BIOSAFETY MANUAL

INDIAN INSTITUTE OF TECHNOLOGY BOMBAY

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Scope

This biosafety manual is applicable for all bio research activities carried out in Institute laboratories.

Introduction

The advancement in the field of bio research has increased the risks to personnel involved in research activities. The bio samples handled may be infected with pathogens and the manipulation of host cells may produce toxic effects. This calls for adoption of good microbiological techniques and appropriate facilities while carrying out bio research activities.

Apart from this, other hazards involved in microbiological laboratories, involves handling of hazardous chemicals, radioactive chemicals, liquid nitrogen, etc.

Classification of infective microorganisms by risk group

Risk Group 1

A pathogen that is unlikely to cause any disease in humans or animals.

Risk Group 2

A pathogen that can cause disease in humans or animals but is unlikely to be a serious hazard.

Effective treatment and preventive measures are available and the risk of spread of infection is limited.

Risk Group 3

A pathogen that can cause serious human or animal disease, but does not ordinarily spread from one infected person to another.

Effective treatment and preventive measures are available.

Risk Group 4

A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly.

Effective treatment and preventive measures are not usually available.

Risk Assessment

Risk assessment must be conducted before the commencement of the research activities to identify the hazards and control measures to be adopted to prevent laboratory associated infections.

The input from the risk assessment can be used in selecting suitable practices, equipments and facility requirements.

The risk assessment must be carried out in the following steps:

- Identify the hazards related with the pathogen/human/animal cells being handled

The hazardous characteristics of the pathogen include

- Routes of transmission
- Infective dose
- Stability in the environment
- Host range and its endemic nature.
- Method of treatment available.

The probable routes of transmission in the laboratory are:

- Exposure of the agent through the skin, eyes and mucous membranes.
- Parenteral inoculation by a syringe needle or other contaminated sharp.
- Bites, scratches from infected animals.
- Ingestion of liquid suspension of an infectious agent.
- By contact of the contaminated hand to mouth exposure.
- Inhalation of infectious aerosols.

- Identify the hazards related to laboratory procedures

Processes that generate aerosols must be taken into consideration as they remain invisible to personnel in the laboratory and there is a risk of infection to everyone present or entering the lab.

The risk involved is greater if the pathogen is capable of being transmitted through the respiratory passage in the form of aerosols.

Experimental animals can transmit zoonotic agents through saliva, urine or feces.

Genetic manipulation could increase an agent's pathogenicity or affect its susceptibility to antibiotics or other effective treatments. The risk involved in experimental alteration of virulence genes may lead to increased risk.

Technical proficiency in the use of microbiological practices, the person's training, experience in handling infectious agents must also be taken into consideration.

Pre existing disease, medications and pregnancy can increase exposure to infants, are examples of some of the conditions that may increase the risk of an individual for acquiring laboratory acquired infections.

- Determine the appropriate biosafety level and determine the precautionary measures to be adopted.

The inputs obtained from risk assessment can be used in determining the biosafety level and associated precautions for the same.

- Review the risk assessment

The risk assessment that is done must be reviewed and modified whenever there is a change in the agent used, modification of practices and equipment or on availability of new information of the hazards.

While carrying out risk assessment, the lab personnel must discuss with the Principal Investigator and Co PI, other experienced personnel having competency in the area, refer literature providing information on the hazards of the agent and exposures that have happened in the past, etc.

The existing system, facilities and control measures available in the laboratory must also be taken into consideration.

Containment

The term containment is used to describe the safe work practices adopted in handling of pathogenic agents in the laboratory to reduce exposure to personnel and contamination of the environment**.**

Types of containment

- Biological containment

Any combination of vector and host which is to provide biological containment must be chosen to limit the infectivity of vector to specific hosts and control the host-vector survival in the environment.

- Physical containment

Physical containment is achieved through

- laboratory practices
- containment equipment
- laboratory facility

Containment equipment includes biosafety cabinets, enclosed containers and other engineering controls to minimize exposures.

