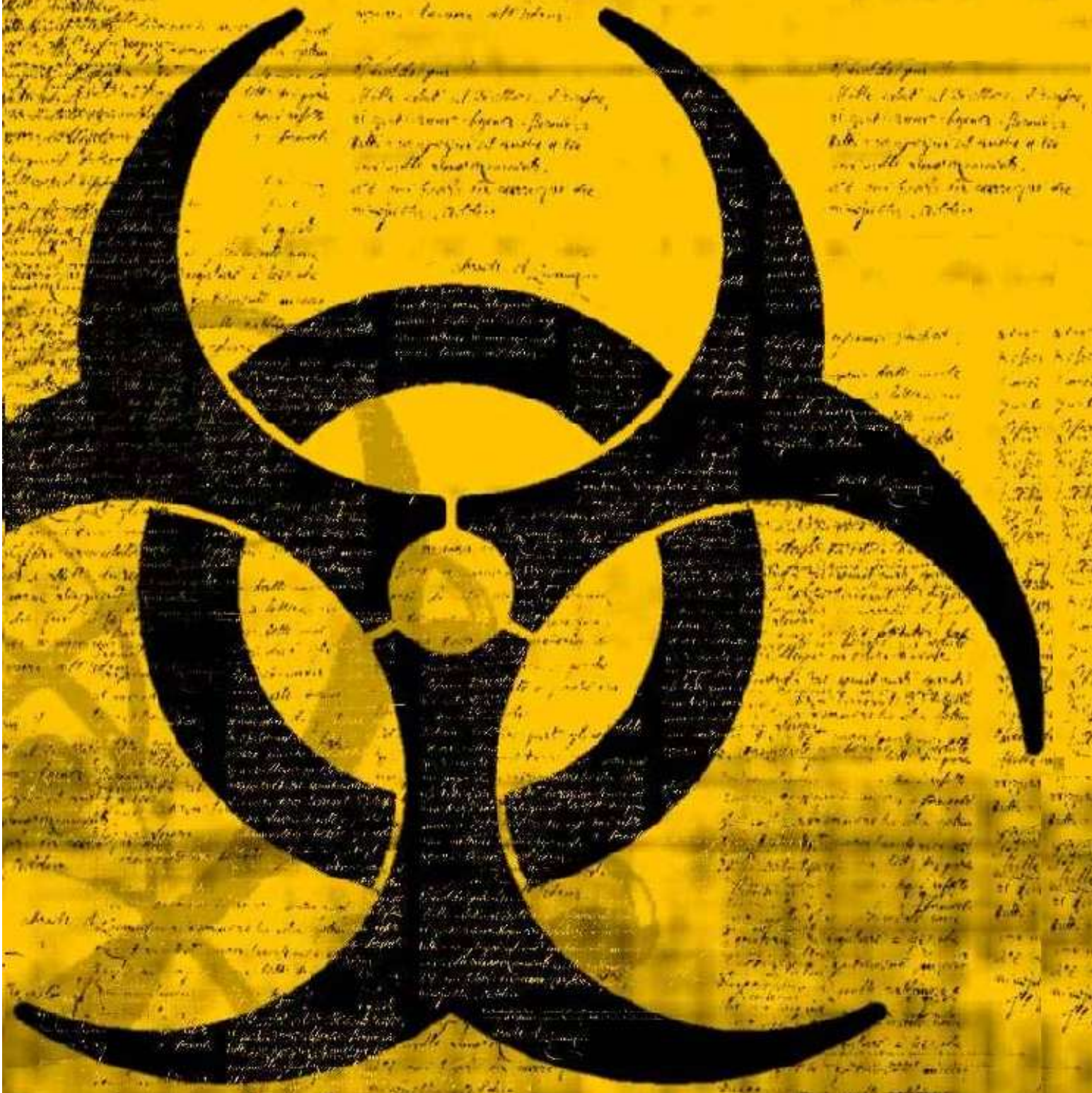
BIOSAFETY MANUAL FOR MEDICALLABORATORIES

SECOND EDITION



SRI LANKA COLLEGE OF MICROBIOLOGISTS
2014

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FOR

MEDICAL LABORATORIES

Second Edition

Sri Lanka College of Microbiologists

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First edition 2002

Second edition 2014

Cover page: Dr. Roshan Jayasuriya

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Sri Lanka College of Microbiologists, 2014

ISBN 978-955-8891-04-9

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Sri Lanka College of Microbiologists

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Biosafety Manual for Medical Laboratories
2014

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FOREWORD

It is with great pleasure that I write the Foreword for the 'Biosafety manual for medical laboratories' reviewed and edited by the Sri Lanka College of Microbiologists. This manual encourages basic concepts in biological safety applicable to medical laboratories in local settings in Sri Lanka.

The first edition of this manual was published in 2002 and it is both appropriate and long overdue for the publication of the second edition. This edition contains several new chapters addressing biological safety and is the outcome of several years of hard work by the working group.

I like to express my sincere gratitude to all contributors who wrote and reviewed chapters.

I wish to congratulate the editors for their commitment and for completion of the task before the end of this year.

I am sure the medical laboratories would find these guidelines appropriate and useful.

Dr. Kumudu Karunaratne
President
Sri Lanka College of Microbiologists
September 2014

PREFACE

It has been more than 12 years since the first edition of “Biosafety Manual for Medical Laboratories” which was published in Sri Lanka by the consultants and senior medical laboratory technologists attached to the Medical Research Institute. Revision of the manual has been a long felt need of the Sri Lanka College of Microbiologists with the expansion of the work in medical laboratories due to the emergence of new infectious agents, availability of new technologies etc.

There has been a significant revision of the previous publication. General designing and organization of a laboratory, laboratory commissioning and certification, safety equipment, biosecurity, biosafety in molecular laboratories and biotechnology procedures are some of the new areas addressed in this issue.

This laboratory manual high lights the importance of continuous training and the team work to minimize the risk associated with laboratory work. The implementation and success of these procedures and the responsibility of the biosafety depends on the combined efforts of all staff categories in the laboratory.

We hope this manual will guide and support establishing a safe working environment in medical laboratories in Sri Lanka.

ACKNOWLEDGEMENTS

The Editors of the Biosafety Manual and the Sri Lanka College of Microbiologists extend their sincere gratitude to all those mentioned below for the guidance and assistance.

- Mrs. Sudharma Karunaratne, Secretary, Ministry of Health
- Dr. P.G.Mahipala, Director General of Health Services, Ministry of Health
- Dr. Ananda Gunasekara, Deputy Director General of Laboratory Services, Ministry of Health
- Dr. Vijith Gunasekara, Director, Laboratory Services, Ministry of Health
- All the contributors
- Ms. Priyanga Opatha for secretarial assistance

Chapter 1

Introduction to Biosafety and Biosecurity

Introduction

A laboratory is a unique environment that requires special practices and containment facilities to protect persons working with pathogenic microbes, chemicals, physical and radioactive hazards.

There are many documented cases of laboratory personnel acquiring diseases due to their work. Most of such published reports were on incidents which occurred during the early part of the twentieth century, associated with contracting infectious diseases such as typhoid, cholera, glanders and brucellosis. Several publications on laboratory acquired infections are available from countries including UK, Denmark and the USA.

The concept of categorizing infectious agents and related laboratory activities into four Risk Groups and four Containment Levels by the Office of Biosafety, Centers for Disease Control and Prevention in 1974 initiated the basis for biosafety in microbiology laboratory practice.

Three main elements of safe containment of infectious agents are good laboratory practices and technique, safety equipment and facility design. These concepts, together with the guidelines for health care workers in the form of standard precautions for blood borne pathogens, have contributed immensely to the safety of the health care worker.

The 'Biosafety Manual for Medical Laboratories' was initially prepared in 2004, by the consultants and senior medical laboratory technologists(MLTT)attached to the Medical Research Institute, Colombo as a biosafety practice manual to be used by all medical laboratories in the country.

This updated 2nd edition of the Biosafety Manual for medical laboratories in Sri Lanka is a long felt need. This manual is intended to supplement and update the knowledge of all health care laboratory workers attached to laboratories throughout the country and of administrators and policy makers who have a responsibility towards laboratory staff.The Biosafety Manual has been developed to guide the all medical laboratories in the state and private sector in their development of biosafety policies and programmes. This also serves as a technical document providing information and recommendations on the design, construction and commissioning of containment facilities.

The manual provides guidance on the use of safe and secure workplace practices, appropriate protective equipment and engineering and administrative controls in the handling of pathogenic microorganisms in the laboratories and during transportation. The expected outcome is to protect

workers, the environment and the community from exposure, infection and subsequent development of disease.

The aim of this biosafety manual is to provide guidance to the workers in the medical laboratories in this country on the principles, objectives and practices of laboratory biosafety, biosecurity and the required infrastructure.

It is the responsibility of all laboratoryworkers,irrespective of their rank or position,toadhere to safety rules and regulations at all times. The managers and laboratory workers should use the information available in this manual to perform their work in an ethical and safe environment in a secure manner.

We hope this manual will achieve its goal of establishing a safe working environment for medical laboratory workers throughout the country, in keeping with the vision of Ministry of Health, Sri Lanka.

Objectives

- To provide guidelines to laboratory staff on the principles, practices and requirements of biosafety
- To enhance awareness of the concept of biosecurity among the laboratory staff

Administration of a Biosafety Programme

Administration of a bio safety programme is through implementation of 4 specific controls. These are engineering or physical facilities, standard operating procedures (SOPs), administrative controls (which include training, immunization of staff, audits and compliance with SOPs) and personal protective equipment (PPE) for exposed staff. Administrative endorsement is essential for implementation of such a programme.

The standards necessary to achieve are in accordance with the Laboratory Biosafety Manual (WHO, 2004) and Laboratory Biosecurity Guidance(WHO, 2006).

Biosafety Committee

All health care institutions should have a biosafety committee, which meets regularly and discusses issues regarding biosafety. Meetings should be held once in 3 months. In events of outbreaks or emergency situations, the committee has to meet more often. Records of the minutes of the meetings should be maintained for5 yearsat least.

The representatives of the biosafety committee are as follows:

- Director of the institution
- Consultant microbiologist
- Biosafety officer
- Senior MLTT from relevant departments
- Infection control nurse
- Maintenance officer

Responsibilities of the committee:

- Formulate policies
- Review existing and proposed new protocols
- Conduct risk assessments
- Plan future activities

Responsibilities of the Director of the institution

- Provision of resources
- Ensure compliance with regulations

Responsibilities of the consultant microbiologist

- Develop and review policies on biosafety
- Development of guidelines
- Supervision of training on biosafety
- Conduct audits
- Appoint the biosafety officer

Responsibilities of the biosafety officer

- Training of staff under the supervision of consultant microbiologist
- Provide continuous education
- Periodic internal biosafety audits
- Investigation and reporting of incidents
- Ensure proper decontamination
    - Spills
    - Equipment prior to servicing
- Ensure proper waste management
- Conduct vaccination programs

Responsibilities of the infection control nurse

- Training on infection control practices
- Implementing protocols and guidelines in infection control
- Surveillance and audit
- Troubleshooting on a daily basis

Responsibilities of the maintenance officer

- Ensure maintenance and repair of safety equipment
- Periodic checks on the emergency eyewash and shower stations
- Ensure proper waste management system

Responsibilities of the senior medical laboratory technologist

- Direct responsibility for compliance
- Ensure training in safety practices
- Supervise performance of the staff
- Report any incidentto the biosafety officer

References

- Biorisk Management: Laboratory Biosecurity Guidance. World Health Organization; September 2006.
- Laboratory Biosafety Manual. 3rd Edition. Malta: World Health Organization; 2004.

Chapter 2

Risk Groups of Microorganisms and Biosafety Levels

Some microorganisms which are present in laboratory specimens are more dangerous to handle and more likely to cause infection in laboratory workers. According to the relative risk of handling, microorganisms are divided in to four Risk Groups. Table I shows the currently used WHO classification of the Risk Groups of microorganisms.

Table I: WHO classification of the Risk Groups of microorganisms (Annexure 1)

Risk Group 1 (no or very low individual and community risk)
A microorganism that is unlikely to cause human or animal disease.
Eg. *Bacillus subtilis*

Risk Group 2 (moderate individual risk and low community risk)
A pathogen that causes human or animal disease but unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection but effective treatment and preventive measures are available and the risk of spread of infection is limited.
Eg. Hepatitis B virus, Human Immunodeficiency Virus (HIV),*Salmonella* spp.

Risk Group 3 (high individual risk and low community risk)
A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are usually available. Eg.*Bacillus anthracis*, Brucella spp., Burkholderia spp., Mycobacterium spp., *Salmonella* Typhi and *Salmonella* Paratyphi, Shigella spp., and endemic fungal species.

Risk Group 4 (high individual and community risk)
A pathogen that usually causes serious human or animal disease and can bereadily transmitted directly or indirectly from one individual to another. Effective treatment and preventive measures are usually not available.
Eg. Ebola virus, Marburg virus

Biosafety or Containment Levels

Different risk groups require different conditions for containment. These are called biosafety or containment levels. The assignment of an agent to a Biosafety Level (BSL) for laboratory work must be based on a risk assessment. Such an assessment will take the risk group as well as other factors into consideration in establishing the appropriate Biosafety Level. For example, an agent that is assigned to Risk Group 2 may generally require BSL-2 facilities, equipment, practices and procedures for safe conduct of work. However, if a particular experiment requires the generation of high-concentration aerosols, then BSL-3 may be more appropriate to provide the necessary degree of safety, since it ensures superior containment of aerosols in the laboratory workplace.

The Biosafety Level assigned for the specific work to be done is therefore, driven by professional judgment based on a risk assessment rather than by automatic assignment of a laboratory Biosafety Level according to the particular risk group designation of the pathogenic agent to be used.

There are four biosafety or containment levels designated 1 – 4.

- Basic – Biosafety Level 1 (BSL-1)
- Basic – Biosafety Level 2 (BSL-2)
- Containment – Biosafety Level 3 (BSL-3)
- Maximum containment – Biosafety Level 4 (BSL-4)

In cases where sufficient information is not available for risk assessment (clinical specimens or epidemiological samples), it is prudent to take a cautious approach to specimen manipulation.

- Standard precautions should always be followed
- Basic containment – BSL-2 practices and procedures should be the minimum requirement
- Transport of specimens should follow national and international rules and regulations

The following information may help in determining the risk of handling these specimens:

- Medical data of the patient
- Epidemiological data (morbidity and mortality data, suspected route of transmission, other outbreak investigation data)
- Information on the geographical origin of the specimen

A laboratory does not have complete control over the specimens it receives and laboratory workers may be exposed to organisms in higher risk groups than anticipated. Therefore, safety guidelines and policies should be strictly adhered to in the laboratory.

In outbreaks of diseases of unknown aetiology, appropriate *ad hoc* guidelines may be generated and posted by national authorities.

Biosafety Level 1

This provides the foundation for all containment laboratories. Biosafety is primarily achieved through a basic level of operational practices (i.e. good microbiological laboratory practices) and physical design features (eg. well-designed, functional laboratory).This is appropriate for handling organisms of Risk Group 1 which do not cause disease in healthy adult humans. Such work can be conducted on an open bench without any containment equipment or facility.

Biosafety Level 2

Diagnostic and healthcare laboratories (public health, clinical or hospital based) must all be designed for BSL-2 or above. This level of safety is applicable to most diagnostic procedures involving clinical specimens containing pathogens of Risk Group 2. Clinical specimens containing a majority of human pathogens may be safely manipulated on the open bench with adherence to standard precautions. However, manipulations which involve techniques with high aerosol potential should be conducted in a biological safety cabinet (BSC). Operational practices for BSL-2 include administrative controls (eg.biosafety programme management, training) and procedures (eg. work practices, PPE use, decontamination) that reduce the risks associated with the activities conducted within the zone. Physical containment features include facility design (eg. location, surface finishes, access control) and biosafety equipment such as primary containment devices (eg. BSC) for certain activities.

Biosafety Level 3

This level is appropriate for diagnostic work involving organisms of Risk Group 3 and a high concentration of Risk Group 2 pathogens. BSL-3 activities should be conducted in a separate room equipped with a class II BSC. Diagnostic work which is likely to produce aerosols and cause infection by the airborne route, are also included in this level. Biosafety and biosecurity at BSL-3 are achieved through comprehensive operational practices and physical containment requirements. BSL-3 requires stringent facility design and engineering controls (eg. inward directional airflow, High Efficacy Particle Air (HEPA) filtration of exhaust air), as well as specialized biosafety equipment (eg. BSCs, centrifuges with sealed rotors), to minimize the release of infectious agents into the surrounding laboratory work area, animal rooms/cubicles, and the environment. BSL-3 requires a high level of operational practices that build on those required at BSL-2 (eg. PPE use, work practices).

Biosafety Level 4

BSL-4 is the highest level of containment available. This is intended for work with organisms in Risk Group 4. The laboratory should be an isolated unit that is functionally and when necessary, structurally independent of all other areas with strictly controlled access. There should bemaximum engineering controls (eg. HEPA filtration of exhaust and supply air), specialized biosafetyequipment(eg. BSC, effluent treatment systems) and other biosafety features (eg. two stages of HEPA filtration of exhaust air). BSL-4 requires the maximum level of operational practices (eg. PPE, work practices, medical surveillance) that build on those required at BSL-3.

Table 2: Relationship of Risk Groups to Biosafety Levels, practices and equipment

Risk Group	Biosafety Level	Laboratory Type	Laboratory Practices	Safety Equipment
1	Basic-Biosafety Level 1	Basic teaching, research	GMT	None, open bench work
2	Basic-Biosafety Level 2	Primary health services, diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus BSC for potential aerosols
3	Containment - BiosafetyLevel 3	Special diagnostic services, research	As Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4	Maximum containment – BiosafetyLevel 4	Dangerous pathogen units	As Level 3 plus airlock entry, shower exit, special waste disposal	Class III BSC or positive pressure suits in conjunction with Class II BSCs, double ended autoclave (through the wall), filtered air

BSC- biological safety cabinet, GMT- good microbiological techniques

Source: Laboratory Biosafety Manual,WHO (2004)

Table 3: Basic requirements for Biosafety Levels

Biosafety Level	**1**	**2**	**3**	**4**
Isolation[a] of laboratory	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation: • inward air flow • controlled ventilation system • HEPA filtered air exhaust	 No No No	 Desirable Desirable No	 Yes Yes Yes/No[b]	 Yes Yes Yes
Double-door entry	No	No	Yes	Yes
Airlock	No	No	No	Yes
Airlock with shower	No	No	No	Yes
Anteroom	No	No	Yes	-
Anteroom with shower	No	No	Yes/No[c]	No
Effluent treatment	No	No	Yes/No[c]	Yes
Autoclave: • on site • in laboratory room • double-ended	 No No No	 Desirable No No	 Yes Desirable Desirable	 Yes Yes Yes
Biological safety cabinets	No	Desirable	Yes (2A)	Yes
Personnel safety monitoring capability[d]	No	Desirable	Yes	Yes

[a]Environmental and functional isolation from general traffic
[b]Dependent on location of exhaust
[c]Dependent on agent(s) used in the laboratory
[d]Window, closed-circuit television, two-way communication

Source: Laboratory Biosafety Manual,WHO (2004)

References

- Biosafety in Microbiological and Biomedical Laboratories. 5th Edition. USA: HHS Publication No. (CDC) 21-1112; 2009.
- Laboratory Biosafety Manual. 3rd Edition. Malta: World Health Organization; 2004.