Personal protective equipment (PPE) must be used where it is not practical to use Biosafety cabinets (BSCs) and also in combination with BSCs.

PPEs include lab coats, coveralls, safety glasses/goggles, face shield, respirators for protection from aerosols, hand gloves, toe covered footwear, etc.

Special laboratory design and construction will act as secondary barrier to protect personnel within the laboratory and outside in case of release of infectious agents.

The facilities vary based on the risk level. Laboratory facilities are designated as

- Basic- Biosafety Level 1
- Basic- Biosafety Level 2
- Containment-Biosafety Level 3
- Maximum Containment-Biosafety Level 4.

The assignment of a biosafety level takes into consideration the pathogenic agent handled, the facilities and equipment available, the practices and procedures required to conduct work safely in the laboratory.

Biosafety levels

The four biosafety levels correspond to four risk groups of pathogens.

The infectivity, severity of disease, transmissibility and whether the agent is exotic or indigenous are taken into account in defining the biosafety levels. A pathogen in the low risk group can be assigned a higher biosafety level if the risk assessment conducted desires so.

Biosafety level 1

The facilities in which work is done with defined strains of microorganisms not known to cause any disease in healthy adult human.

Requires no special facility or equipment, but the laboratory personnel must be trained and supervised.

The standard practices, safety equipment and facility requirements for Biosafety level 1 are as follows.

Standard microbiological practices

- Access to the laboratory must be controlled.

- Hands must be thoroughly washed after handling of pathogens and also at the end of the work while leaving the laboratory.

- Food items must not be stored or consumed in the laboratory.
- Mouth pipetting must not be practiced in the lab, mechanical pipetting devices must be used.
- Work practices followed must reduce the risk of injuries while handling sharps.
- Broken glass pieces must be handled only with forceps or dustpan and brush.
- Perform the procedures in a manner such as to reduce splashes or aerosols.
- Decontaminate the work area after the completion of work or after any spill or splash.
- Materials/waste to be decontaminated must be segregated in leak proof bins.
- The lab in charge must ensure that the personnel working are aware of the potential hazards and precautions to be taken and they are updated whenever there are changes in procedures.

Safety equipment (primary barriers and personal protective equipment)

- Special containment devices or equipment are normally not required.
- Lab coats, eye protection and toe covered footwear to prevent contamination must be used.
- Respiratory protection must be adopted if there is a risk of spread of infection through inhalation.
- Face shields must be used to protect the personnel from splashes.
- Hand gloves must be selected based on the risk involved and same must be inspected for damage before use.

- Hands must be washed at the end of the work even if hand gloves were worn.
- Do not reuse disposable gloves.
- Bench top must be impervious to water and resistant to chemicals.
- Protective clothing used inside the lab must not be worn outside.
- The sink must be provided near the exit and preferably must be hands free.

Biosafety level 2

-Applicable in facilities in which work is done with the moderate-risk agents which are associated with human disease of varying severity.

-Requires specific training in handling pathogens and to be supervised by competent personnel.

-The pathogenic agents must be handled in biosafety cabinets and access to the laboratory must be controlled.

-For biosafety level 2 the practices and equipment used, must include those mentioned under biosafety level 1 in addition to the following.

Standard microbiological practices

- Laboratory personnel must be specifically trained in handling the pathogenic agents. They must be supervised by personnel who are competent in working with the pathogenic agents.
- Access to the laboratory must be restricted when work is being carried out.
- The international biohazard warning symbol must be displayed on the doors of the labs where microorganisms of Risk Group 2 or higher risk groups are handled. Name of the PI and contact details must also be displayed.

BIOHAZARD

ADMITTANCE TO AUTHORIZED PERSONNEL ONLY
Biosafety Level: _____
Responsible Investigator: _____
In case of emergency call: _____
Daytime phone: _____Home phone: _____
Authorization for entrance must be obtained from the Responsible Investigator named above.

Special practices

- Personnel entering the laboratory must be adviced of the potential hazards.
- Medical surveillance and immunization must be provided to the lab personnel.
- Spills involving contaminated material must be decontaminated and cleaned up.
- Equipment in the laboratory must be subjected to maintenance as per the manufacturer's recommendation.
- Equipment must be decontaminated after repair and before reuse.

Laboratory facilities (Secondary barriers)

- The lab doors must be self closing type and there must be provision for locking the same.
- Handling of pathogens must be done inside the biosafety cabinet.

- BSC must be located away from doors and windows that could be opened which could create disruptions in the airflow.
- An eye wash station must be installed in the laboratory to flush the eyes in case of contamination.

- Liquid disinfectant trap must be provided to vacuum lines.

Biosafety level 3

-Suitable for facilities in which work is done with exotic agents with risk of infection by aerosols and which can cause diseases with serious or lethal consequences.

-Personnel handling the pathogens require specific training and be supervised by personnel experienced in the same.

-Laboratory must be designed especially for handling the pathogen, with biosafety cabinets and access to be strictly controlled.

-The following standard and special safety practices, equipment, and facility requirements apply to BSL 3 in addition to those required for BSL 1 and BSL 2.

Safety equipment (Primary Barriers and Personal Protective Equipment)

- All manipulations involving infectious pathogens must be carried out in BSC II or III.
- Personnel shall use wrap around gowns or coveralls.
- Reusable clothing to be decontaminated before being laundered.
- Eye and face protection must be decontaminated before use.

Laboratory facilities (Secondary barriers)

- Laboratory must be separated from areas where there is continuous movement of personnel.

- Access must be restricted and through two self closing doors with a change room in between.
- The space between the exits and windows must be capable of sealing for fumigation.
- Vacuum lines must be protected with high efficiency particulate air (HEPA) filters.
- The ventilation system must provide directional airflow by drawing air from clean areas to contaminated areas.

Biosafety level 4

The practices, equipment and facilities are applicable to work with dangerous pathogenic agents which pose a high individual risk of life threatening disease.

In addition to the requirements of BSL I, II and III, the following measures apply to BSL 4.

- Personnel must be well trained in laboratory procedures.
- Work to be carried out in specially designed laboratories with stringent safety measures including Class III safety cabinet and positive pressure suit.
- Access to be strictly limited.
- Access to the laboratory must be through an airlock fitted with air tight doors.
- A shower to be provided to decontaminate the surface of the pressure suit before the person leaves the work area.
- The exhaust air from the suit area must be filtered by two sets of HEPA filters.
- Double door autoclave for decontamination of disposable waste materials from the suit area to be used.

Biosafety cabinets (BSCs)

- BSCs are designed to protect the operator, the laboratory environment from exposure to infectious aerosols which can be generated during the manipulation of infectious microorganisms.

- BSCs must be used whenever infectious materials are handled. If there is a risk of airborne infection such materials can be centrifuged outside the BSC if sealed safety cups are used and the same is loaded and unloaded in the BSC.

Examples of procedures that produce vigorous aerosols are

- centrifugation
- blending
- vigorous shaking or mixing
- intranasal inoculation of animals
- harvesting of infectious tissues from animals
- when using loops
- streaking agar plates
- pipetting
- making smears
- opening cultures
- homogenizing and vortexing infectious materials

- The personnel in the laboratory will be unaware of the generation of aerosols as the particles will remain invisible to the unaided eyes. One or several pathogens may be present in the respirable aerosol particles.

- These small aerosol particles can remain airborne for a long period of time and can disperse around the laboratory environment. Larger aerosol particles can fall over and contaminate the work area.

- The infectious aerosols can be inhaled by the user and it can cause cross contamination.

- BSC can prevent laboratory acquired infections and cross contaminations if used properly.