Chapter 3

General Designing and Organization of a Laboratory

Laboratory Design and Facilities

In designing a laboratory and when assigning certain types of work, special attention should be paid to work related conditions/activities that are known to pose safety problems.
These include:

- Formation of aerosols
- Work with large volumes and/or high concentrations of microorganisms
- Overcrowding and too much equipment
- Infestation with rodents and arthropods
- Inappropriate workflow

Laboratory Design

- Adequate space must be provided for safe conduct of laboratory work and for cleaning and maintenance. It is recommended that each laboratory worker is assigned 50ft2 of bench space and 150-200 ft^2 of floor space within a laboratory to provide a safe work area. Ideally, a minimum space of 5 feet should be allowed between the workers and any object behind the worker to provide reasonable maneuverability.

- In the planning of new facilities, consideration should be given to the provision of mechanical ventilation systems that provide an inward flow of air without recirculation. If there is no mechanical ventilation, it should be possible to open the windows, which should be fitted with arthropod-proof screens.

- Airflow within the microbiology laboratory should be directional airflow from the corridor into the microbiology laboratory and from there into separate and enclosed specialized laboratories such as tuberculosis, mycology and virology.

- Walls, ceilings and floors should be smooth, easy to clean, impermeable to liquids and resistant to the chemicals and disinfectants normally used in the laboratory. They should be painted with washable, non-porous paint.

- Doors should have vision panels and self-closing and preferably be fire proof.

- Floors should be non-slippery.

- Illumination should be adequate for all activities. Undesirable reflections and glare should be avoided.

- There should be a reliable and adequate electricity supply and emergency lighting to permit safe exit. A stand-by generator is recommended for the support of essential equipment, such as incubators, biological safety cabinets, freezers, etc., and for the ventilation of animal cages. Electrical outlets are recommended at each work station.

- A dependable supply of good quality water is essential. There should be no cross connections between sources of laboratory and drinking water supplies. An anti-backflow device should be fitted to protect the public water system.

- Wash basins with running water should be provided in each laboratory room, preferably near the exit door. Hands-free sinks (foot-pedal operated) are required for BSL-3 facilities and are recommended for BSL-2 facilities.

- There should be a reliable and adequate supply of gas.

- Work benches that have storage shelves above the center of the bench are preferred. Laboratory shelving should NOT be installed at heights which require workers to reach 30 cm (1 foot) above shoulder height when standing on the floor or on a 30 cm step stool.

- Bench tops should be impervious to water and resistant to disinfectants, acids, alkalis, organic solvents and moderate heat. The laboratory should be designed so that it can be easily cleaned. Bench tops must be a seamless one piece design to prevent contamination. Laminated bench tops are not suitable. Openings for electrical, plumbing and gas must be completely and permanently sealed. If the bench abuts a wall, connection to the wall has to be seamless or the junction must be sealed.

- Laboratory furniture should be sturdy.

- Open spaces between and under benches, cabinets and equipment should be accessible for cleaning.

- Storage space must be adequate to hold supplies for immediate use, to prevent clutter on bench tops. Additional long term storage space should be located outside the laboratory working areas.

- Space and facilities should be provided for the safe handling and storage of solvents, radioactive materials and compressed and liquefied gases.

- The laboratory must have a dedicated autoclave for on-site decontamination of waste before disposal. Locate the autoclave in a well -ventilated area or ensure it is exhausted through a capture hood above it. A dedicated drainage system for disposal of laboratory waste is recommended.

- At BSL-2, an autoclave or other means of decontamination should be available in appropriate proximity to the laboratory.

- Safety systems should cover fire, electrical emergencies, emergency shower and eyewash facilities.

- First aid areas or rooms suitably equipped and readily accessible should be available.

- Facilities for eating, drinking, resting and storing outer garments and personal items should be provided outside the laboratory working areas.

Specialised Laboratories

- The laboratory should be separated from the areas of general traffic
- There should be double-door entry with airlock
- Airlock with shower is preferred for BSL-3
- Controlled ventilation system with inward airflow and HEPA filtered air exhaust system is recommended
- Windows must be closed, sealed and break resistant
- The laboratory room must be sealable for decontamination. Air-ducting systems must be constructed to permit gaseous decontamination
- Surfaces of walls, floors and ceilings should be water resistant and easy to clean. Openings through these surfaces (eg. for service pipes) should be sealed to facilitate decontamination of the room(s)
- Facilities must be available for effluent treatment

- Autoclave for the decontamination of contaminated waste material should be available in the containment laboratory. A double-ended autoclave is needed and it should be placed in a dedicated area
- If infectious waste has to be removed from the containment laboratory for decontamination and disposal, it must be transported in sealed, unbreakable and leak proof containers according to national or international regulations, as appropriate
- Personnel safety monitoring should be in place depending on the work carried out

References

- Biosafety in Microbiological and Biomedical Laboratories. 5th Edition. USA: HHS Publication No. (CDC) 21-1112; 2009.
- Laboratory Biosafety Manual. 3rd Edition. Malta: World Health Organization; 2004.
- Miller JM, Astles R, Baszler T, Chapin K, Carey R. et al. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. Recommendations of a CDC-convened, Biosafety Blue Ribbon Panel. USA: Centers for Disease Control and Prevention, MMWR Supplement; January 6, 2012;61(01):1-101.
- National guidelines on laboratory safety. Ministry of Healthcare and Nutrition, Sri Lanka; 2007.

Chapter 4

Laboratory Commissioning and Certification

Laboratory Commissioning

Laboratory commissioning is the systematic review and documentation process verifying that specified laboratory structural components, systems and/or system components have been installed, inspected, functionally tested and verified according to national or international standards. Laboratories designated as BSL 1–4 will have different and increasingly complex commissioning requirements. Geographical and climatic conditions also have to be considered in laboratory commissioning.

The commissioning process and acceptance criteria should be established early, preferably during the planning phase of the construction and it should be continued during the warranty period. This provides the institution and the surrounding community with a greater degree of confidence that the structural, electrical, mechanical and plumbing systems, containment and decontamination systems and security and alarm systems will operate as designed.

Laboratory commissioning is conducted by a team consisting of the head of the institution, consultant microbiologist, the institution's biosafety officer, senior MLT and a representative of the maintenance staff. They should ensure that the finished containment zone, equipment and containment systems will operate in accordance with the specifications.

The following laboratory systems and components should be included in a commissioning plan depending on the containment level of the facility.

Ventilation

- Heating, ventilation (supply and exhaust) and air conditioning (HVAC) systems
- HEPA filtration systems
- HEPA decontamination systems
- HVAC and exhaust air system controls and control interlocks
- Air-tight isolation dampers
- Airlock door control interlocks
- Air-tight door seals
- Breathing air systems

- Cascading pressure differential verification of laboratories and support areas
- Barrier pass-through penetration

Power supply

- Normal power systems
- Emergency power systems
- Emergency lighting systems
- Lighting fixture penetration seals
- Electrical and mechanical penetration seals
- Telephone systems

Water

- Domestic water backflow prevention devices
- Processed water systems (i.e. reverse osmosis, distilled water)
- Water detection systems (eg. in case of flooding inside containment zone)

Gas supply

- Medical laboratory gas systems
- Liquid nitrogen system and alarms

Laboratory instruments

- Laboratory refrigeration systems
- Boilers and steam systems
- Fire detection, suppression and alarm systems
- Biological safety cabinets
- Autoclaves

Waste management system

- Waste management
- Liquid effluent treatment and neutralization systems
- Chemical decontaminant systems

Others

- Decontamination shower and chemical additive systems
- Local area network (LAN) and computer data systems
- Structural integrity verification: concrete floors, walls and ceilings
- Barrier coating verification: floors, walls and ceilings

Laboratory Certification

This is a regular on-going quality and safety assurance activity by systematic examination of all safety features and processes within the laboratory (engineering controls, personal protective equipment and administrative controls).

All laboratories should be regularly certified and will have to ensure the following:

- Proper engineering controls are being used and are functioning adequately as designed
- Appropriate site and protocol specific administrative controls are in place
- Personal protective equipment is appropriate for the tasks being performed
- Decontamination of waste has been adequately considered and proper waste management procedures are in place
- Proper procedures for general laboratory safety, including physical, electrical and chemical safety are in place

Institutions may employ personnel with appropriate skills required for conducting audits, surveys or inspections associated with the certification process. Findings of the audit, survey or inspection should be discussed with laboratory staff and management. Certification of the laboratory is not completed and the laboratory should not be declared functional until deficiencies have been adequately addressed.

References

- Biosafety in Microbiological and Biomedical Laboratories. 5th Edition. USA: HHS Publication No. (CDC) 21-1112; 2009.
- Laboratory Biosafety Manual. 3rd Edition. Malta: World Health Organization; 2004.

Chapter 5

Good Laboratory Practices

Most laboratory injuries and work-related infections are caused by human error, poor laboratory techniques and misuse of equipment. Good laboratory practices will minimize such injuries and infections.

Safety procedures and precautions are meant to

1. restrict micro-organisms present in specimens or cultures to the container in which they are carried so that the personnel and the environment are protected from the harmful effects of the micro-organisms
2. prevent environmental micro-organisms (normally present on hands, hair, clothing, laboratory benches or in the air) from contaminating the specimens or cultures and thus interfering with the results

Safety procedures to be adopted in the laboratory should be aimed at

- protecting the staff
- protecting the clinical samples
- protecting the environment and general public

GeneralMeasures for Laboratory Safety

- Proper layout of the laboratory, enabling each activity to be carried out in a designated area
- Restricted entry
- Adhere to all rules and recommendations in the laboratory
- Be concernedabout safety
- Be alert for unsafe conditions
- Take effective and corrective actions immediately
- Proper disposal of waste
- Availability of first aid
- Report all accidents to the supervisor and/or safety officer
- Availability of fire extinguishers and alarms

General rules that should be observed in the laboratory

- Do not smoke, eat or drink inside the laboratory
- Do not apply cosmetics or contact lensesinside the laboratory
- Flat-heeled, covered foot wear is preferable
- Wear laboratory coats/gowns within the laboratory and remove before leaving
- Cover cuts and abrasions with non-absorbent dressings
- Observestandard precautions when handling potentially infectious material
- Wear gloves during handling and processing of specimens
- Mouth pipetting is strictly prohibited
- Wash hands after handling infectious material and before leaving the laboratory
- All containers containing infectious material should be clearly labelled and dated
- Keep paper work (specimen request forms, reports, books etc.) away from infected areas
- Do not store food items in the laboratory refrigerators

Laboratory access

- Universal biohazard symbol should be displayed at special laboratories
- Access to the laboratory must be restricted to authorized staff only
- Appropriate signs should be displayed directing all visitors to a reception area

Work area

- Keep the work area clean and tidy
- Decontaminate at least once a day after work and whenever any infectious material is spilt

Clothing

- Laboratory coats should be worn while working
- Open backed, full button down coats with long sleeves are recommended
- Laboratory coats should not be worn outside the laboratory area
- Wash hands after removing the coat
- Laboratory coats should be cleaned regularly

Procedure Related Biosafety Measures

Specimen handling

- All specimens and laboratory materials must be regarded as potentially hazardous and dangerous. All procedures and manipulations of potentially infectious material should be performed carefully to minimize creation of droplets and aerosols

- Specimen containers may be of glass or plastic. Secondary containers, such as boxes, should be used to avoid accidental leakage or spillage. They should be fitted with racks so that the specimen containers remain upright
- Personnel who transport, receive and unpack specimens should be aware of the potential health hazards and should be trained in standard precautions
- Stoppers from vacuum collecting tubes must be removed carefully to prevent generation of aerosols
- Separation of serum
    - Wear laboratory coats, gloves and eye protection
    - Carefully pipette but do not pour blood and serum
    - Minimize splashes and aerosols by gentle handling
    - Completely submerge pipettes in 0.1% sodium hypochlorite solution after use and leave for 20-30 minutes before disposal or washing and sterilization for reuse
    - Place the discarded specimen tubes containing blood clots (with caps replaced) in suitable leak proof containers for autoclaving and/or incineration
    - Clean any splashes and spillages with suitable disinfectants (Refer page 42)
- Safety precautions for contaminated/leaking specimens
    - Seek advice from the consultant in charge and the senior MLTbefore attempting retrieval
    - Label another suitable container with the patient's details
    - Wear disposable gloves and place contaminated container on a tray and place in biosafety cabinet. Remove cap/lid and transfer remaining specimen to the labelled new container using a Pasteur pipette
    - Dispose of the soiled container and lid in a biohazard bag
- Contaminated request forms
    - Wear disposable gloves for handling
    - Put contaminated forms in biohazard bag or transparent plastic sheet protector
    - Write details in a separate sheet for working purposes

Bacteriological transfer loops

- Microbiological transfer loops should have a diameter of 2–3 mm and be completely closed to avoid the premature shedding of their loads. The shanks should not be more than 6 cm in length to minimize vibration
- In an open bench, transfer loops should be held in a vertical position to the flame of a Bunsen burner until red hot, for disinfection
- Inside a BSC
    - Disposable transfer loops are preferable
    - If the BSC has the in-built facility for flaming, reusable transfer loops can be used
    - Bunsen burners cannot be used in a BSC due to disruption of airflow

- Disposable loops should be discarded in to 2% phenol or 0.1% sodium hypochlorite after use and discarded as clinical waste

Use of pipettes and pipetting aids

- Pipetting by mouth is strictlyprohibited. A pipetting aid should be used
- Syringes with hypodermic needles should not be used for pipetting
- Pipettes should be plugged with cotton wool to reduce contamination of devices
- Avoid blowing through liquid containing infectious agents
- Do not mix infectious materials by alternate suction and expulsion through a pipette
- Do not forcibly expel material from a pipette
- Do not bubble air through liquids using a pipette
- To avoid dispersion of infectious material dropped from a pipette, an absorbent material should be placed on the working surface and it should be disposed as infectious waste after use
- Completely submerge the contaminated pipettes in 0.1% sodium hypochlorite for 20-30 minutes before disposal or re-sterilization

Fixed smears for microscopy

- Take care when drying sputum smears to avoid creating aerosols
- Fixing and staining of smears (eg. blood, sputum and faecal samples) for microscopy does not necessarily kill all infectious organisms. Smears should be handled as infectious samples.Handle them with forceps, store appropriately and decontaminate and/or autoclave before disposal

Avoiding ingestion of and contact with infectious materials

- Large particles and droplets (>5µm in diameter) released during microbiologicalmanipulation settle rapidly on bench surfaces and on the hands of the operator
- Wear disposable gloves and avoid touching mouth, eyes and face
- Do not place articles in the mouth (pens, pencils, etc.)while in the laboratory
- Shield or protect the face, eyes and mouth during any operation that may result in the splashing of potentially infectious materials

Avoiding injection of infectious materials

- Accidental injection may result from sharps injuries eg. with hypodermic needles, glass Pasteur pipettes or broken glass. Perform procedures carefully, in order to prevent accidental

inoculation resulting from broken or chipped glassware

- Discard disposable sharp articles directly after use into puncture-proof/puncture-resistant containers fitted with covers (sharps bin)

Handling syringes and needles

- Minimize the use of syringes and needles (eg. pipettes can be used - instead of syringes and needles).Engineered sharp safety devices can be used instead of syringes and needles
- Disposable syringes and needles or sterilized glass equipment should be used for venepuncture and aspiration of fluids from patients
- Wear gloves during the procedure
- Avoid quick and unnecessary movements while holding the syringe
- Make sure the needle is secured properly
- Do not bend, recap or remove the needle from syringe by hand
- Used needle and syringe should be promptly placed in a sharps bin (Refer page 47)

Opening of ampoules and containers with infectious materials

- Care should be taken when vials, flasks, petri dishes, culture tubes or embryonated eggs containing infectious materialare opened, as the contents may be under reduced pressure and the sudden inrush of air may disperse some of the materials into the atmosphere
- Ampoules should always be opened in a biological safety cabinet
- Wear a laboratory coat and gloves
- Open containers with clinical specimens in a well lit and designated area
- Use absorbent paper towels to facilitate clean up and reduce aerosols
- Decontaminate the outer surface of the ampoule. Hold the ampoule in alcohol-soaked cotton to protect hands. Remove the top gently and treat as contaminated material

Use of biological safety cabinets – refer chapter 6

Use of centrifuges

- Equipment must be used according to manufacturer’s instructions
- Staff must be adequately trained prior to using the equipment
- Centrifuges should be placed at a level where the interior can be visualized
- Buckets suitable for the centrifuge tubes should be used
- The buckets must be loaded, equilibrated, sealed and opened in a biological safetycabinet
- Sealable centrifuge buckets (safety cups) must be used for micro-organisms in Risk Groups 3 and 4
- Buckets and rotors should be inspected regularly for damage and wear

- Thick walled glass or plastic centrifuge tubes must be used. These have to be inspected for damage before use
- Maintain appropriate speed to minimize aerosolization of infectious agents
- Buckets should be stored in an inverted position
- Tube compartments and all accessible surfaces should be cleaned weekly with non-corrosive disinfectant (eg. 3%-5% phenolic disinfectant)

Use of homogenizers, shakers, blenders and sonicators

- Laboratory blenders and stomachers should be used. Do not use domestic homogenizers in laboratory as they may leak or release aerosols
- Ensure that caps and cups or bottles are in good condition and free from flaws or distortion. Caps should be well-fitting and gaskets should be in good condition
- Pressure builds up in the vessel during the operation of homogenizers, shakers and sonicators. Aerosols containing infectious materials may escape from between the cap and the vessel. Plastic vessels are recommended as glass may break, releasing infectious material
- Cover the homogenizers, shakers and sonicators with a strong plastic casing while in use and disinfect it after use
- Open the containers in a biological safety cabinet at the end of the operation
- Provide hearing protection for people using sonicators

Storage of biohazardous/infectious material

- All incubation, refrigeration, cold rooms and freezer units used for storage of infectious material must display the biohazard symbol, contents and limitation of storage
- Contact details of the consultant or senior MLT should be displayed near or on the storage units
- All biohazard or infectious material should be appropriately stored in designated areas
- All biohazard or infectious material must be covered or stored in suitable containers to prevent the possibility of any leakage, spillage or the formation of airborne particles
- All containers should be shelved safely and excessive stacking should be avoided
- All specimens that pose a high risk should be incubated, refrigerated and stored in sealed, locked containers that are clearly labelled
- All containers with biohazard and infectious material must be labelled with
    - Name/description of content
    - Hazard warning
    - Handling instructions
    - Date of preparation and storage
    - Contact number and name of person responsible for the stored material

Use of refrigerators, low temperature freezers and ultra-low temperature freezers

- Ensure all installation requirements have been met
    - An electrical connection with a ground pole appropriate to the voltage and frequency of the equipment
    - If more than one unit is installed to the same electrical circuit, verify that the capacity is adequate for supplying the required amount of power
    - Never connect a unit to an overloaded outlet or one with voltage deficiencies
    - Leave free space around the equipment (15cm at sides and at the back) to facilitate ventilation
    - Avoid installing under direct sunlight or near a heat source
    - When activating the unit, wait for the unit to reach the desired temperature before storing any product
    - Select the temperature at which the alarm should be activated
- Load the unit as per capacity established by the manufacturer (allow for free air circulation to maintain uniform temperature)
- Label all containers stored in refrigerators clearly with the scientific name of the contents, the date stored and the name of the individual who stored them
- Maintain an inventory of the freezer's contents
- Wear appropriate personal protective equipment (laboratory coat, dry insulated gloves) when retrieving items
- Avoid keeping the door open for long periods
- Maintain a temperature chart daily or twice daily, if necessary (validate with a calibrated thermometer every six months)
- Clean the interior every quarter. Defrost and clean every 6 months
- Wear face protection and heavy duty rubber gloves during cleaning. After cleaning, the inner surfaces of the refrigerator should be disinfected
- Clean the condenser every 6 months and verify the door gasket quarterly
- Remove any ampoules or tubes that have broken during storage
- Autoclave and discard the unlabelled and obsolete materials

Storage in liquid nitrogen (LN)

- Laboratory staff should receive special training to handle LN canisters
- Store LN canisters in a well-ventilated area at room temperature
- Maintain records on LN levels and dates of refilling
- Do not overfill the canisters, splash or spill LN
- Use a wheeled platform when transporting LN canisters
- Ampoules should be stored only in the gaseous phase above the liquid nitrogen. They should never be immersed in liquid nitrogen because cracked or imperfectly sealed ampoules may break or explode on removal

- Wear appropriate personal protective equipment (laboratory coat, face shield, dry insulated gloves) when handling canisters
- The outer surfaces of ampoules should be disinfected when they are removed from storage
- Transport storage vials using a rack and a secondary container
- Do not warm frozen vials in your hand
- Keep records of inventory of vials in LN storage (cell type, canister no, cane no, vial no, date of freezing, date thawing, name of the responsible person etc.)

References

- Biosafety in Microbiological and Biomedical Laboratories. 5th Edition. USA: HHS Publication No. (CDC) 21-1112; 2009.
- Laboratory Biosafety Manual. 3rd Edition. Malta: World Health Organization; 2004.

Chapter 6

Safety Equipment

Hazardous aerosols are an important source of infection. Aerosols can be generated by many laboratory operations such as blending, mixing, grinding, shaking, stirring, sonication and centrifugation of infectious materials. Therefore, care should be taken to prevent the formation and dispersion of aerosols.

Even when safety equipment issued, these procedures should be carried out in an appropriate biological safety cabinet, whenever possible. The operator should be adequately trained in the proper handling of the equipment to prevent or minimize the risk of transmission of infection. Also the equipment should be tested and certified on a regular basis for safety and performance.

6.1. Biosafety Cabinets

Biological safety cabinets (BSC) are designed to protect the operator and laboratory environment from exposure to infectious aerosols and splashes that may be generated while handling infectious agents. The laboratory staff should read and follow the manufacturer's instructions and recommendations. They should be educated about the use and limitations of biological safety cabinets and be aware that the cabinet will not protect the operator from spillage, breakage or poor technique.

- **Location** – The BSC should be located in an area remote from traffic and more than 10 feet away from the door way and potentially disturbing air currents. A 30cm clearance should be provided behind and on each side of the cabinet. A clearance of 30–35cm above the cabinet is required for accurate air velocity measurement and for exhaust filter changes.

- **Standard operating procedures** - Standard operating procedure guidelines should be printed and placed near the cabinet for referral.

- **Planning of work** – Prepare a checklist of the required material and place them inside the cabinet before starting work. This is to reduce interruption of air currents.

- **Operation –** The cabinet should be turned on for 5 minutes before beginning work and after completion of work to allow contaminated air to be removed from the cabinet environment.

Check gauges and monitors to ensure that the equipment is functioning properly before commencement of work.

- **Use of biological safety cabinets**

    - Do not use the cabinet unless it is working properly
    - Do not open the glass viewing panel when the cabinet is in use
    - Avoid using Bunsen burners in the cabinet as the heat will distort the airflow and may damage the filters. An electric micro-incinerator is permissible, but the use of sterile disposable instruments is advisable
    - Do not block the air grills with notes, pipettes or other materials, as this will disrupt the airflow causing potential contamination of the material and exposure of the operator

- **Material placement –** Keep only materials actively in use inside the work area. The surface of equipment and reagent containers to be used inside the cabinet should be decontaminated with 70% alcohol before placing inside the BSC. Work may be performed on absorbent towels soaked with disinfectant to capture splatters and splashes.

  All material should be placed as far back in the cabinet, towards the rear edge of the work surface, without blocking the rear grill. Aerosol generating equipment (eg. mixers, centrifuges etc.) should be placed towards the rear of the cabinet. Sterile equipment and contaminated equipment should be placed separately inside the BSC. Bulky items, such as biohazard bags, discard pipette trays and suction collection flasks should be placed to one side of the interior of the cabinet.

- **Personal protective equipment –** Along sleeved laboratory coat should be worn when working in the BSC. Gloves should be pulled over the wrists of the gown. Masks and safety glasses should be used as and when required.

- **Work inside the cabinet –** Work should be carried out at least 4 inches from the front grill or rear part of the working surface. The procedure should be visible to the operator through the viewing panel. Wait for about 1 minute after placing hands and arms inside the cabinet before starting the procedure. Movements inside the cabinet should be minimal, slow and unhurried so as not to disturb the air currents. The operator should keep the head out of the hood/cabinet while working. The work should flow from clean to contaminated areas across the work surface.

- **Ultraviolet lights** - Ultraviolet lights are not required in BSCs. If they are used, they must be cleaned weekly to remove any dust and dirt. These must be turned off while the room is occupied, to protect eyes and skin from inadvertent exposure. The light intensity should be checked when the cabinet is recertified to ensure that light emission is appropriate.

- **Spills –** The spill should be cleaned up according to protocol (page no 42) The BSC should be kept switched on during the procedure.

- **Cleaning and disinfection -** All items within the BSC should be decontaminated prior to removal, when work is completed, while the cabinet is running. The interior surfaces of BSCs should be wiped with 70% alcohol after completion of work, including the work surface, back and interior of glass.

- **Maintenance -** All repairs of BSCs should be done by a qualified technician. Any malfunction in the operation should be reported and repaired as soon as possible. BSCs must be decontaminated by a qualified professional before filter changes and before being moved.

Types of Biological Safety Cabinets

Class 1 BSC

This is an open fronted ventilated cabinet that provides personal protection to the operator but not the product. Room air is drawn in through the front opening at a minimum velocity of 0.38 m/s, which passes over the work surface. This air is not circulated but expelled through a HEPA filter to protect the environment. This type is usually used when handling toxic chemicals and radioactive material.

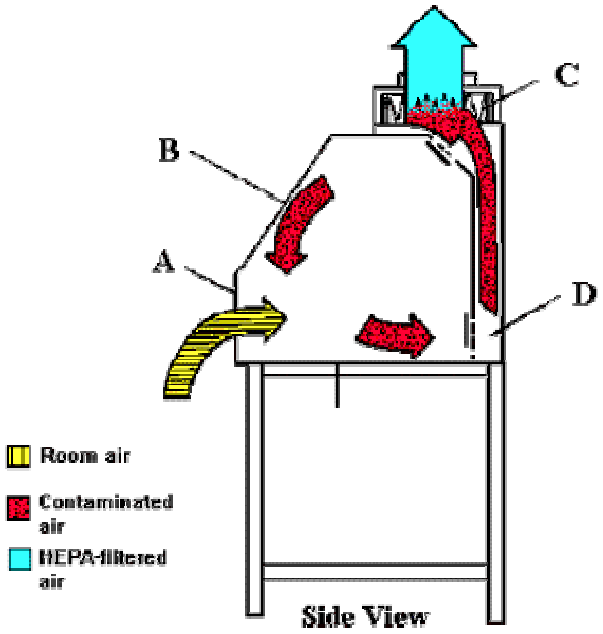

Figure 1: Class 1Biological Safety Cabinet

Class II BSC

This is designed to provide personnel and environmental protection and also to protect work materials from contaminated room air. All Class II cabinets are designed for work involving microorganisms assigned to Biosafety Levels 1, 2 and 3. They are suitable for cell culture propagation and may also be used for the formulation of non-volatile anti-neoplastic or chemotherapeutic drugs.

The Class II biological safety cabinet has three key features

A. A front access opening with carefully maintained inward airflow

B. HEPA-filtered, vertical, unidirectional airflow within the work area

C. HEPA-filtered exhaust air to the room or exhaust to a facility exhaust system

Class II cabinets have been sub-classified to Class A1, A2, B1 and B2 based on airflow patterns, velocities, HEPA air filter position, ventilation rates and exhaust methods.

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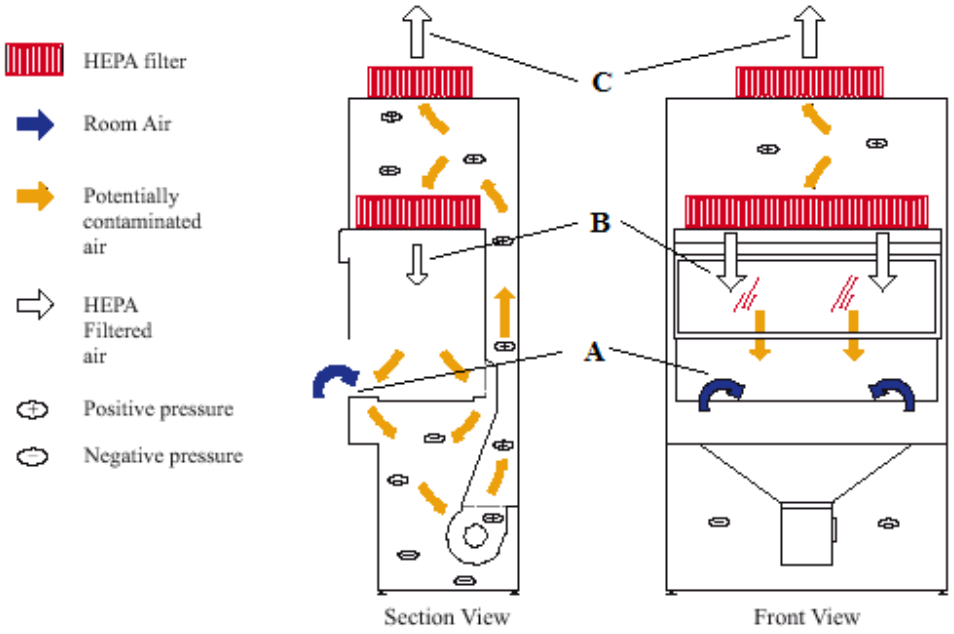


Figure 2:Class II Biological Safety Cabinet

Class II type A1 BSC

This type is designed to provide a minimum airflow velocity of 0.38 m/s. Any aerosol particles generated at the work surface are immediately captured and the highest level of product protection is provided. About 70% of the air re-circulates through the supply HEPA filter back into the work zone; the remaining 30% passes through the exhaust filter into the room or to the outside.

Class II type A2BSC

The Class II Type A2 BSC (formerly A/B3), has a minimum inflow velocity of 0.5m/s. A negative air pressure plenum surrounds all contaminated plenums that are under positive pressure. All the other specifications including air circulation are identical to those of a Type A1 cabinet.

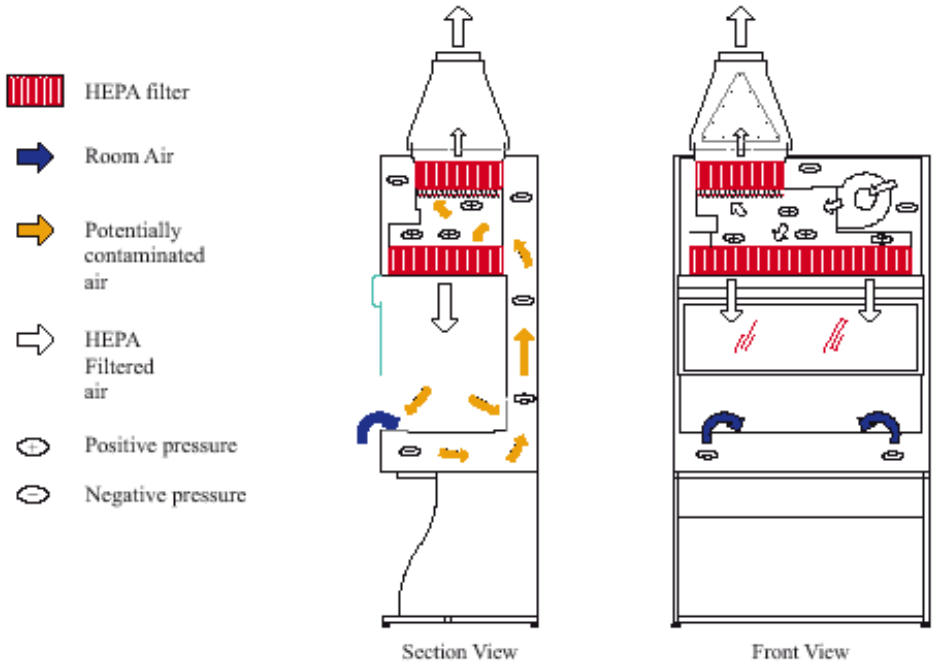


Figure 3: Class II type A2 Biological Safety Cabinet

Class II type B1BSC

The Class II Type B1 BSC has a minimum inflow velocity of 0.5m/s. Compared to type A1 and A2 cabinets, 60% of air from the rear grille is exhausted and only 40% is recirculated. Since exhaust air is drawn from the rear grille, work with chemicals should be conducted in the rear of the cabinet. All biologically contaminated plenums are surrounded by negative pressure plenums.

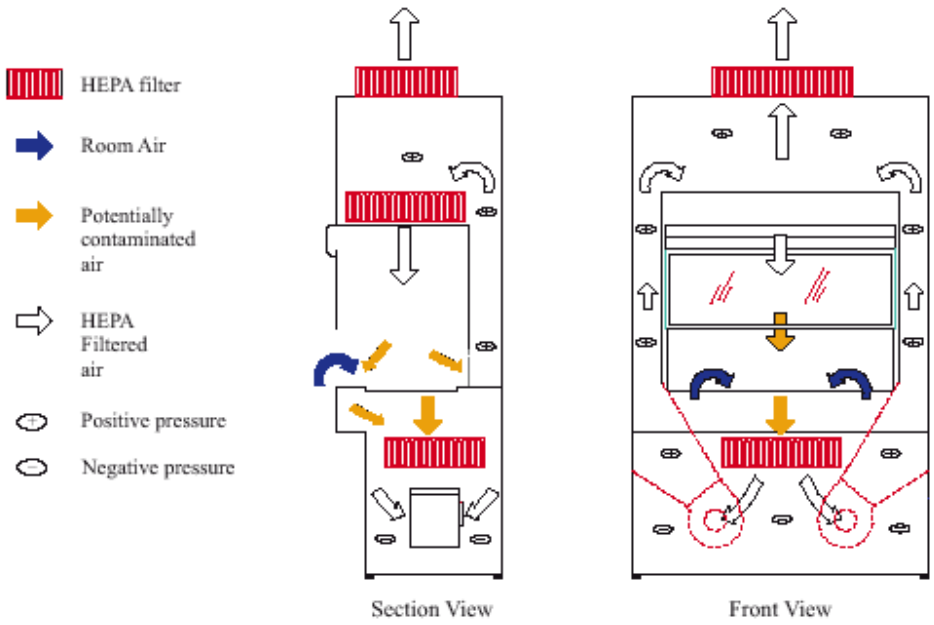


Figure 4: Class II type B1 Biological Safety Cabinet

Class II type B2BSC

This type has a minimum inflow velocity of 0.5m/s with total exhaust and without recirculation of air. Therefore, this type is mainly found in toxicology laboratories, where the ability to safely use hazardous chemicals is important. All ducts and plenums are under negative pressure or surrounded by directly exhausted negative pressure ducts or plenums.

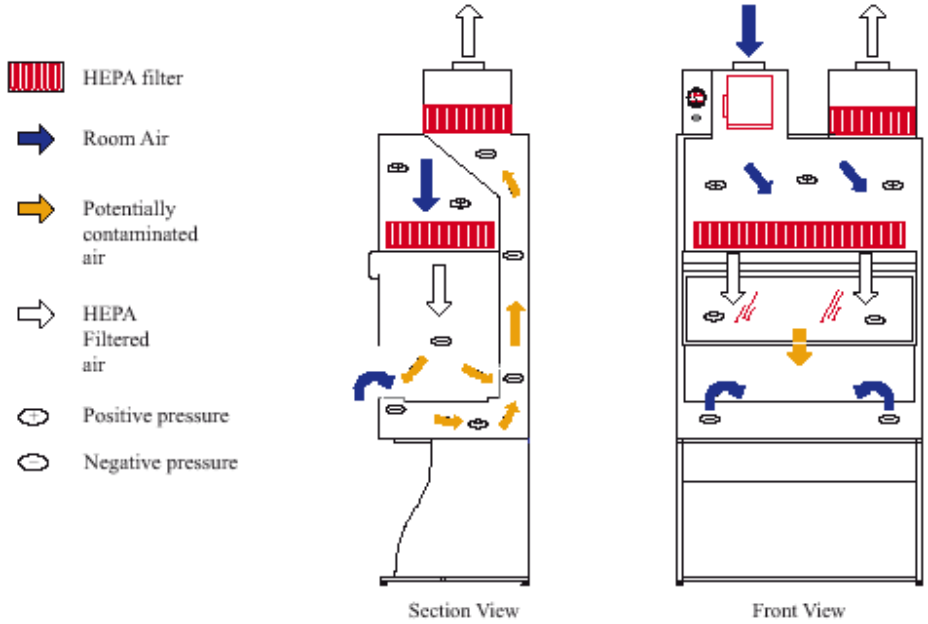


Figure 5: Class II type B2 Biological Safety Cabinet

Class III BSC

A class III BSC provides highest level of personnel protection. This type is generally only installed in maximum containment laboratories and specifically designed for work with BSL-4 pathogenic agents.