- They consist of high efficiency particulate air (HEPA) which traps 99.97% of particles greater than 0.3 micron in diameter from air.

- Horizontal and vertical outflow cabinets or clean air work stations must not be used in place of BSC.

- Carrying out regular maintenance as per the manufacturer's instructions helps in maintaining the efficiency of the filter.

BSCs are of three types

- Class I
- Class II
- Class III

Class I Biosafety cabinet

- The air is drawn through the sash opening over the work surface and through the HEPA filter contained in the exhaust. Only filtered air is passed out of the filter.

- The continuous flow of air into the sash offers protection to the user from the aerosols that are generated.

- The disadvantage is that it does not provide protection from contamination of the sample that is being handled as the room air passes over it.

Class II Biological safety cabinets

- The Class II BSC differs from Class I BSC in that it circulates HEPA filtered air over the work surface in addition to protection to user.

- Class II BSCs are of four types A1, A2, B1 and B2.

- They can be used for working with infectious agents in Risk Groups 1, 2, 3 and 4.

- Use of risk group 4 organisms require for the user to wear positive pressure suit.

Type A1 BSC

- The room air is drawn through the front grill by the fan. Air then flows through a HEPA filter and provides particulate free air over the work surface.

- Air flow so provided helps to reduce turbulence and minimise chances of cross contamination.

- The air that flows over the work surface is split into two parts, one of which flows into the front grill and the other into the rear grill.

- A Class II Type A1 BSC is not to be used for work involving volatile toxic chemicals as chemical vapours can build up in the cabinet and the laboratory through the recirculated and exhausted air.

Type B1 BSC

- This type of BSC can be used for handling small quantities of hazardous chemicals such as organic solvents or carcinogens.

- Blowers draw in room air and a portion of recirculated air, through the front HEPA filters immediately below the work surface.

- The particulate-free air flows through the plenum at each side of the cabinet and then downwards over the work area. Some cabinets use additional HEPA filters to remove particulates that may be generated by the blower motor.

- 70 percent of the down flow air exits through the rear grille, passes through the exhaust HEPA filter, and is discharged from the building. Remaining 30 percent of the down flow air is drawn through the front grill.

- The activities that generate hazardous chemical vapours must be conducted toward the rear of the cabinet, as the air that flows to the rear grille is discharged into the exhaust system.

Type B2 BSC

- No air is recirculated within the cabinet, all are totally exhausted. The cabinet provides biological and chemical containment. But the chemicals that can cause damage to the filter medium must not be used.

- The blower draws room air at the top of the cabinet through the HEPA filter and is passed over the work area. The exhaust system draws air through both the rear and front grills and all air is exhausted as it passes through the HEPA filter.

Type A2 BSC

- All positive pressure contaminated plenums within the cabinet are surrounded by a negative air pressure plenum thus ensuring that any leakage from a contaminated plenum will be drawn into the cabinet and not released to the environment.

- Minute quantities of volatile toxic chemicals or radionuclides can be used in a Type A2 cabinet only if it exhausts to the outside via a properly functioning canopy connection.

Class III Biosafety cabinet

- The BSC is used for working with highly infectious pathogenic agents and provides maximum protection for the environment and the worker.

- The BSC is gas tight and has a non opening view window.

- Materials are passed into the cabinet through a dunk tank that is accessible through the cabinet floor or through a double door pass through box.

- Both supply and exhaust air are HEPA filtered on a Class III cabinet. Exhaust air is passed through two HEPA filters before it is discharged directly to the outdoors.

- The manipulation of the materials inside the cabinet is done through a long heavy duty rubber gloves attached to the cabinet. Gloves help in preventing direct contact with the hazardous materials that are being handled.

- Class III BSC is used while working with Risk Group 3 & Risk Group 4 agents.