It is totally enclosed, maintained under negative pressure, supply air is HEPA filtered and exhaust air passes through two HEPA filters. The work is done through long sleeved rubber gloves attached to ports in the cabinet. All materials enter and leave through a connected double-door autoclave. Flammable gases should not be used in these cabinets.

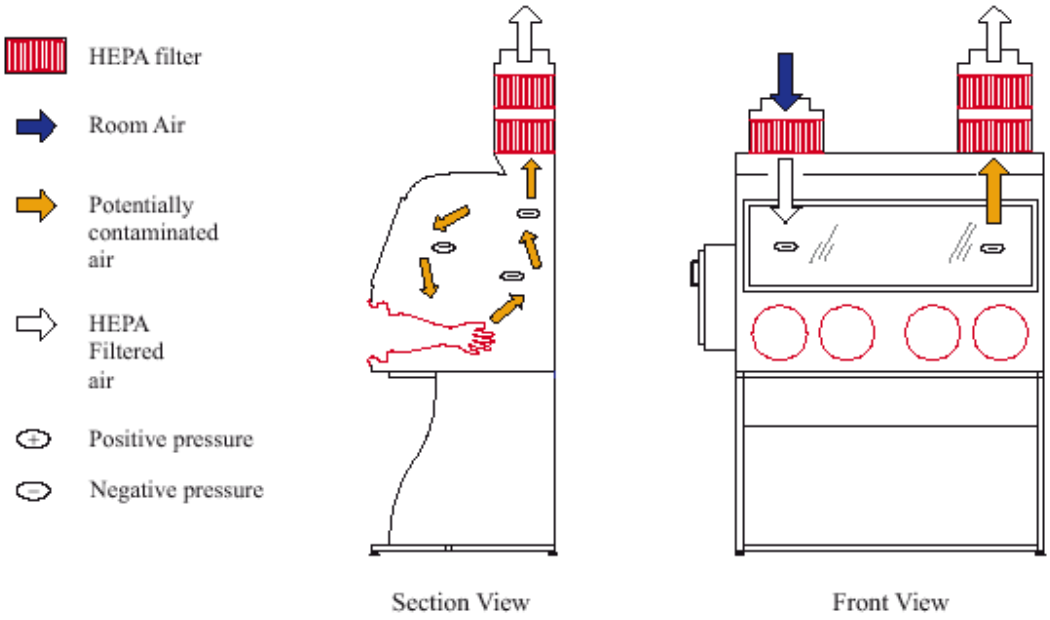


Figure 6: Class III Biological Safety Cabinet

Table 4:Selection of a BSC by the type of protection needed

Type of Protection	**BSC Selection**
Personnel protection, microorganisms in Risk Groups 1–3	Class I, Class II, Class III
Personnel protection, microorganisms in Risk Group 4, glove-box laboratory	Class III
Personnel protection, microorganisms in Risk Group 4, suit laboratory	Class I, Class II
Product protection	Class II, Class III only if laminar flow included
Volatile radionuclide/chemical protection minute amounts	Class IIB1, Class IIA2 vented to the outside
Volatile radionuclide/chemical protection	Class I, Class IIB2, Class III

Other Types of Cabinets

Laminar flow cabinets

These are designed to prevent contamination of biological samples. Air is drawn through a HEPA filter and flows in a smooth, laminar stream towards the user. There are many different types of cabinets with a variety of airflow patterns and acceptable uses. Laminar flow cabinets are designed to protect the work only and offer no protection to the workers. These are useful in the preparation of culture media and sterile solutions.

PCR hoods/PCR workstations

This is used in biology and genetic laboratories to prevent cross contamination between samples. HEPA filtered air is directed downwards over the work area to protect work from particulate contamination. It is equipped with UV light to deactivate DNA and RNA contaminants.

6.2. Personal Protective Equipment (PPE) and Clothing

These act as barriers to minimize the risk of exposure to aerosols, splashes and accidental inoculation.
Clothing and equipment should be selected according to the potential risk of transmission of organisms handled and the nature of work performed.

Laboratory coats, gowns, coveralls, aprons

- Long sleeved, back or side opening gowns or coveralls provide better protection than laboratory coats when working at the biological safety cabinet and in microbiology laboratories
- Laboratory coats should be buttoned completely when working in the laboratory
- An apron should be worn over the laboratory coat or gown, for additional protection against spillage of chemicals or biological materials whenever necessary
- Protective clothing should not be worn outside the laboratory areas
- Laundering services should be provided

Goggles, safety spectacles, eye and face shields

- Select appropriate equipment to protect the eyes and face from splashes and impacting objects depending on the activity performed
- Ordinary spectacles and contact lenses do not protect the wearer from splashes. Such persons should wear goggles over them for protection

- Wear shatter-proof plastic face shields for splash and impact protection or when handling potentially explosive material
- Do not wear goggles, safety spectacles or face shields outside the laboratory areas

Respirators

These should be used when carrying out aerosol generating procedures with highly infectious respiratory pathogens. eg. cleaning of a spill containing TB bacilli

- The choice of respirator will depend on the type of hazardous procedure. There are different types of respirators from N95 masks to masks with filters and self-powered air purifying respirators. Select appropriate type of interchangeable filter for the correct type of respirator
- Respirators should fit well over the operator's face in order to achieve optimal protection.
- Do not wear respirators outside the laboratory areas
- Surgical masks are not recommended for respiratory protection as they are designed solely for patient protection and do not provide protection to workers

Gloves

- Wear disposable gloves (latex, vinyl or nitrile gloves) to prevent contamination of hands while performing laboratory procedures
- Hands must be washed before and after removing gloves
- Ensure correct removal and decontamination of gloves before disposal and discard as infectious laboratory waste
- Wear stainless steel mesh gloves when there is a potential exposure to sharp instruments such as during postmortem examinations
- Do **not** wear gloves while handling writing materials, telephones, computers etc.
- Do not wear gloves outside the laboratory areas

6.3. Emergency Showers and Eyewash Stations

Accidental exposure to infectious or chemical material may cause injuries to eyes, face and other parts of a human body. Emergency eyewash and shower stations provide a flush away effect of hazardous substances. Eye and face wash stations are designed to flush both eyes and face simultaneously at a velocity low enough to be non-injurious to the user.

The emergency shower and eyewash stations are supplementary to recognised engineering controls, safety procedures and personal protective equipment.

- Locate the emergency eyewash and shower station on the same level as close to the hazard as possible, requiring no more than 10 seconds to reach

- Ensure these stations are located in an area where further contamination of hazardous material will not occur
- Keep the passageway to the emergency shower and/or eyewash station free of obstructions (i.e. doors etc.) that may inhibit the immediate use of it (no stairs to travel between the workstation and the emergency equipment)
- Make sure the emergency eyewash and shower station does not come into contact with any electrical equipment that may become a hazard when wet
- Equip the emergency shower and eyewash station with an audible or visual alarm or both to alert other personnel when it is activated
- Exposure to infectious substances - flush the affected area for a minimum of 15 minutes using a large amount of clean flushing fluid under low pressure
- Exposure to chemicals - flushing or rinsing time should be modified according to the identity and properties of the chemical

  Given below is a general recommendation. For specific activity on exposure, please refer to specific Material Safety Data Sheet (MSDS) of the chemical

  - mildly irritating chemicals - minimum 5 minutes
  - moderate-to-severe irritants - minimum 20 minutes
  - non-penetrating corrosives - 20 minutes
  - penetrating corrosives – minimum 60 minutes

Emergency showers

The emergency showers should be constructed to deliver a stream of water with a diameter of at least 50.8 cm to ensure that the water will come into contact with the entire body, not just the top of the person's head. According to the American National Standard Institute (ANSI) guidelines the shower head should be between 208.3 and 243.8 cm from the floor. The minimum volume of spray should be 75.7 litres/minute for a minimum time of 15 minutes.

The shower should be activated in less than 1 second and should remain operational without the operator's hand on the valve (or lever, handle, etc.). This valve should be within easy access. If enclosures are used, ensure that there is an unobstructed area of 86.4 cm in diameter.

Eyewash stations

The eyewash stations should deliver fluid to both eyes simultaneously at a volume of 1.5 litres/minute for 15 minutes. The volume should not be at a velocity that may injure the eyes. The

eyewash unit should be stationed at a suitable height which will deliver the jet of water to a person's eyes.

The unit should be activated in less than 1 second and should remain operational without the operator's hand on the valve (or lever, handle, etc.) with the valve being located in an easily operated place. The user should be able to open their eyelids with their hands.

- Soap, nailbrushes and towels should be provided with the emergency shower and eyewash stations
- Inspect and maintain emergency shower and eyewash facility every six months and operate weekly to verify operation, to ensure operational performance in an emergency

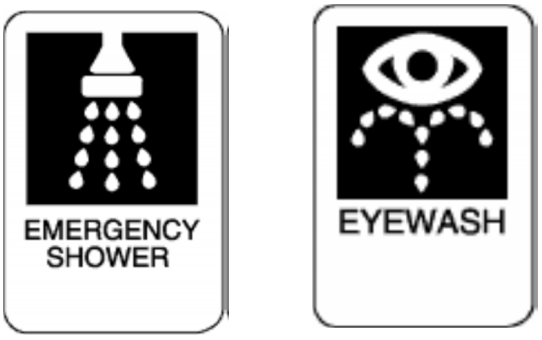


Figure 7: Signage for emergency shower and eye wash

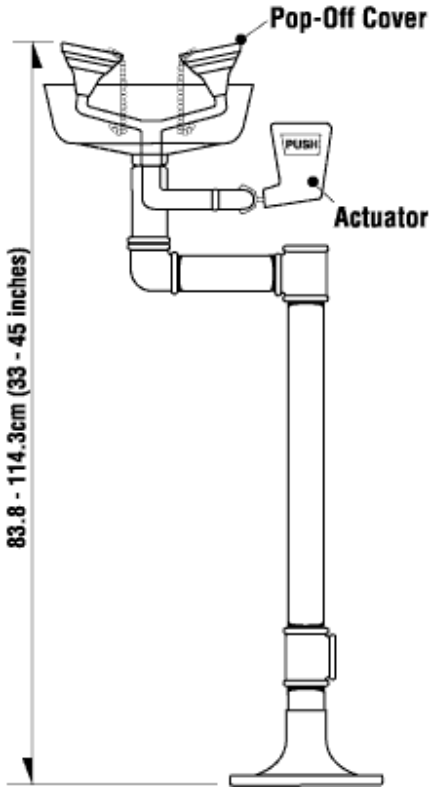


Figure 8: Plumbed eye wash station

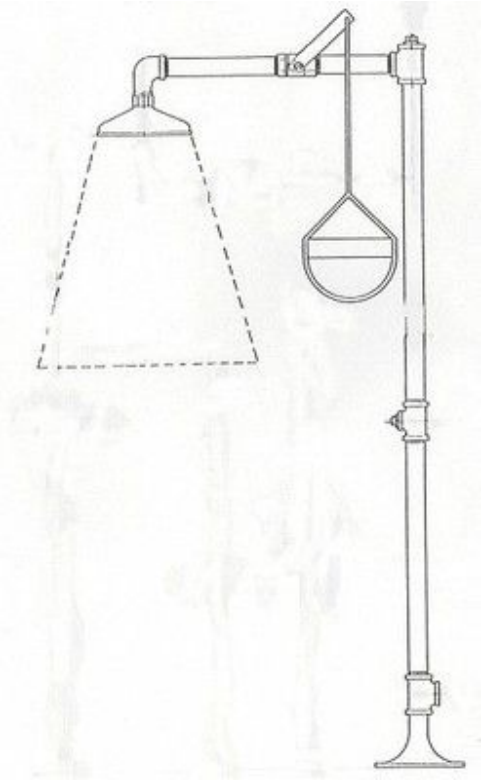


Figure 9: Plumbed emergency shower

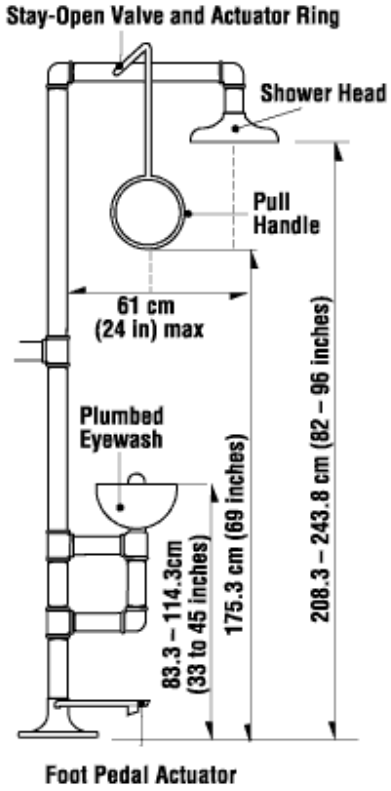


Figure 10:Emergency shower and eye wash unit

References

- Biosafety in Microbiological and Biomedical Laboratories. 5th Edition. USA: HHS Publication No. (CDC) 21-1112; 2009.
- Emergency Eyewash and Shower Equipment Standard Selection, Use, Installation and Maintenance. Version 1.12. Canada: University of Toronto; August 2013.
- http://www.biocompare.com/Lab-Equipment/21291-PCR-Workstat Accessed on 02.02.2014
- http://www.eyewashdirect.com/ANSI-Eyewash-Z358-Eyewash-Standard-Guide-s/31.htm accessed on 15.05.2014
- http://oes.tamu.edu/web/guidelines/Emergency Showers and Eyewash Stations_CCOSH.pdf. Accessed on 17.06.2014
- Laboratory Biosafety Manual. 3rd Edition. Malta: World Health Organization; 2004.

Chapter 7

Emergency Procedures in Microbiology Laboratories

Hazards in Laboratories

- General hazards
  - Cut injuries
  - Burns
  - Electrical shocks
  - Explosions
  - Fires

- Infections
  - Inhalation
  - Direct inoculation
  - Splashing on mucous membranes
  - Contact with infected material on cuts, scratches or abraded skin
  - Accidental swallowing

- Chemical hazards
  - Poisoning (by ingestion, inhalation, skin or mucous membrane absorption)
  - Irritation
  - Burns
  - Asphyxiation
  - Allergies
  - Explosions

- Special hazards
  - Radioactivity
  - Carcinogens
  - Mutagens
  - Teratogens

Management of Exposure to Hazards

- **Puncture wounds, cuts and abrasions**
  - Remove protective clothing, wash hands and the affected area with soap and water and clean with70% alcohol

- **Splash of blood and/or body fluids on intact skin**
  - Immediately wash the affected area thoroughly with soap and running water

- **Splash of blood and/or body fluids to eyes**
  - Irrigate the eyes gently but thoroughly for at least 15 minutes
  - Use the eye wash station if available

- **Splash of blood and/or body fluids to mouth or nose**
  - Immediately spit out the blood or fluids and rinse the mouth several times with water
  - Blow the nose and wash with water
  - Do not use disinfectant

- **Ingestion of potentially infectious material**
  - Protective clothing should be removed and medical attention should be sought immediately
  - The ingested substance should be identified and reported to the supervisor

Note: In all the above situations, the cause and the circumstances of the incident and the organisms involved should be reported immediately to the supervising officer.

Medical attention should be sought as necessary and medical records should be maintained.

Release of potentially infectious aerosols (outside a BSC)

- Switch off fans and air conditioners
- All persons should immediately vacate the affected area
- Signs should be posted indicating that entry is forbidden
- The laboratory supervisor and the biosafety officer should be informed at once
- Any exposed persons should be referred for medical advice
- No personnel should enter the room for one hour, to allow aerosols to be carried away and heavier particles to settle. If the laboratory does not have a central air exhaust system, entry should be delayed accordingly
- After the appropriate time, decontamination should proceed, supervised by the biosafety officer
- Appropriate protective clothing and respiratory protection should be worn for decontamination

- Post-incident discussions should be carried out to take preventive actions and those should be documented

Handling a breakage in a centrifuge

- If a breakage is known or suspected while the centrifuge is running, the motor must be switched off. The lid should not be opened for 30 minutes to allow settling of aerosols
- If a breakage is discovered after the lid is opened, it should be immediately closed and kept for 30 minutes
- Thick rubber gloves, laboratory coat, aprons and mask should be worn for cleaning
- Forceps or cotton swabs held in forceps should be used to pick up glass debris
- All broken tubes or glass fragments should be placed in a sharps container for disposal
- Plastic buckets and all non-metal removable parts should be soaked in 1% hypochlorite solution (10,000 ppm) for at least 20 minutes followed by rinsing residual hypochlorite
- Metal centrifuge buckets should be soaked in 2% gluteraldehyde for 20 minutes or in 70% alcohol for at least one hour
- The bowl and rotor must be swabbed with 70% alcohol and allowed to dry in air
- All swabs should be treated as infectious waste

Management of spills

- Wear heavy duty rubber gloves, laboratory coat, apron and mask. Boots might be required depending on the amount of spill
- Cover spill with absorbent material (cloth, wadding or paper towels)
- For blood spills, soak with freshly prepared 1% hypochlorite solution (10,000 ppm). For blood-free samples such as urine, stools, pus, broth culture - soak with 3-5% phenol
- Leave for at least 30 minutes then mop up with absorbent material
- Any broken glass or other sharps should be cleared using a dustpan, tongs or a piece of stiff cardboard
- After debris is removed and decontaminated, the area should be washed with a general purpose detergent and clean water and allowed to dry
- Place contaminated material accordingly in-to infectious waste container/sharps bin for disposal
- If laboratory forms or other printed or written material are contaminated, the information should be copied onto another form and the original should be discarded into the infectious waste container

Emergency Equipment

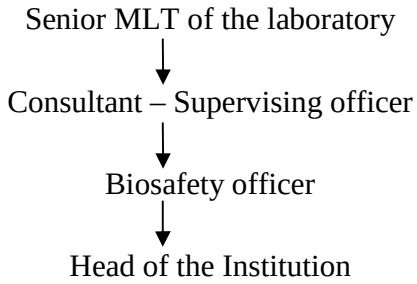
The following emergency equipment must be available

- First-aid kit, including universal and special antidotes
    - Sterile dressings, plaster, bandages, cotton wool, antiseptic (70% alcohol, povidone iodine), pair of scissors, forceps, paracetamol
- Spill kit
    - Gown, heavy duty gloves, disposable gloves, eye shield/goggles, absorbent material (wadding or gauze), freshly prepared 1% hypochlorite solution, general purpose detergent, forceps, dust pan and brush, yellow bags for infectious waste
- Eyewash station
- Emergency shower
- Appropriate fire extinguishers

The following are also suggested but may vary according to local circumstances

- Full protective clothing (one-piece coveralls, gloves and head covering forincidents involving micro-organisms in Risk Groups 3 and 4)
- Full-face respirators with appropriate chemical and particulate filter canisters
- Room disinfection apparatus eg. sprays and formaldehyde vaporizers
- Stretcher
- Tools eg. hammers, axes, spanners, screwdrivers, ladders, ropes
- Hazard area demarcation equipment and notices

Line of Communication in an Emergency



Emergency Services: Whom to Contact

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

1. The institution or laboratory (the address and location may not be known in detail by the caller or the services called)
2. Director of the institution
3. Head of the laboratory (eg. consultant microbiologist)
4. Senior MLT
5. Biosafety officer
6. Medical officer
7. Fire Service Department
8. Hospitals/ambulance services
9. Police
10. Water, gas and electricity services

References

- Biosafety in Microbiological and Biomedical Laboratories. 5th Edition. USA: HHS Publication No. (CDC) 21-1112; 2009.
- Hospital Infection Control Manual. Colombo: Sri Lanka College of Microbiologists; 2005.
- Laboratory Biosafety Manual. 3rd Edition. Malta: World Health Organization; 2004.
- National guidelines on laboratory safety. Ministry of Healthcare and Nutrition, Sri Lanka; 2007.

Chapter 8

Disposal of Infectious and Non-infectious Waste

Infectious and non-infectious waste produced in the laboratory should be collected and disposed separately. All infectious waste should be rendered non-infectious prior to disposal.

Waste should be segregated according to the colour code provided by the Ministry of Health document, National Colour Code for Health Care Waste, March 2006.

Yellow	– Infectious waste
Yellow and Red stripes	– Sharps
Black	– General waste
Green	– Food
Red	– Glass
Blue	– Paper (non-infectious)
Orange	– Plastic/polythene

Infectious waste

- Clinical specimens – blood/serum, pus, sputum, body fluids, urine and stools
- Contaminated items
    - specimen containers/glassware/animal cages
    - used syringes and needles
    - soiled dressings/cotton wool
    - used culture media
    - other items contaminated with infectious material eg. paper, polythene

Non-infectious waste

- Food
- Paper, cardboard and polythene wrappers
- Empty plastic and glass bottles not contaminated with infectious material
- Disposable items which are not contaminated with blood and/or body fluids

Disposal of infectious waste

These should be rendered non-infectious by autoclaving. It is recommended that a separate autoclave be made available to all laboratories for handling infectious material.

Whenever possible, fluid waste should be sent to a closed drainage system. Once fluids are rendered non-infectious, they could also be disposed via the general drainage system (i.e. pouring into a sink).It is preferable to have a dedicated sink for this purpose. However, draining high level disinfectants in to the common sewerage systems would disturb the natural process of decomposition by bacteria. Hence, the best method is to dilute the used disinfectants first and then discard them to a separate drainage system. Untreated clinical samples (eg. blood, serum, urine) can be poured into a dedicated sink.

- Histology specimens/laboratory animal carcasses
    - These should be disposed according to existing local regulations. Disposal by incineration or burial are recommended methods. Burial should be done in a deep pit under supervision

- Containers
    - Disposable containers eg. cardboard containers - by incineration
    - Plastic containers should be rendered non-infectious by autoclaving and disposed as non-infectious waste. Recycling of plastic material is recommended. Do not dispose plastic containers to municipal dumps

- Laboratory glassware
    - Reusable glassware (eg. tubes, pipettes and bijou bottles)
      These should be decontaminated by autoclaving and washed using a brush and a general purpose detergent to remove all organic material
    - Disposable glassware (eg. specimen containers)
      These could be incinerated after collecting into a leak-proof container. If an incinerator is not available, they may be buried after autoclaving or boiling (Crushing before burying is recommended to prevent reuse)

- Culture media
    - All used media should be rendered non-infectious by autoclaving prior to disposal. After autoclaving they may be disposed as general waste or through a dedicated drainage disposal system

Disposal of sharps

Do's

- Dispose used needles and syringes or any sharp object to a specially designed leak-proof sharps bin. The bin should have an opening on top sufficient only to dispose the used object conveniently
- The sharps bin should be available at the point of use
- When the sharps bin is ¾ full, seal the opening and dispose
- Use heavy duty rubber gloves when handling the sharps bin
- Store the sharps bins in an identified, dedicated place until disposal
- Incinerate or burn the sharps bins under the supervision of a safety officer or any other person to whom the responsibility is delegated
- Report any needle stick or sharps injury to the superior officer

Don'ts

- Never recap used needles
- Do not forcibly insert sharps into the sharps bin
- Do not overfill the sharps bin
- Do not attempt to retrieve any item from the sharps bin
- Do not attempt to reopen the sharps bin

Disposal of non-infectious waste

They should be collected appropriately into the bins lined by polythene liners according to the colour code and disposed to the municipal garbage collecting system.

References

- Ayliffe GAJ, Fraise AP, Geddes AM, Mitchell K. Control of Hospital Infections: A Practical Handbook. 4th Edition:CRC Press; 2000.
- Environmental Management Framework for Healthcare Waste and Infrastructure Development-Draft. Second Health Sector Development Program. Ministry of Health. 08/11/2012. Page 22
- Laboratory Biosafety Manual. 3rd Edition. Malta: World Health Organization; 2004.
- Prüss-Ustun A, Emmanuel J, Rushbrook P, Zghondi R, Stringer R. et al. Safe management of wastes from health-care activities.2nd Edition. Bulletin of the World Health Organization;2013.

Chapter 9

Safe Transport of Infectious Material

Transport of infectious and potentially infectious material is subject to national and international regulations. Transport regulations are important to prevent or minimize the risk of exposure to microorganisms that may escape from broken or leaking sample containers or samples that are improperly packed which may cause infection in transport staff, other passengers and the community. Therefore, the packaging of infectious substances for transport must be designed to minimize the potential for damage during transport. In addition, the packaging must ensure the integrity of the materials for timely and accurate processing of specimens.

International Transport Regulations

The regulations for the transport of infectious materials by any mode of transport are based upon the United Nations Model Regulations on the Transport of Dangerous Goods. These recommendations are developed by the United Nations Committee of Experts on the Transport of Dangerous Goods (UNCETDG). The International Air Transport Association (IATA) issues Infectious Substances Shipping Guidelines annually. WHO serves in an advisory capacity to the UNCETDG.

Laboratory personnel must ship infectious substances according to applicable transport regulations. Under the Dangerous Goods Classification, Class 6; division 6.2, addresses seven groups of infectious substances and their transport regulations.

For the purpose of transport, specimens in a clinical laboratory are classified as:

1. Infectious substances (Category A: UN 2814)
2. Biological substances (Category B: UN 3373)
3. Biological products
4. Genetically modified microorganisms and organisms
5. Medical and clinical waste
6. Exempt human/animal patient specimens(No UN number)
7. Exceptions

The packages must be properly marked and labelled for all who may come in contact with the package during the shipping process, including laboratory and transport staff.

International transport

Shippers have to be trained by IATA for transport of dangerous goods including infectious substances. Most of the commercial courier companies have been trained by IATA for this purpose.

Basic triple packaging system provides three layers of containment to protect the substances being shipped. These layers are primary, secondary and outer containers. In addition, packaging requirements for each category mentioned above will be different. The maximum net quantity of infectious substances per package will differ according to the mode of transport and the type of infectious substance.

The labels and markings of the outer container of the triple package will differ according to the transport category of the specimen. This is to identify the mode of transport of the dangerous goods clearly.

Furthermore, the documents required for each category is different. The Air Way bill, invoice and import permit from the country of destination will be required for all shipments by air. A shipper should complete the Declaration of Dangerous goods form for all shipments under category A.

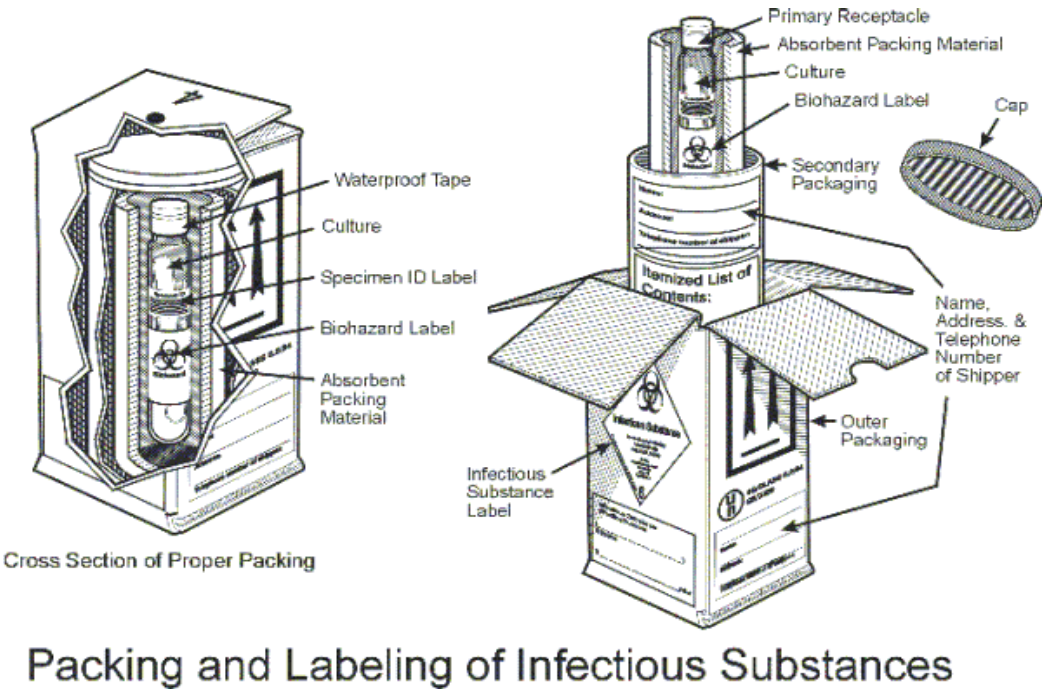


Figure 11: Packing and labeling of infectious substances

Carbon dioxide, solid (dry ice) – label and marking

Dry ice is classified as Class 9 under the Dangerous Goods Classification. The label for dry ice should be placed on the outside of any package which contains dry ice.

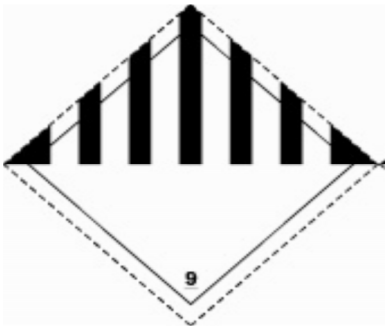


Figure 12: Carbon dioxide, solid (dry ice) label

The net weight of the dry ice must be marked on the outside of any package containing dry ice.

UN1845 dry ice
Net quantity _____kg

Because of the differences in the hazards posed by these infectious substances, there are variations in the packaging, labelling and documentation requirements for transport.

Reference

- Biosafety in Microbiological and Biomedical Laboratories. 5th Edition. USA: HHS Publication No. (CDC) 21-1112; 2009.
- Guidance on regulations for the transport of infectious substances. 2011-2012. International Health regulations coordination. World Health Organization; 2011.
- Laboratory Biosafety Manual. 3rd Edition. Malta: World Health Organization; 2004.

Chapter 10

Safety of Laboratory Workers

Screening, Immunization and Training on Laboratory Safety

A. Awareness programme

All new recruits to a laboratory should follow an awareness programme on laboratory safety

B. Screening

They should ideally undergo an initial assessment of immune status for the following diseases.

- Hepatitis B
- Rubella
- Measles
- Chickenpox
- Tuberculosis

C. Immunization

All laboratory workers should be immunized against Hepatitis B infection. In addition, appropriate vaccines should be given according to current vaccination protocols, depending on the area of work eg. workers in rabies or polio laboratories.

- **Hepatitis B vaccination**
  - **Pre-exposure prophylaxis to Hepatitis B virus infection**
    - All laboratory workers should be vaccinated against Hepatitis B at recruitment
    - Three doses of hepatitis B vaccine are recommended at 0, 1and 6 months
    - Immune status should be tested 6 -8 weeks after completion of the full course
    - The minimum protective level of Hepatitis B surface antibody (HBsAb) titre is ≥10 mIU/ml
    - If seroconversion has been achieved following a full course of Hepatitis B vaccine, regular testing and booster doses are not recommended

- **Post exposure management to Hepatitis B virus infection**
  - First aid
    - Wound - wash with soap/antiseptic and water, do not squeeze the wound
    - Splash into eye or mucous membrane – aggressive flushing with water
  - Inform supervisor and infection control officer/biosafety officer
  - Make an entry in the Laboratory Accidents Register
  - Hepatitis B surface antigen (HBsAg) testing of source/index case
  - Get hepatitis B vaccination history and check HBsAb level of health care worker (HCW)
  - Management depends on results of the HBsAg of the source and HBsAb of the HCW

Table 5: Post exposure management of health-care personnel for Hepatitis B

Postexposure management of health-care personnel after occupational percutaneous and mucosal exposure to blood and body fluids, by health-care personnel Hep B vaccination and response status

Health-care Worker status	Postexposure testing		Postexposure prophylaxis		Postvaccination serologic testing†
	Source patient (HBsAg)	HCW testing (anti-HBs)	HBIG*	Vaccination	
Documented responder§ after complete series (≥3 doses)	**No action needed**				
Documented non-responder¶ after 6 doses	Positive/unknown	—**	HBIG x2 separated by 1 month	—	No
	Negative	**No action needed**			
Response unknown after 3 doses	Positive/unknown	<10mIU/mL**	HBIG x1	Initiate revaccination	Yes
	Negative	<10mIU/mL	None		
	Any result	≥10mIU/mL	**No action needed**		
Unvaccinated/incompletely vaccinated or vaccine refusers	Positive/unknown	—**	HBIG x1	Complete vaccination	Yes
	Negative	—	None	Complete vaccination	Yes

Abbreviations: HCP = health-care personnel; HBsAg = hepatitis B surface antigen; anti-HBs = antibody to hepatitis B surface antigen; HBIG = hepatitis B immune globulin.

* HBIG should be administered intramuscularly as soon as possible after exposure when indicated. The effectiveness of HBIG when administered >7 days after percutaneous, mucosal, or non-intact skin exposures is unknown.

† Should be performed 1–2 months after the last dose of the Heptitis B vaccine series (and 4–6 months after administration of HBIG to avoid detection of passively administered anti-HBs) using a quantitative method that allows detection of the protective concentration of anti-HBs (≥10 mIU/mL).

§ A responder is defined as a person with anti-HBs ≥10 mIU/mL after ≥3 doses of HepB vaccine.

¶ A non-responder is defined as a person with anti-HBs <10 mIU/mL after ≥6 doses of Hep B vaccine.

** HCW who have anti-HBs <10mIU/mL, or who are unvaccinated or incompletely vaccinated, and sustain an exposure to a source patient who is HBsAg-positive or has unknown HBsAg status, should undergo baseline testing for HBV infection as soon as possible after exposure, and follow-up testing approximately 6 months later. Initial baseline tests consist of total anti-HBc; testing at approximately 6 months consists of HBsAg and total anti-HBc.

- **Hepatitis C virus infection**
  - **Post exposure management**
    - First aid
      - Wound - Wash with soap/antiseptic and water
      - Splash into eye or mucous membrane – aggressive flushing with water
    - Inform supervisor and infection control officer/biosafety officer
    - Hepatitis C antibody testing of source/index case
    - If source is Hepatitis C antibody positive
      - Baseline hepatitis C antibody and ALT testing of HCW
      - HCV RNA testing of HCW at 6 weeks and 12 weeks
      - Follow up hepatitis C antibody and ALT testing of HCW at 12 weeks, 24 weeks and 1 year
    - If source is HCV antibody negative
      - Baseline serum sample
      - HCV antibody testing if liver signs/symptoms develop
    - If source is unknown
      - Test for HCV antibody at 12 weeks and 24 weeks

- **HIV infection**
  - **Post exposure prophylaxis (PEP)**
    - Determine the risk associated with exposure

Factors to be considered in assessing exposures

Type of exposure

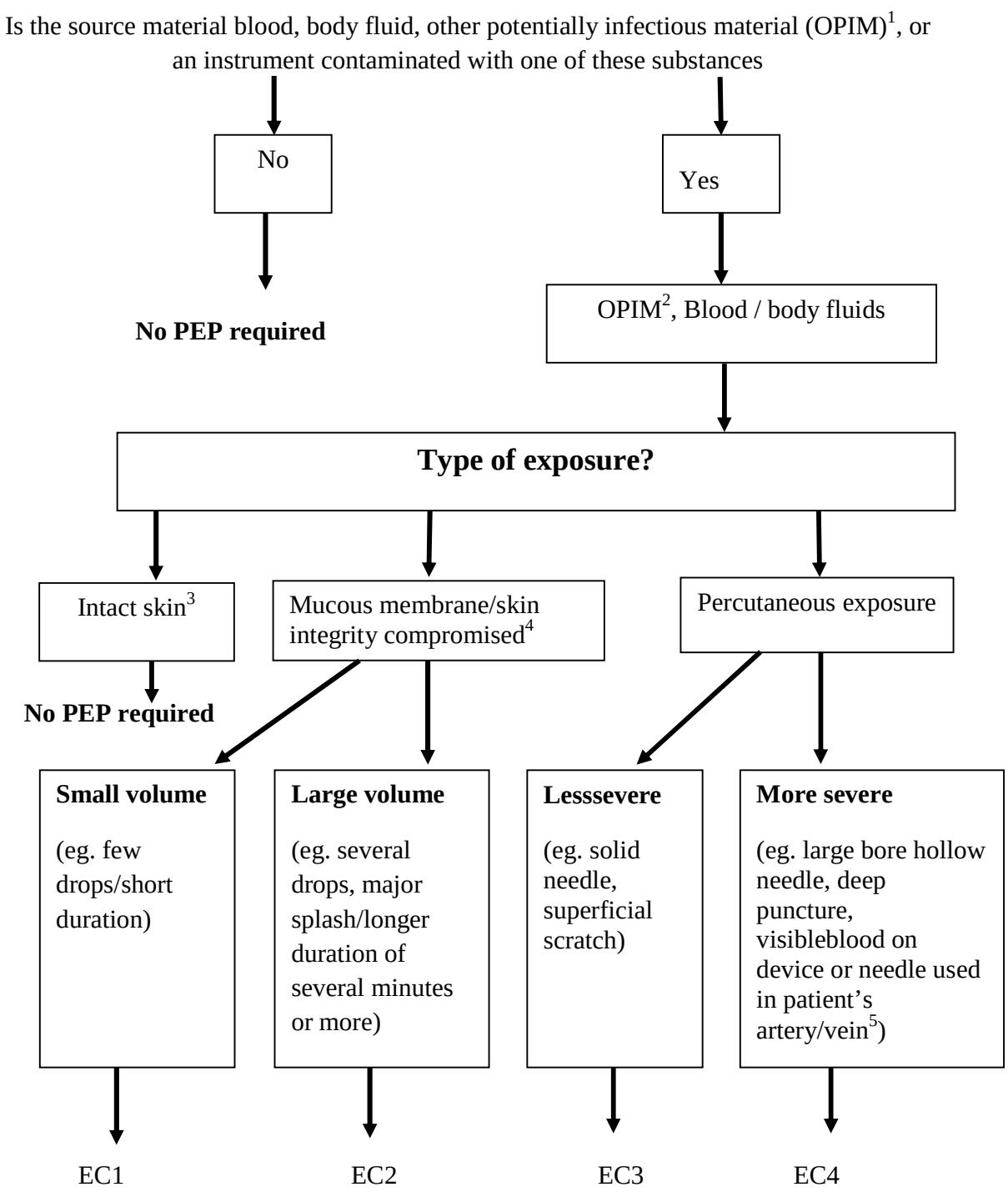
- Percutaneous injury
- Mucous membrane exposure
- Non-intact skin exposure

Type and amount of fluid/tissue

- Blood
- Fluids containing blood
- Other potentially infectious material (OPIM) (cerebrospinal, synovial, pleural, peritoneal, pericardial, and amniotic fluids, semen, vaginal secretions)

- Determine the Exposure Code according to the flow chart below

EXPOSURE CODE (EC)



1. Semen or vaginal secretions, cerebrospinal, synovial, pleural, pericardial or amniotic fluids or tissue
2. Exposure to OPIM must be evaluated on a case by case basis. In general, these body substances are considered a low risk for transmission in health care settings. Any unprotected contact to concentrated HIV in a research laboratory or production facility is considered an occupational exposure that requires clinical evaluation to determine the need of PEP.
3. Contact with intact skin is not normally considered a risk for HIV transmission. However, if the exposure was to blood and the circumstances suggest a higher volume exposure (eg. An extensive area of skin was exposed or there was prolonged contact with blood), the risk for HIV transmission should be considered.
4. Skin integrity is considered compromised if there is evidence of chapped skin, dermatitis, abrasion or open wound.
5. The combination of these severity factors (eg. Large bore needle and deep puncture) contribute to an elevated risk for transmission if the source person is HIV positive.