Selection of a biological safety cabinet

The selection of BSCs must be based on the type of protection needed. This includes

- Product protection
- Personnel protection
- The risk level of pathogens handled.
- Whether there will be use of radionuclide's or volatile chemicals

- Volatile toxic chemicals must not be used in Class I, Class II A1 or Class II A2 cabinets.

- Class II B1 BSCs can be used when minute amounts of chemicals or radionuclides are to be used.

- Class II B2 BSC, is to be used when significant amounts of radionuclides and volatile chemicals are to be used.

Using biological safety cabinets in the laboratory

Location

- Frequent air currents generated in front of the BSC, due to movement of people, open windows, or opening or shutting of doors affect the integrity of air inflow.

- BSCs must be located in a remote location free from disturbing air currents.

- Clearance of a minimum 30cms must be provided behind and sides of BSC for maintenance purpose.

Operation

- Improper use of BSCs will diminish the benefit of protection provided by the same.

- Care must be taken to move the arms in and out slowly and perpendicular to the front opening.

- All necessary items must be placed inside the BSC before commencing the work so that the movement of arms can be reduced without affecting the integrity of front air flow.

Material placement

- Equipment/material to be placed inside the cabinet must be decontaminated with 70% alcohol before they are placed inside the cabinet.

- If there are chances of spill, the work must be carried out in trays which are soaked with disinfectants.

- Equipment (centrifuges, mixers, etc.) or materials placed inside the cabinet must be placed as far back as possible and without blocking the rear grill.

- The autoclavable bags for collecting biowaste during the work must not be placed outside the cabinet, but towards a corner of the cabinet to avoid frequent in and out movement of the hands.

- Any repairs on BSC must only be done by the authorized technician. The maintenance and testing of the BSC must be done as per the manufacturer's recommendation.

Ultraviolet lights

- Ultraviolet lights, if used, must be cleaned weekly to remove any dust or dirt that may block the effectiveness of the light.

- Ultraviolet lights must be turned off when the laboratory is occupied to protect the eyes and skin from inadvertent exposure.

Open flames

- Open flames must not be used inside the BSC as they disrupt the airflow patterns and can be a source of ignition when volatile or flammable substances are used.

Spills

- Any spills within the BSC must be cleaned up immediately while the cabinet is operating. All materials which came in contact with the spill must be decontaminated.

Cleaning and disinfection

- When work is completed, all items used inside the BSC must be decontaminated before they are taken outside.

- The BSC must be decontaminated before and after the work.
70% Alcohol can be used to wipe down the sides, back and the interior of the sash.

- If bleach is used, then a second wiping must be done with sterile water.

- The BSC must be left running at least for 5 minutes before it is switched off in order to purge the inside air.

Personal protective equipment

- Lab coats and hand gloves and safety glasses must be worn while working with biosafety level 1 and level 2. A face shield may be used depending on requirement.

- Lab gowns or pressure suits must be worn for Bio safety level 3 and 4. Gloves must be pulled over the wrists of the gown rather than worn inside.

- If any spill enters the front or rear grilles, the drain valves must be closed and decontaminating solution must be poured into the drain pans.

- Contaminated liquid must be decontaminated before disposing into the sewer.

Blood borne pathogens

Blood borne pathogens are microorganisms that are present in human blood which can cause diseases in human beings. e.g., HIV, HBV, etc.

Precautions

- Contaminated needles and other contaminated sharps must not be bent or recapped.

- Contaminated sharps must always be stored in puncture resistant containers which are properly labelled.

- Handling of blood samples and other infectious materials must be done in a manner to prevent splashing and spraying.

- At the end of the work, hands must be washed immediately after the removal of gloves or other personal protective equipment.

- In case of contact with blood or other infectious material the hands or the portion of the skin must be immediately washed with soap and water.

- The personal protective equipment used while working with blood samples must not be left on work benches. It must be stored at a designated place for decontamination or disposal.

- Hand gloves must be used whenever there is a chance of contact with blood samples or other specimens.