Evaluation of exposure sources

Test **known sources** for HBsAg, anti-HCV, and HIV antibody

- Consider using a rapid HIV-antibody test
- If the source person is not infected with a blood-borne pathogens, base line testing or further follow-up of the exposed person is not necessary

For sources whose **infection status remains unknown** (eg. the source person refuses testing)

- Consider medical diagnoses, clinical symptoms and history of risk behaviors

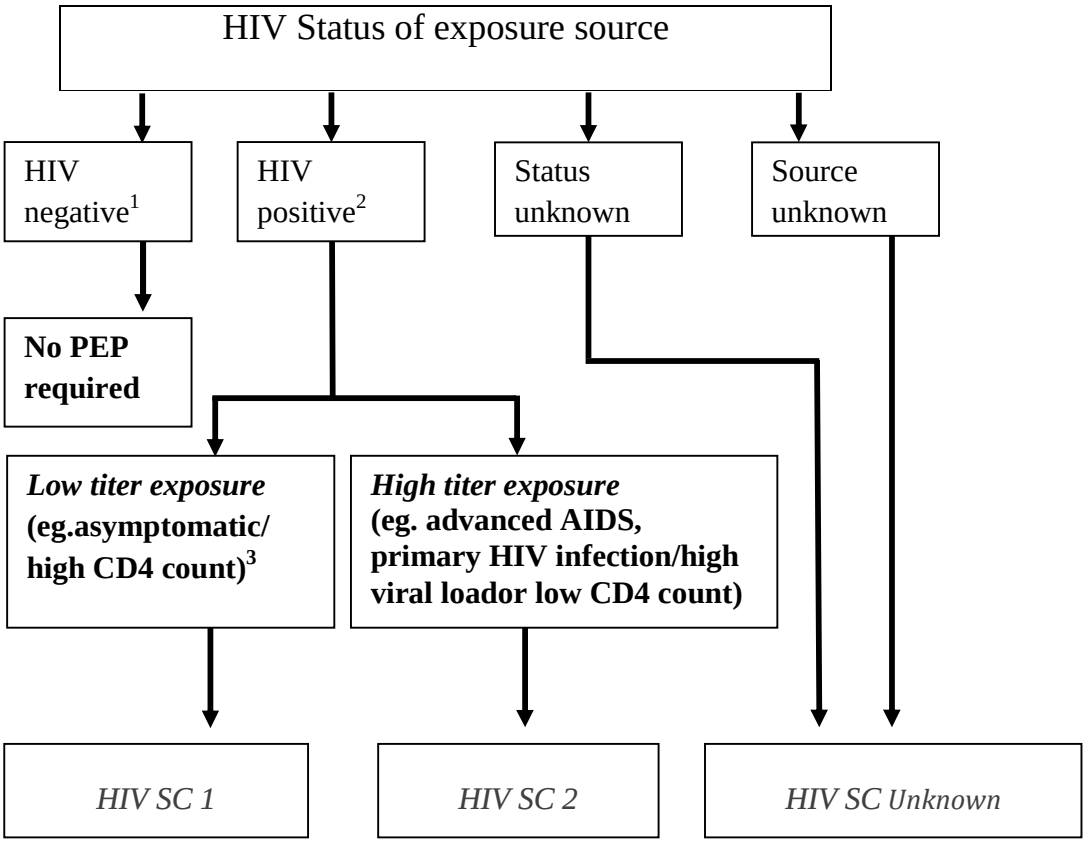
For **unknown sources**, evaluate the likelihood of exposure to a source at high risk of infection

- Consider likelihood of blood-borne pathogen infection among patients in the exposure setting

- Evaluate exposure source

For HIV determine the exposure source code according to the flow chart below

SOURCE CODE (SC)



1. A source is considered negative for HIV infection if there is laboratory documentation of a negative HIV antibody, HIV polymerase chain reaction (PCR) or HIV p24 antigen test results from a specimen collected at or near the time of exposure and there is no clinical evidence of recent retroviral like illness.
2. A source is considered infected with HIV (HIV positive) if there has been a positive laboratory results for HIV antibody, HIV PCR, HIV p24 antigen or physician diagnosed AIDS.
3. Examples are used as surrogates to estimate the HIV titre in an exposure source for purposes of considering PEP regimens and do not reflect all clinical situations that may be observed. Although a high HIV titre (HIV SC2) in an exposure source has been associated with an increased risk for transmission, the possibility of transmission from a source with low HIV titre also must be considered.

- **Postexposure management of HCW potentially exposed to HIV**
  - Determination of PEP Recommendation

    The health care worker should be referred to a consultant venereologist for recommendation of PEP for HIV exposure.

    For further information contact National STD/AIDS Control Program (NSACP) on a working day.

    Telephone no: 0112667163

- **Rubella/Chickenpox/Measles**
  Vaccination against the above diseases for the non-immune laboratory workers should be encouraged.

- **Tuberculosis**
  A Mantoux test should be performed for
  - those who are **without** a definite BCG scar **or**
  - documentary evidence of a previous BCG **or**
  - documented positive Tuberculin (Mantoux) test within the last five years

Immunization with BCG vaccine is advised if the Mantoux test is negative and there is no documentary evidence of BCG vaccination.
BCG vaccination is rarely recommended for adults over the age of 16 years because the efficacy is questionable. However, it can be given to adults aged between 16 and 35 years who are at occupational risk of tuberculosis.

Other vaccines
Vaccination against polio, hepatitis A and typhoid should be considered for those who handle such infectious agents.

D. Training

The safety officer should ensure that all laboratory workers are given regular in-service training on laboratory safety by organizing discussions, meetings and workshops. These training programmes should address areas such as,

- good laboratory practices
- safe handling and maintenance of equipment
- reporting of any laboratory accidents etc.

References

- Guidelines for the management of potential exposures to hepatitis B, hepatitis C, HIV and recommendations for post-exposure prophylaxis. Saskatchewan Subcommittee on HIV/AIDS. January 2004.
- Guidance on TB Pre-Employment Screening in new NHS Employees. North West Regional TB Group. The Guideline Group. 2008.
- Health clearance for tuberculosis, hepatitis B, hepatitis C and HIV: New healthcare workers. Department of Health. Health Protection Division, General Health Protection. London. March 2007.
- Henderson DK. Managing occupational risks for Hepatitis C transmission in the health care setting. Clinical Microbiology Reviews. 2003;16(3):546-568
- http://www.nhs.uk/Conditions/vaccinations/Pages/bcg-tuberculosis-TB-vaccine.aspx. Accessed on 28.03.2014
- Immunizations of healthcare and laboratory staff: the green book. Chapter 12. 2013. London. Public Health England.
- Management of health care worker exposures to HIV and recommendations to post exposure prophylaxis. General circular letter number 02-36/2001. Ministry of Health services and nutrition. 12 March 2001.
- Ramsay ME on behalf of the PHLS Advisory Committee on Blood Borne Viruses. Guidance on the investigation and management of occupational exposure to hepatitis C. Communicable Diseases and Public Health Guidelines. 1999;2(4):258-262
- Schillie S, Murphy TV, Sawyer M, Ly K, Hughes E et al. CDC Guidance for Evaluating Health-Care Personnel for Hepatitis B Virus Protection and for Administering Post exposure Management. Recommendations and Reports. MMWR. December 20, 2013/62(rr10);1-19
- Tuberculosis: Clinical diagnosis and management of tuberculosis, and measures for its prevention and control. National Collaborating Centre for Chronic Conditions and the Centre for Clinical Practice at NICE. National Institute for Health and Clinical Excellence. 2011.
- Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendations for Post exposure Prophylaxis – U.S. Department of Health and Human Services. CDC. Updated U.S. Public Health Service MMWR, Recommendations and reports. June, 2001;(50)RR-11.

Chapter 11

Documentation and Document Control

The international standard organization - ISO 15189:2012 on 'Medical laboratories-Requirement for quality and competence' states: the laboratory shall control documents required by the quality management system and shall ensure that unintended use of any obsolete document is prevented.

The laboratory shall have a documented procedure to ensure that the following conditions are met:

- All documents, including those maintained in a computerized system, issued as part of the quality management system are reviewed and approved by the authorized personnel before issue
- All documents are identified to include:
    - a title
    - a unique identifier on each page
    - date of the current edition and/or edition number
    - page number of total number of pages (eg. Page 1 of 5, 2 of 5 etc.)
    - authority for issue
- Current authorized editions and their distribution are identified by means of a list (eg. document register, log or master index)
- Only current, authorized editions of applicable documents are available at point of use
- Where a laboratory's document control system allows for the amendment of documents by hand, pending the re-issue of documents, the procedures and authorities for such amendments are defined, amendments are clearly marked, initialed and dated, and a revised document is issued within a specified time period
- Changes to documents are identified
- Documents remain legible
- Documents are periodically reviewed and updated at a frequency that ensures that they remain fit for purpose
- Obsolete controlled documents are deleted and marked as obsolete
- At least one copy of an obsolete controlled document is retained for a specified time period or in accordance with applicable specified requirements

There are 3 categories of documents.

1. Policy documents
    - Biosecurity plan to describe how the institution will mitigate risks including: a written security plan and incident response plans
    - Written protocols for employee training on potential hazards, the biosecurity program and incident response plans

2. Documents of procedures
    - Standard operating procedures

3. Working documents
    - Accident and incident reporting form
    - Records of personal health evaluation including immunization history of vaccine preventable diseases, history of any other medical conditions that predispose to acquiring infectious diseases
    - Documents with regard to biosafety and biosecurity when handling dangerous biological materials

All samples received in the laboratory should be documented at all levels

- reception counter
- processing section
- report issuing counter

Measures should be in place, so that any sample received in the laboratory is traceable at any given time. Highly infectious samples should be flagged at the receiving counter, to warn any person handling the sample to take extra precautionary measures.
All accidents and incidents where breach of biosafety has occurred should be reported immediately to the biosafety officer/SMLT and the consultant.

Regular safety audits should be carried out quarterly or semi-annually by the biosafety officer and the consultant. Deficiencies should be identified and corrective action should be taken.

The following documents are necessary to maintain laboratory biosafety

- Immunization history of vaccine preventable diseases, history of any medical condition that can predispose to acquiring infectious diseases of the staff members
- Records of personal health evaluation before and after placement of staff members on specific work
- Written recommendations for control of infectious diseases in laboratory staff
- SOPs on regular in-service training and education on laboratory safety practices
- Accident and incident reporting forms
- Audit tools to carry out audits in safety

Inventory and accountability
Material accountability procedures should be established to track the inventory, storage, use, transfer and destruction of dangerous biological materials. Dangerous biological materials should be defined and records should be maintained and updated.

Information security
This is to protect information from unauthorized release and ensure that the appropriate level of confidentiality is preserved. It is important that access to sensitive information be controlled. Policies for properly identifying and securing sensitive information such as electronic files and removable electronic media should be developed.

Transport policies
When transporting biological agents material transport policies should include accountability measures for the movement of materials within and outside the facility via appropriate documentation.

References

- Biosafety in Microbiological and Biomedical Laboratories. 5th Edition. USA: HHS Publication No. (CDC) 21-1112; 2009.
- Laboratory Biosafety Manual. 3rd Edition. Malta: World Health Organization; 2004.
- Medical laboratories-Requirement for quality and competence. 3rd Edition. Austrian Standards Institute. International Standard; ISO 15189:2012.

Chapter 12

Laboratory Safety Audits

A laboratory safety audit is a methodical examination and critical review of laboratory safety. During this procedure, safety practices and equipment are evaluated. General safety, life safety, biological, chemical and radiation safety will be discussed. Laboratory audits should be scheduled on a regular basis, announced or unannounced.

Regular safety audits should be conducted by a designated safety officer at quarterly interval, accompanied by the SMLT and a building maintenance officer. A written report with suggestions for corrective action should be sent to the laboratory supervisor. Support from higher management is essential for an audit to have the desired effect of improving employee safety, as well as instituting compliance with applicable regulations.

A checklist should include the following biosafety elements

- autoclave repair and operational records
- proper use of PPE
- no food or drink in the laboratory
- proper disposal of laboratory waste
- biohazard signs
- use of in-line HEPA filters on laboratory vacuum outlets
- cleaning and disinfection of work surfaces at appropriate intervals
- pest control

Additional biosafety elements of audit may include other activities depending on the centre.
The following documents should be assessed

- weekly cleaning of floor and sink drains
- weekly flushing of eyewash station
- monthly assessment of emergency communication devices
- testing the operating status of alarms, emergency lights, and emergency exit lights
- availability of laboratory standard operating procedures, laboratory biosafety manuals and laboratory personnel training records

Following four events warrant conducting a formal, unscheduled audit of a laboratory

1. Accident or injury in the workplace
2. Follow-up to implementation of new biosafety regulations or procedures
3. A new funding source requesting documentation of workplace safety
4. Establishing a test for a new infectious agent in the laboratory

Reference

- Laboratory Biosafety Manual. 3rd Edition. Malta: World Health Organization; 2004.

Chapter 13

Biosecurity

Biosecurity refers to the protection of microbial agents from loss, theft, diversion or intentional misuse. Biosafety and biosecurity are related, but not identical concepts. Biosafety programs reduce or eliminate unintentional exposure of individuals and the environment to potentially hazardous biological agents.

Global events in the recent past have highlighted the need to protect laboratories and the material they contain from being intentionally compromised in ways that may harm people, livestock, agriculture or the environment.

It is necessary to expand the traditional approach to biosafety through the introduction of laboratory biosecurity measures.

A specific laboratory biosecurity programme must be prepared and implemented for each facility according to the requirements of the institution, the type of laboratory work conducted and the local conditions.

The relevant officers for the biosecurity programme include head of the institution, consultant microbiologist, biosafety officer, SMLT, maintenance staff, law enforcement personnel (police etc.) and security staff if appropriate.

Biosecurity measures do not have to be sophisticated. Accountability of infectious agents and toxins in the laboratory, authorized entry, protection of relevant sensitive information and secure storage of infectious agents should be instituted to prevent intentional misuse. These measures are in place in most laboratories that apply good laboratory management practices and have appropriate biosafety programs.

This can be achieved by

- updated inventory of microorganisms and storage location
- identification of personnel with access
- control documentation for sensitive information including infectious agents
- documentation of internal and external transfers within and between facilities and line of authority
- inactivation and/or disposal of the materials

An institutional laboratory biosecurity protocol should be established for identifying, reporting, investigating and remediating breaches in laboratory biosecurity, including discrepancies in inventory results. The involvement and roles and responsibilities of public health and security authorities in the event of a security infraction must be clearly defined.

Laboratory biosecurity training distinct from laboratory biosafety training should be provided to all personnel. Such training should help personnel understand the need for protection of such materials and the rationale for the specific biosecurity measures.

In summary, security precautions should become a routine part of laboratory work, just as having aseptic techniques and other safe microbiological practices. Biosecurity management should not unduly interfere with the day-to-day activities of laboratory personnel or be an impediment to conducting research and legitimate access to important research and clinical materials must be preserved.

Reference

- Biosafety in Microbiological and Biomedical Laboratories. 5th Edition. USA: HHS Publication No. (CDC) 21-1112; 2009.
- Laboratory Biosafety Manual. 3rd Edition. Malta: World Health Organization; 2004.

Chapter 14

Introduction to Biosafety in Molecular Biology and Biotechnology

Molecular biology laboratory is a place where work is being done to advance understanding of biological processes at the molecular level providing the knowledge needed to diagnose infectious and genetic diseases. A diagnostic molecular biology laboratory conducts polymerase chain reaction by conventional or real time technique. All biosafety and biosecurity measures practiced in a microbiology laboratory should be practiced in the molecular biology laboratory.

In addition to these, the staff in a molecular biology laboratory should practice a few additional measures.

- Standard microbiological practices should be performed
- Many chemical agents, physical agents, solvents and staining reagents are hazardous substances and known or suspected carcinogens. Guidelines for handling such substances should be available
- Solutions containing ethidium bromide can be degraded chemically, or collected and incinerated. While deactivating, it can be neutralized and poured down the drain with excess water. Deactivation may be confirmed using UV light to detect fluorescence
- The voltages used in electrophoretic machines can cause electrocution. Care should be taken when handling gels in such machines
- The transilluminator for visualizing gels uses UV light. Goggles or UV safe face shields should be worn when using the transilluminator

Recombinant DNA technology involves combining genetic material from different sources, thereby creating genetically modified organisms (GMOs). Recombinant DNA technology has already had an enormous impact on biology and medicine.
Experiments involving the construction or use of GMOs should be conducted after performing a biosafety risk assessment. The pathogenic properties and any potential hazards associated with such organisms may be novel and not well-characterized.

The following should be evaluated before such experiments and procedures

- The properties of the donor organism
- The nature of the DNA sequences that will be transferred
- The properties of the recipient organism
- The impact on the environment

These factors should help determine the Biosafety Level that is required for the safe handling of the resulting GMO, and identify the biological and physical containment systems that should be used. Safety guidelines help to ensure that safety is built into the facilities and procedures of the operations. In addition to general requirements such as pressure differential zones, air filtration and containment systems, waste treatment systems and manufacturing procedures must be standardized by using good manufacturing practices, standard operating procedures and validated quality control procedures.

Risk Assessments for Genetically Modified Organisms

Risk assessments for work with GMOs should consider the characteristics of donor, recipient or host organisms.

- Hazards arising directly from the inserted gene of donor (organism)
  Assessment is necessary in situations where the product of the inserted gene has known biologically or pharmacologically active properties which may give rise to harm
    - toxins
    - cytokines
    - hormones
    - gene expression regulators
    - virulence factors or enhancers
    - oncogenic gene sequences
    - antibiotic resistance
    - allergens

- Hazards associated with the recipient (host)
    - susceptibility of the host
    - pathogenicity of the host strain, including virulence, infectivity and toxin production
    - modification of the host range
    - immune status of the recipient
    - consequences of exposure

References

- 90/219/EEC on the contained use of genetically modified microorganisms. Official Journal of the European Communities. 1998;L330:13–31
- European Council. Council Directive 98/81/EC of 26 October 1998 amending Directive
- Guidance on the use, testing and maintenance of laboratory and animal flexible film isolators. Advisory Committee on Dangerous Pathogens. Health and Safety Executive. London; 1990.

- http://web.princeton.edu/sites/ehs/chemwaste/etbr.html accessed on 23.09.2014
- http://www.slideshare.net/AISHMALIK/biosafety-and-ethics-in-molecular-biology-laboratory accessed on 06.10.2014.
- Maintenance and distribution of transgenic mice susceptible to human viruses: memorandum from a WHO meeting. Bulletin of the World Health Organization. 1993; 71:497–502.
- O'Malley BW Jr, Li D, Buckner A, Duan L, Woo SL, et al. Limitations of adenovirus-mediated interleukin-2 gene therapy for oral cancer. Laryngoscope. 1999;109(3):389–395.

Chapter 15

Safe Handling of Chemicals

Laboratory workers must be aware of the health risks of various chemicals which are used in the laboratory. Periodical awareness programmes on the safe handling of chemicals should be conducted for the staff. Manufacturer's material safety data sheets (MSDS) should be easily accessible to the laboratory staff.

The Material Safety Data Sheet (MSDS)

The MSDS provides information about the chemicals, its properties, use and their safe handling. Most MSDS will provide the following information about the chemical

- the name of the chemical/chemical formula
- product code
- key ingredients
- the physical description and properties of the chemical (eg. colour, odour, melting point, boiling point)
- hazard information about the chemical and its effects on humans
- safety equipment to use when handling the chemical
- safe methods to store and transport the chemical

The laboratory should maintain an up-to-date file of MSDS for all chemicals and reagents used by the laboratory.

Classification of chemicals

Examples of classes that must be considered in safety planning include

- oxidising agents and reducing agents
- corrosives such as acids and bases
- water reactive chemicals
- air reactive chemicals
- highly toxic chemicals
- explosive chemicals
- radioactive chemicals

Labelling of chemicals

All chemicals must be correctly and securely labelled.

- Most manufactured chemicals are already labelled and the label should include
  - name of the chemical
  - concentration (strength) of the chemical
  - information about hazards associated with the chemical eg.poison, carcinogen, flammable, corrosive, oxidizing
  - name of the manufacturer
  - date of manufacture
  - shelf-life

- On reception of the chemical to the laboratory following should be added
  - date received
  - date of initial opening

Care must be taken **not** to obliterate the information given on the label**.**

- All reagents prepared in the laboratory should be labelled with
  - chemical name and formula
  - concentration
  - date of preparation
  - name of the person who prepared the reagent
  - storage classification
  - hazard warning label (available from a safety supplier)
  - reference to original source of chemical (eg. manufacturer etc.)

Protective clothing

Depending on the nature of the chemical exposure, use of protective clothing, overcoats, masks, gloves and goggles is recommended. Fume hoods must be used when handling vaporizing toxic chemicals.

Storage of chemicals

- Chemicals should be stored in minimum amounts in the laboratory for daily use

- Bulk stocks should be stored in specially designated rooms or buildings, which should have concrete floors with sills at doorways to retain spills

- Storage cupboards and cabinets should have a list of chemicals displayed outside

- Chemicals must be stored at an appropriate temperature and humidity level. They should not be stored near heat sources, such as steam pipes or laboratory ovens and should not be exposed to direct sunlight

- Inflammable substances should be stored in separate rooms which are equipped with continuously running exhaust fans. Electrical switches should be situated outside the room and lighting should be in enclosed compartments

- Chemicals should not be routinely stored on the bench tops. Each chemical should have a specific storage area. Bottles containing strong acids and alkalis and all large bottles with chemicals should be stored at floor level and in drip trays

- Fume hoods should not be used as general storage areas for chemicals. This may seriously impair the ventilating capacity of the hood

- Care should be taken when chemicals are stored
    - They should not be stored in alphabetical order. This may lead to incompatible chemicals (page no 74) being stored near each other
    - They should not be stored according to poorly chosen categories. Eg. All acids should not be stored together
    - Flammable materials must never be stored in domestic-type refrigerators. Only explosion-proof or flammable material refrigerators should be used for storage of these chemicals within a laboratory environment

- All containers stored within the refrigerator should be tightly capped to keep vapours from interacting with each other and must be properly labelled

- Visual inspection of the material and its container should be done routinely. Indications for disposal include
    - cloudiness of liquids
    - colour change
    - evidence of transformation of liquids to solids or solids to liquids
    - evidence of pressure build-up within the bottle
    - deterioration of container

- Chemicals that are no longer to be used should be properly disposed

- Adequate security must be provided so that unauthorized personnel do not have access to hazardous material stores

The hazard symbols

They are provided as a guide for quick recognition of the acute hazards associated with chemicals. Laboratory workers should be familiar with the hazard symbols used in the labels of chemical containers.

Flammable chemicals
These chemicals need to be kept away from heat and substances that may ignite or explode. They are stored in specially designed cupboards or cabinets. Fire-extinguisher should be available

Oxidizing substances
Oxidizing substances can ignite flammable and combustible material and worsen existing fire. Should be kept away from flammable, combustible and spontaneously combustible materials

Corrosive chemicals
They can react violently and explosively with contact of some types of chemicals. These substances destroy living tissue and equipment. Inhalation of vapours and contact with skin and eyes should be avoided.

Toxic chemicals
These chemicals are known to be carcinogenic or teratogenic (eg. Sodium azide). Inhalation, swallowing or contact with skin should be avoided. When an exposure is reported, contact a physician immediately.

Explosive chemicals
These chemicals may undergo a rapid chemical change producing large amounts of heat and gases, when subjected to an initiating stimulation, such as heat, impact, contamination or friction. Only non-metallic materials should be used around explosive chemical. They should be stored with height less than 6 feet (eg. ether, perchloric acid, picric acid and picrates).

Environmentally toxic chemicals
These chemicals contain compounds which are directly harmful to the environment. They should be released to the environment after treatment.

Radioactive chemicals

These are substances which have measurable radioactivity. Use proper protective shields and equipment when handling. The area must be labelled with radioactive warning signs.

Figure 13: Signs of different types of chemicals

Incompatible chemical groups

Many common laboratory chemicals react dangerously if they come into contact with each other. The following substances in the two columns below should not be in contact together.

Table 6: Incompatible chemical groups

Substance category	Incompatible substances
Alkali metals, eg. sodium, potassium, caesium and lithium	Carbon dioxide, chlorinated hydrocarbons, water
Halogens	Ammonia, acetylene, hydrocarbons
Acetic acid, hydrogen sulfide, aniline, hydrocarbons, sulfuric acid	Oxidizing agents, e.g. chromic acid, nitric acid, peroxides, permanganates

Specific Chemicals

Acetic acid ⟷ Chromic acid, Nitric acid, Hydroxyl compounds, Ethylene glycol, Perchloric acid, Peroxides, Permanganates

Acetone ⟷ concentrated Sulfuric and Nitric acid mixtures

Acetylene ⟷ Copper (tubing), Halogens, Silver, Mercury and their compounds

Ammonia (anhydrous) ⟷ Mercury, Halogens, Calcium hypochlorite and Hydrogen fluoride

Ammonium nitrate ⟷ Acids, metallic powders, flammable liquids, Chlorates, Nitrates, Sulfur and finely divided organic or combustible compounds

Aniline	⟷	Nitric acid and Hydrogen peroxide
Carbon (activated)	⟷	Calcium hypochlorite, Oxidizing agents
Chlorates	⟷	Ammonium salts, acids, metallic powders, Sulfur, finely divided organic or combustible compounds and Carbon
Chlorine	⟷	Ammonia, Acetylene, Butadiene, Benzene and other petroleum fractions, Hydrogen, Sodium carbide, Turpentine, and finely divided metals
Chromic acid	⟷	Acetic acid, Naphthalene, Camphor, Alcohol, Glycerol, Turpentine and other flammable liquids
Copper	⟷	Acetylene, Azides and Hydrogen peroxide
Cyanides	⟷	Acids and Alkalis
Flammable liquids	⟷	Ammonium nitrate, Chromic acid, Hydrogen peroxide, Nitric acid, Sodium peroxide and Halogens
Hydrocarbons (general)	⟷	Fluorine, Chlorine, Formine, Chromic acid, Sodium peroxide
Hydrogen peroxide	⟷	Chromium, Copper, Iron, most other metals, their salts, flammable liquids and other combustible products, Aniline, and Nitromethane
Hydrogen sulfide	⟷	fuming Nitric acid and oxidizing gases
Iodine	⟷	Acetylene and Ammonia
Mercury	⟷	Aceylene, Fulminic acid, Hydrogen
Nitric acid	⟷	Acetic, Chromic and Hydrocyanic acids, Aniline, Carbon, Hydrogen sulfide, fluids, gases and other substances that are readily nitrated
Oxygen	⟷	oils, greases, Hydrogen and flammable liquids, solids and gases
Oxalic acid	⟷	Silver and Mercury

Perchloric acid	⟷	Acetic anhydride, Bismuth and its alloys, Alcohol, paper, wood and other organic materials
Phosphorus pentoxide	⟷	water
Potassium permanganate	⟷	Glycerol, Ethylene glycol, Benzaldehyde and Sulfuric acid
Sodium azide	⟷	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 a plumber is working on it
Sulfuric acid	⟷	Chlorates, Perchlorates, Permanganates and water

The list is not exhaustive and the absence of any particular chemical does not imply that it is non-hazardous. All chemicals should be treated with caution.

Handling of compressed gases

Gas cylinders should be properly chained and stored in a well-ventilated area. The metal cap which is removed when the regulator is installed should always be in place when the gas cylinder is not in use. Cylinders should be transported, chained.

Disposal of chemical waste

The disposal of chemical waste should be done safely, in compliance with government regulations and legislation. Please refer to the MSDSs and other sources for safe methods of disposal of specific chemicals.

Routes of exposure to chemicals

Route of exposure describes the way the chemical enters the body. Chemicals may have serious effects by one route, and minimal effects by another. Exposure to hazardous chemicals may occur by

- inhalation – absorption through the respiratory tract
- ingestion–absorption through the digestive tract
- dermal contact – absorption through the intact skin/eye

- injection–directly into the blood stream
    - needlestick
    - through broken skin

Exposure to chemicals

- Chemical spillage
  The following equipment should be available in dealing with chemical spillages.
    - protective clothing (heavy duty rubber gloves, overshoes or rubber boots, respirators)
    - scoop and dust pans
    - forceps for broken glass
    - mops, cloth and paper towels
    - buckets
    - soda ash (sodium carbonate or sodium bicarbonate for neutralizing acids)
    - sand
    - non-flammable detergents

  Chemical spillages are dealt with, by appropriate neutralization process according to the manufacturer's instructions.
        - Acids and corrosive chemicals - cover with soda ash (sodium carbonate)
        - Alkalis - cover with sand/ammonium chloride

  A large chemical spillage
    - Stop all the activities and equipment
    - The room should be evacuated and the windows should be opened
    - If the spill material is flammable all open flames in the room concerned and adjacent rooms should be extinguished and all electrical equipment that could spark should be switched off
    - If volatile, flammable, or toxic material spill, shut off flames and spark-producing equipment at once and evacuate and inform the supervisor

  A small chemical spillage
    - Clean up small spills immediately
    - Inform the supervisor

- Chemical Burns

  If hazardous chemicals should come into contact with skin or eyes, follow the first aid procedures below.

Skin

- Remove garments as required and rinse the affected area with large quantities of water for at least 15 minutes (sink, shower or hose)
- Do not apply burn ointments/spray to affected areas
- Seek medical advice without delay

Eyes

- Rinse area of eyes, eyelids and face thoroughly with lukewarm water for at least 15 minutes at the eye wash station
- Seek medical advice without delay

References

- Carl AB, Edward RA, David EA. Tietz Textbook of Clinical Chemistry General Laboratory technics and Procedures. 2nd Edition. St Louis: Elsevier Sounders; 1986.
- Carl AB, Edward RA, David EA. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th Edition. St Louis: Elsevier Sounders; 2006.
- Furr AE, CRC Hand book of Laboratory Safety. 5th Edition. Cleveland: CRC Press; 2000.
- Globally harmonized system of classification and labelling of chemicals (GHS): 4th Edition. Europe: United Nations Economic Commission; 2011.
- Kaplan LA, Pesce A. Clinical Chemistry Theory Analysis and Correlation. 3rd Edition. Philadelphia: CV Masby Company; 1996.
- Kaplan LA, Pesce A. Clinical Chemistry Theory Analysis and Correlation. 4th Edition. Philadelphia: CV Masby Company; 2003.
- Laboratory Biosafety Manual. 3rd Edition. Malta: World Health Organization; 2004.
- Pipitone DA. Safety in academic Chemistry Laboratories. American Chemical society committee on Chemical Safety. 4th Edition. Washington: Wiley inter-science Publication; 2012.
- Stephen KH. Chemical safety in the Laboratory. Cleveland: CRC Press; 1993.
- Varly H, Alan HG, Bell M. Practical Clinical Biochemistry. 5th Edition. London: Butterworth Heinemann Ltd, CBS Publisher; 1980.

Chapter 16

Electrical and Fire Safety

Electrical Safety

All electrical installations and equipment should be inspected and tested regularly by qualified personnel. The laboratory staff should be made aware of the electrical hazards.

- All the electrical appliances should be earthed
- Do not overload electrical outlets
- Ensure that electrical outlets have safety caps
- Keep electrical cords away from gas and water
- Do not use extension cords in the laboratory
- Keep sparking equipment away from flammable substances
- Do not keep electrical equipment switched on and unattended

Fire Safety

Laboratory fires present additional risks to workers, fire fighters and the environment due to possible dissemination of infectious, radioactive and toxic materials.

Fires are grouped into four classes, according to the type of material involved. Different types of extingushes are needed to extingush different types of fire. This may determine whether to extinguish or contain the fire.

Class A fire - ordinary combustibles such as wood, cloth and paper
Class B fire - flammable liquids, oil and grease
Class C fire - fires involving live electrical equipment
Class D fire - combustible metals such as magnesium, potassium and sodium

Laboratory fuels and sources of ignition

The most hazardous materials are

- methanol, ethanol and other alcohols
- diethyl ether, toluene, acetone and various combinations of these
- other chemicals such as alcohol-based stains, fixatives and ethanol or propanol disinfectants
- fuel gases such as butane, methane and propane (supplied as gases or as liquid petroleum gas - LPG), acetylene and hydrogen supplied in compressed gas cylinders

The obvious ignition sources are

- oxidant gases
- Bunsen burners or other naked flames
- kerosene and gas operated ovens
- appliances with hot surfaces or heating elements
- boilers, incinerators
- lighters and matches used by smokers

Ignition sources that are not immediately obvious are

- arcing or sparking when electrical circuits are broken eg. switches in lighting or power supplies
- temperature control (thermostat) devices
- static electricity discharges to earth
- overheating in faulty electrical equipment
- overloading of electrical circuits by connecting too many appliances to a single socket outlet

The basic aims of a fire prevention strategy are

- avoiding formation of any flammable gas or vapour mixture
- preventing contact between any flammable gas or vapour-air mixture and any ignition source

Adopt following measures to minimize fire hazards

- Use of non-flammable materials or substances
- Where this is not possible, use of materials or substances that offer the least fire hazard
- Use smallest possible quantities of flammable material. Stock the minimum amounts in the laboratory
- Prevent accumulation of flammable gases and vapours by adequate ventilation (natural or mechanical)
- Containers and vessels containing liquids or gases should be kept securely capped or closed
- Whenever possible, handle flammable substances in an exhaust cupboard or hood or in a well-ventilated area of the laboratory
- Use lipped trays to prevent or restrict the spillage of highly flammable liquids
- Never smoke in the laboratory
- Allow at least two meter distance between open flames
- Install fire alarms
- Display fire action notices in all laboratories
- Post fire evacuation plans showing the nearest exit in case of fire. Mark fire exits prominently and keep them free of obstacles
- Educate the laboratory staff on how to activate fire alarms and what action to take when the alarms are heard
- Employees should be trained to use fire extinguishers
- Conduct fire drills every 6 months
- Inspect fire extinguishers every 6 months

Fire safety equipment

- Fire alarms
  - Fire alarms are designed so that all laboratory personnel and occupants of the building are alerted by an audible warning
  - All employees should become familiar with the exact location of the alarm pull stations nearest to their laboratory
  - The fire alarms should be tested regularly
  - Installation of sprinkler systems, smoke detectors and heat detectors are recommended

- Fire extinguishers
  These are portable devices used to put out fires of limited size**.** Each class of fires requires its own type of fire extinguisher.

  - For class A fires: ordinary combustibles such as wood, cloth and paper
    These are usually water based. Water provides a heat-absorbing (cooling) effect on the burning material to extinguish the fire. Stored-pressure extinguishers use air under pressure to expel water. Pump-tank extinguishers are operated by a hand pump.