- Care must be taken if there are any wounds or abrasions on the skin; these must not be exposed to the samples.

- Personal protective equipment must be inspected before use to ensure that they are not torn or damaged.

- The work surfaces and equipments used for handling blood samples must be cleaned and decontaminated.

- Contaminated broken glassware must not be picked up with hands. It must be cleaned up only by using a brush and dust pan, tongs or forceps.

- The containers used for storing blood contaminated waste must be of closed type.

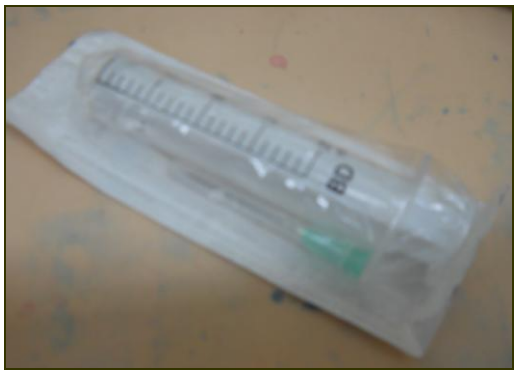
- Contaminated laundry must be washed separately.

- In case of any injury or incident involving the handling of blood borne pathogens same must be reported to the PI.

- Personnel who are working with blood samples, human tissues/cells must receive vaccination for Hepatitis B.

Needle stick injuries

- Needle stick injuries are wounds caused due to accidental puncture of the skin while using hypodermic syringes or other needle equipment.



- Personnel who use, disassemble and dispose needles, are susceptible to such injuries.

- Injury from syringe needles can happen at any stage during the handling of syringe needles. Equipment design, the nature of task done and the procedures followed in use and disposal are some of the factors that influence the occurrence.

- If needles are not disposed off properly or if they are disposed off with ordinary routine waste, there is a chance that people who handle them suffer injury.

- Needle stick injuries can transmit infectious diseases. Acquired Immune Deficiency Syndrome, Hepatitis B and Hepatitis C are a few examples.

- The risk of infection after exposure to infected blood varies by blood borne pathogen.

Equipment design

- Use of needles with safety shields or needle-free systems with self-sealing ports would help to prevent many of these injuries.

Decontamination

Decontamination of work surfaces, equipments used and laboratory environment helps to reduce the chances of transmission of infectious agents to the personnel working in the laboratory and outside. Decontamination also helps to reduce cross contamination.

Methods of decontamination

Sterilisation

Sterilisation helps to kill all microorganisms, including bacterial endospores. Same is accomplished by using heat, ethylene oxide gas, etc.

The commonly adopted method for decontamination in the laboratory is sterilisation by steam autoclaving.

Autoclaving

Autoclaving is the most effective and reliable means of sterilising laboratory materials.

The following cycles can be followed to ensure sterilisation of correctly loaded autoclaves:

1. 3 min holding time at 134 deg C
2. 10 min holding time at 126 deg C
3. 15 min holding time at 121 deg C
4. 25 min holding time at 115 deg C

Precautions in the use of autoclaves

- Autoclaves must only be operated by trained personnel.

- Routine maintenance of the equipment must be carried out as per manufacturer's instructions.

- The materials must be placed in containers that permit good heat penetration. Do not place sealed containers in autoclaves as they can explode. Use non glass containers wherever possible.

- For autoclaves without an interlocking safety device the door of the autoclave must be opened only after the temperature falls below 80 deg C.

- Place plastic bags in secondary containers to contain leakage.
- Heat resistant hand gloves with long sleeves and visors for face protection must be used while opening the autoclave. Open the autoclave after 10 minutes at the end of the cycle to allow cooling.

- In any routine monitoring of autoclave performance, biological indicators must be placed at the centre of each load to determine proper operating cycles.

Disinfection

Disinfection eliminates nearly all pathogenic microorganisms but not all bacterial spores on inanimate objects.