  - For Class B fires: flammable liquids, oil and grease
    Class B fires are extinguished by excluding air, by slowing down the release of flammable vapours, or by interrupting the chain reaction of the combustion.
    Three types of extinguishing agents, carbon dioxide gas, dry chemicals and foam are used for fires involving flammable liquids, grease and oils.

  - For Class C fires: fires involving live electrical equipment
    The extinguishing agent in class C fire extinguishers must be electrically non-conductive. Both carbon dioxide and dry chemicals can be used in electrical fires. An advantage of carbon dioxide is that it leaves no residue after the fire is extinguished. When electrical equipment is not energized, extinguishers for Class A and B fires may be used.

  - For Class D fires: combustible metals such as magnesium, potassium and sodium
    A heat-absorbing extinguishing medium is needed for fires involving combustible metals. Also, the extinguishing medium must not react with the burning metal. The extinguishing agents known as dry powders cover the burning metal and provide a smothering blanket.

The extinguisher label gives operating instructions and identifies the class or classes of fire on which the extinguisher may be used safely.

Figure 14: Fire extinguisher sign

Table 7: Fire extinguisher colour code

Type of extinguisher	Colour
Water	Red
Dry powder	Blue
Foam	Cream
Carbon dioxide	Black
Vapourising liquid	green
Wet chemical	Yellow

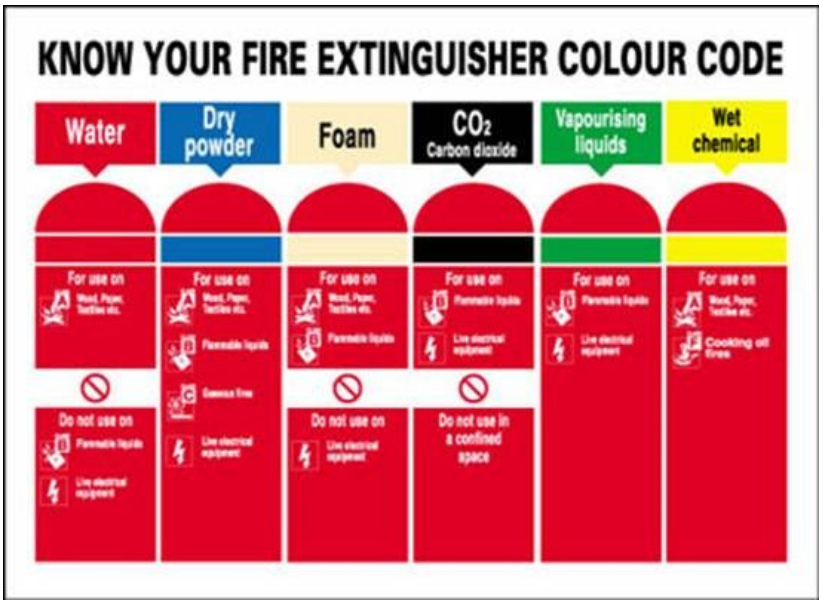


Figure 15: Fire extinguisher colour code

Combination type ABC extinguishers are found in most laboratories.

All laboratories are required to have fire extinguishers, which have to be clearly labelled and accessible. Usually a notice is placed above the fire extinguisher indicating clearly and briefly which type of fire it can put out.
Fire extinguishers may go unused for many years, but they must be maintained in a state of readiness. For this reason, periodic inspection and servicing are required and that responsibility rests with the laboratory.

Following steps should be followed in case of fire

- Raise the alarm by breaking a red fire alarm call point (if available)
- Rescue any injured individual
- Extinguish the fire, if possible
- Leave by the nearest exit and proceed to the assembly point
- Do not stop to collect belongings or work items
- Do not use the lift
- Contain the fire by closing doors and windows to limit spread of fire as you pass by, as long as this does not delay your exit
- Report to the Head of the Institution/Fire Service Department immediately
  (Fire Service Department – Telephone no: 0112422222, Emergency-Colombo 110)
- Return only after receiving authority from the Fire Service Department officer or the Departmental Fire Safety Manager

Reference

- Biosafety in Microbiological and Biomedical Laboratories. 5th Edition. USA: HHS Publication No. (CDC) 21-1112; 2009.
- Laboratory Biosafety Manual. 3rd Edition. Malta: World Health Organization; 2004.

Chapter 17

Radiation Protection and Safety Programmes in Health Care Laboratories

The Head of the Institution should obtain a license for transport, storage and processing of radioisotopes from the Atomic Energy Authority (AEA) of Sri Lanka by forwarding an application to

Atomic Energy Authority
No- 60/460, Baseline Road, Orugodawatta, Wellampitiya
Tel : 0112533427-8 or 0112533449
Fax : 0112533448
E-mail: officialmail@aea.gov.lk

Always follow the guidelines established by the AEA.
The Radiation Protection officer appointed by the AEA (a trained member of the staff) is responsible for the environment and personnel protection within the institution.

Workplace monitoring

Area and air borne monitoring methods, frequency of measurements, reference levels and action to be taken if the limits are exceeded should be established in the laboratory.

Personnel monitoring

- Radiation workers should be provided with adequate information regarding health risks due to exposure to radioisotopes
- Radiation protection officer shall ensure that all personnel are adequately trained in correct operating procedures
- Thermoluminescent Dosimeters (TLD), Direct reading Dosimeters should be made available to all the radiation workers. AEA provides this service at a nominal fee
- Radiation workers should always wear the protective clothing (masks, goggles, laboratory coats, aprons, foot wear with front cover, gloves and TLDs)
- Periodic checking of the personnel monitoring service by the AEA should be made available and action to be taken in situations where the dose exceeds the reference levels should be established
- The policies regarding female workers who become pregnant should be established

- A programme of health surveillance based on occupational health to assess the initial and continuing fitness of workers for their intended tasks should be established

Facilities and equipment

- Approval of the design of the laboratory should be obtained from the AEA. The thickness of the walls, type of ceiling, drainage ducts (sinks, delay tanks), storage, processing areas and air conditioning system should be specified and approved by the AEA
- The radiation signal should be displayed at the entrance
- Entry into the laboratory should be restricted to authorized personnel
- Appropriate radiation monitoring and measuring equipment should be made available (survey meters, contamination monitors, dose calibrators) and they should be calibrated and certified by the AEA yearly
- Personnel protection equipment (lead shields, lead bricks, fume cupboard, vial shields, remote handling tools, forceps) should be made available at their work stations

Standard conditions and limitation of accumulation and disposal of radioactive waste

The Head of the institution shall ensure,

- that the accumulation and disposal of the radioactive waste is supervised by a person (Radiation Protection officer of the institution) who is competent and would be able to secure compliance with the limitations and conditions specified by the AEA
- that the name of the person supervising the accumulation and disposal is clearly displayed in the laboratory to prevent any loss or escape and access to unauthorized persons
- that the waste be accumulated in suitable containers with the word "Radioactive" clearly marked on it and with the ‘ionizing radiation symbol’ pasted onto the container. User should follow reasonably practical methods of disposal approved by the AEA regarding liquid, solid and gaseous radioactive waste as specified in the license

Figure 16: Ionizing radiation sign

- that eating, drinking, smoking and application of cosmetics are strictly prohibited in the laboratory

Management of radiation accidents

User shall ensure that all the laboratory personnel are well trained in handling emergency procedures according to the code of practice published by the AEA.
Specific instructions given by the AEA should be displayed in the laboratory to manage the following

- minor spillage
- major spillage
- loss of shielding from a sealed radioactive source
- lost or stolen radioactive source
- accidental exposure of a person to ionizing radiation
- external and internal contamination of a person with radioactive material
- transport of radioactive material

Emergency kits for decontamination of personnel, working areas and inanimate objects should be made available in the laboratory. All the accidents be recorded and the AEA should be informed immediately by a telephone message.

Transportation of radioisotopes

- Radioactive material should be transported in the official vehicle of the institution. It should be accompanied by a responsible officer who is capable of handling radio-isotopes safely
- A contamination monitor specific for the radio-isotopes being transported should be carried along with the package
- A document containing following information of the radioisotope should be carried by the officer who accompanies the package
    - activity
    - sealed/unsealed
    - solid/liquid/gas
- If there is a leakage of contents during transport or loss of radio-active material, the AEA radiation protection officer should be contacted immediately

References

- Laboratory Biosafety Manual. 3rd Edition. Malta: World Health Organization; 2004.
- Radiation Accident procedures: code of practice draft - No. 1 – 1991. Atomic Energy Authority of Sri Lanka.

Chapter 18

Pest Management

Pest management is an important part in the management of a microbiology laboratory. Many kinds of pests, such as flies, ants, termites, rats and cockroaches can mechanically transmit disease pathogens and compromise the laboratory environment.

The most common approach to pest control is the use of pesticides. Pesticides can be effective but they have limited long-term effect when used alone. Therefore, it is necessary to employ a comprehensive program approach that integrates housekeeping, maintenance and pest control services.

The facility should be designed with features to exclude pests and minimize pest habitats by ensuring proper sanitation, reducing clutter and performing repairs.

Safe use of pesticides

- Proper pesticide for the specific purpose should be chosen
- Pesticides should be transported and stored safely
- Proper techniques, equipment and protective attire for use of pesticides, should be made available to the staff
- Safe disposal of surplus pesticides is very important

References

- Laboratory Biosafety Manual. 3rd Edition. Malta: World Health Organization; 2004
- Metcalf RL, Luckmann WH. Editors. Introduction to Insect Pest Management. 3rd Edition. USA: John Wiley & Sons Inc.; 1994.

Annexure 1

Risk Groupsof Microorganisms

Risk group 1

Examples of RG1 Agents:
Bacillus subtillis(asporogenic) *Bacillus lichenformis* *Escherichia coli* K-12 Adeno-associated virus (AAV) types 1 through 4

Note: Agents not listed on Risk Groups 2, 3, and 4 are not automatically or implicitly classified in Risk Group 1. A riskassessment must be conducted based on the known and potentialproperties of the agents and their relationship toagents that are listed.

Risk group 2*

Bacteria	*Abiotrophia spp.* *Acinetobacter*spp. *Actinobacillus*spp. *Actinomyces*spp. *Aeromonashydrophila* *Arcanobacteriumhaemolyticum* *Bacillus cereus* *Bartonellahenselae, B. quintana, B. vinsonii* *Bordetella pertussis* *Borreliarecurrentis, B burgdorferi* *Burkholderia*spp. (except *B. mallei),* *Burkholderiapseudomallei* *Campylobaeter coli. C. fetus.* C.*jejuni* *Capnocytophagacanimorsus* *Chlamydia* spp. (except C. *psittaci)* *Clostridium* spp. *Corynebacteriumdiphtheria,* C. *renale,* C.*pseudotuberculosis* *Dermatophiluscongolensis* *Edwardsiellatarda* *Eikenellacorrodens* *Enterococcus* spp. (Vancomycin-resistant strains) *Erysipelothrixrhusiopathiae*	*Kingellakingae* *Klebsiella*spp. *Legionella* spp. *Leptospirainterrogans*(all serovars) *Listeria spp,* *Moraxella* spp. *Mycobacterium*spp. other than *M. tuberculosis complex* *Mycoplasma pneumoniae* *Neisseria gonorrhoeae* *N. meningitidis* *Nocardia*spp. *Oligella*spp. *Pasteurella*spp. *Pseudomonas* spp. *Rhodococcusequi* *Salmonella* serovars *Salmonella* Paratyphi A and B *Salmonella* Typhi *Serratia*spp. *Shigella*spp. *Sphaerophorusnecrophorus* *Staphylococcus aureus* *Strenotrophomonasmaltophilia*

	Pathogenic *Escherichia coli* – all enterophathogenic,enterotoxigenic, entroinvasive and strainsbearing K1 antigen, including *E. coli* O157:H7 *Fusobacterium*spp. *Gardnerellavaginalis* *Haemophilusinfluenzae, H. ducreyi* *Helicobacter pylori*	*Streptobacillusmoniliformis* *Streptococcus pyogenes* *S. pneumoniae* *Treponemapallidum* *Ureaplasmaurealyticum* *Vibrio cholera, V. parahaemolyticus, V. vulnificus* *Yersinia* spp. (except *Y. pestis)*
Viruses	*Adenoviridae*–human Adenovirus all types *Arenaviridae* - Arenavirus Lymphocytic choriomeningitis (LCM) non-neurotropic strains - Tacaribc virus complex *Caliciviridae* - Feline calicivirus - Norovirus - Sapporo-like - Largovirus - Rabbit haemorrhagic disease *Coronaviridae* - Coronavirus other than SARS coronavirus - SARS coronavirus (tests not involving replication) *Flaviviridae* • Flavivirus - Dengue 1, 2, 3 and 4 - Japanese encephalitis (Nakayama strain) - Kokobera - Kunjin - Murray Valley encephalitis - West Nile (Sarafend strain) - Saumarez Reef - Yellow fever (strain 17D) • Hepacivirus - Hepatitis C *Hepadnaviridae* - Duck hepatitis B - Hepatitis B *Herpesviridae* • Alphaherpesvirinae - Simplex	*Parvoviridae* - Human parvovirus *Picornaviridae* Cardiovirus - Encephalomyocarditis virus Hepatovirus - Hepatitis A virus Human Enterovirus - Coxsackievirus - Echovirus - Enterovirus - Poliovirus 1, 2 and 3 Parechovirus Rhinovirus *Poxviridae* Orthopoxvirus - Vaccinia Parapoxvirus - Orf *Reoviridae* Orbivirus - Bluetongue viruses (endemic strains) - Epizootic haemorrhagic disease viruses of deer (endemic strains) Rotavirus - Rotavirus *Retroviridae*(serology, other tests on samples) Oncovirinae - Human lymphotropic virus 1 - Human lympbotropic virus 2 Lentivirinae - Human immunodeficiency virus

	- Varicellac - Betaherpesvirinae - Cytomegalovirus • Gammaherpesvirinae - Herpes 6 and 7 - Lympbocryptovirus (EB-like viruses) *Orthomyxoviridae* Influenza (all strains and candidate vaccine strains) *Paramyxoviridae* • Paramyxovirinae Morbilivirus - Measles Rubulavirus - Menangle - Mumps - Human parainfluenza 2 and 4 Avulavirus - Newcastle disease (non-virulent enzootic strains) - Avian paramyxoviruses 2 to 9 Respirovirus - Sendai - Human parainfluenza 1 and 3 • Pneumovirinae Pneumovirus - Respiratory syncytial • Metapneumovirinae Metapneumovirus - Avian metapneumovirus - Human metapneumovirus	*Togaviridae* Alphavirus - Barmah Forest - Ross River - Scmliki Forest Arterivirus - Equine viral arteritis Rubivirus - Rubella Unclassified - Hepatitis D, Hepatitis E
Parasites	*Ancylostomaduodenale* *Ascarislurnbricoides* *Babesiadivergens* *Babesiamicroti* *Brugia*spp. *Clonorchissinensis* *Cryptosporidium* spp. *Echinococcus*spp. *Entamoebahistolytica* *Giardia duodenalis*(also known as *Giardia lambliaand Giardia intestinalis)* *Hymenolepisdiminuta* *Hymenolepis nana* *Leishmania*(mammalian) spp.	*Loa loa* *Naeglariafowleri* *Necatoramericanus* *Opisthorchis*spp. *Plasmodium* (human and simian) *Strongyloidesstercoralis* *Taeniasaginata* *Taeniasolium* *Toxocaracanis* *Toxoplasma gondii* *Trichinellaspiralis* *Trypanosomabrucei*subspp. *Trypanosomacruzi* *Wuchereriabancrofii*

Fungi	*Aspergillusfimligatus*and *A.flavus* *Candida albicans* *Cladophialophora*spp. *Cryptococcus gattii* *Cryptococcus neoformans* *Epidermophytonfloccosum*	*Exophiala (Wangiella) dermatitidis* *Fonsecaeapedrosoi* *Microsporum*spp. *Scedosporium*spp. *Sporothrixschenckii* *Trichophyton*spp.

*This list is not exhaustive.

Risk group 3*

Bacteria	*Bacillus anthracis* *Bartonellabacilliformis* *Burkholderia mallei* *Brucella*spp. *Chlamydia psittaci* *Coxiellaburnetii* *Francisellatularensis* *Mycobacterium tuberculosis* complex (except BCG) *Rickettsia* spp. *Yersinia pestis*
Viruses	*Arenaviridae* • Arenavirus - Lymphochoriomeningitis (LCM) neurotropic strains *Bunyaviridae* • Group C - Oropouche - Phlebovirus • Hantavirus - Hantaan and related viruses *Coronaviridae* - SARS coronavirus (from cultures and concentrates) *Flaviviridae* • Flavivirus - Japanese encephalitis, St Louis cncephalitis, Tick-borne viruses, West Nile, Yellow fever *Orthomyxoviridae* Avian influenza (exotic pathogenic strains),Influenza (highly pathogenic strains) *Paramyxoviridae* • Paramyxovirinae Rubulavirus - Mapuera Avulavirus - Newcastle disease (exotic strains) *Retrroviridae*(from cultures and concentrates)

	• Oncovirinae - Human Iymphotropic virus 1 - Human lymphotropic virus 2 • Lentivirinae - Human immunodeficiency virus *Rhabdoviridae* • Lyssavirus - Australian bat lysavirus - Rabiesfixed strain (CVS II) *Togaviridae* • Alphavirus - Chikungunya - Eastern equine encephalitis - Western equine encephalitis - Venezuelan equine encephalitis
Parasites	None
Fungi	*Blastomycesdermatitidis* *Coccidioidesimmitis* *Coccidioidesposadasii* *Histoplasma*spp. *Paracoccidioidesbrasiliensis* *Penicilliummarneffei*
Prions	Gertsmann-Straussler syndrome Kuru and Creutzfeldt-Jakob agents

*This list is not exhaustive.

Risk group 4*

Bacteria	None
Viruses	*Arenaviridae* • Arenavirus - Guanarito - Junin - Lassa - Machupo - Mopeia viruses - Sabia *Runyaviridae* • Nairovirus - Crimean-Congo hemorrhagic fever - Hazara *Filoviridae* – Ebola, Marburg

	Flaviviridae • Flaviviruses Absettarov, Central Europeanencephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever disease,Russian spring summer encephalitis, Tick-borne encephalitis *Herpesviridae* • Alphaherpesvirinae - Herpes virus simiae (8 virus) *Paramyxoviridae* • Paramyxovirinae Henipavirus – Hendra, Nipah
Parasites	None
Fungi	None

*This list is not exhaustive.

References

- Safety in laboratories, Part 3: Microbiological aspects and containment facilities (AS/NZS 2243.3:2010) prepared by the Joint Standards Australia/Standards New Zealand Committee CH-026; 2010.
- Caltech Environment, Health and Safety - Biohazardous Agents Classification, California, USA