The effectiveness of a disinfection procedure depends on the following factors:

- type and number of pathogens present and also the presence of bacterial spores
- presence of organic matter (e.g., soil, blood)
- type of equipment or material to be disinfected
- temperature

The material safety data sheet (MSDS) of the disinfectant must be referred before use.

Decontamination of large spaces

Proper sealing of the space has to be ensured before commencing fumigation. Opening in the wall if any must also be sealed.

Formaldehyde

- It is available in flakes, or as solution of the gas in water with a stabilizer.

- Humidity must be controlled and a relative humidity of 80% must be maintained.

- Formaldehyde gas kills all microorganisms and spores at temperatures above 20 ^{0} C.

- The flakes or solution is heated to liberate the gas which is used for decontamination and disinfection of BSC or lab rooms.

- Formaldehyde is a suspected carcinogen. Fumes can cause irritation of mucous membranes.

- Warning signs to prevent personnel from entering the room must be displayed.

- After fumigation the lab room must be ventilated thoroughly before personnel are allowed to enter.

- Personnel entering the fumigated room must wear safety goggles, chemical cartridge respirator and lab coat.

Decontamination of surfaces

- Disinfectants that are commonly used for the decontamination of surfaces include sodium hypochlorite solution, alcohols, phenols, etc.

- The concentrations and exposure time vary as per the recommendations given by the manufacturer.

- The decontamination procedure to be followed will depend upon the type of experimental work and the type of infectious agent being worked with.

Chemical germicides

Chlorine (sodium hypochlorite)

- Chlorine in the form of bleach is highly alkaline and can corrode metals.

- Bleach is commonly used as a general purpose disinfectant for using with non metallic contaminated materials.

- Higher concentrations of sodium hypochlorite can be extremely corrosive as well as irritating to personnel and use of the same must be limited to situations where there is an excessive amount of organic material or very high concentrations of micro organisms.

- Chlorine is a toxic gas. The disinfectant must be stored in well ventilated areas only.

- Bleach must not be mixed with acids as it will release chlorine gas.

Alcohols

- For better effectiveness, they must be used at concentrations of about 70% in water.

- The main advantage of aqueous solution of alcohol is that they do not leave any residue.

- 70% (v/v) aqueous solution of ethanol can be used on work surfaces of laboratory benches and bio safety cabinets, and to soak small pieces of instruments.

- Ethanol is not effective against spores and may not kill all types of non lipid viruses.

- Alcohols are volatiles and flammable and must not be used near open flames or other sources of ignition.

- Alcohols must only be stored in closed containers and it must not be left open to prevent the release of vapours.

- After use the bottles must be placed in cupboards meant for the same.

- All bottles containing alcohol must be properly labelled and stored away from sources of ignition.

Laboratory animal facility

Requirements for laboratory animal facility have been included in the Task Force Report on requirements for setting up a central animal facility at the Institute.

Working with human, mammalian and cancer cells

- Working with human and other primate cells and primary mammalian cells involves risk of laboratory acquired infection.

- Improper handling can result in the transmission of the pathogens which may be present in animal cells, also blood borne pathogens can be transmitted while working with primary human cells, tissues and body fluids.

- Human cells/tissues and body fluids can contain, blood borne pathogens like, Hepatitis B Virus, Human Immunodeficiency Virus, Hepatitis C Virus, etc.

- Other primate cells and tissues can contain viral agents as well as cells carrying viral genomic material also present potential hazards to laboratory workers.

- **The human and animals cells and body fluids must always be considered as potentially hazards and handled in a manner as if they are infected with pathogens.**

Recommended practices

- A risk assessment must be carried out before commencement of laboratory work based on the origin of the cells or tissues as well as the source from which it has been isolated.

- Biosafety level 2 practices and facilities must be adopted while handling human and animal samples.

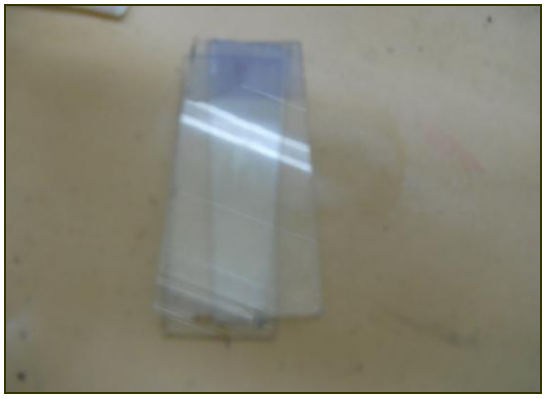
- All work must be conducted in a Bio Safety Cabinet and the wastes generated must be autoclaved and incinerated.

- Personnel protective equipment such as laboratory coats, gloves and eye protection must be worn always in the lab.

- If there is a risk of splashing, a face shield must be used.

- Open toed footware must not be used in the lab.

- Precautions for handling sharps must be followed as mentioned in the above sections.



- Personnel working with human cells and body fluids require Hepatitis B immunisation.

- Medical attention must be sought immediately after and exposure and lab in charge must be informed about it.

Emergency procedures

Puncture wounds, cuts and abrasions

- The protective clothing must be removed and the affected area must be washed with soap and water and seek medical attention.

- The details of pathogens being handled must be reported.

Infectious aerosol release outside a biological safety cabinet

- In case of release of any infectious aerosols outside the lab, lab must be vacated immediately. In case of exposure, the respective persons must take medical treatment immediately.

- The lab in charge must be intimated of the incident.

- Signs must be displayed restricting the entry of personnel. The room must be decontaminated by personnel wearing proper personnel protective equipment.

- No one must be allowed to enter the room till the aerosols are carried away or settled down.

Spillage of infectious substances

- In case of small spills, the spilled infectious material must be covered with cloth or tissue paper and the disinfectant must be poured over it and left for appropriate time.

- The contaminated materials can then be cleared. Do not pick up the contaminated glass pieces with hand, instead the same must be handled with forceps.

- The contaminated area must then be swabbed with disinfectant.

- The dust pans if used must be autoclaved or placed in an effective disinfectant.

- The contaminated material after disinfecting must be placed in the respective containers.

- Hand gloves must be worn during the procedure.

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

- If a breakage occurs while the machine is running, it must be switched off and the machine must not be opened for at least 30 minutes to allow the aerosols to settle.

- Thick rubber gloves must be worn if there is any chance of injury.

- Forceps must be used for removing glass particles if any.

- All broken tubes, fragments, buckets, trunnions and the rotor must be placed in a noncorrosive disinfectant.

- The centrifuge bowl must be swabbed with the same disinfectant with the suitable concentration and then swabbed again, washed with water and dried.

- All materials used in the clean-up must be treated as infectious waste.

Biowaste diposal

Disposal involves proper segregation of waste.

- The wastes that are generated in the laboratory must be segregated in the following manner

- Non contaminated waste that can be disposed off as general waste.
- Contaminated sharps like syringes, knives, broken glass pieces or other sharp objects, to be collected in puncture proof sharp containers.
- Infectious materials for decontamination by autoclaving and reuse.
- Contaminated material for autoclaving and incineration.
- Non contaminated sharps.

Materials to be autoclaved must be collected in autoclavable bags (red colour) and those for incineration must be collected in yellow bags with bio hazard sign.

Any cleaning of infected materials must be done only after autoclaving/disinfection.

All contaminated materials must be autoclaved in bags designated for the same.

Sharps must be collected in puncture proof containers.

All containers must be properly labelled for easy identification.

Collection of biohazard waste for incineration is coordinated by the Public Health Office.

Human and animal cells/tissues, cancer cells, body fluids and related solid wastes generated during the research must be incinerated (Requirement as per Bio-Medical Waste (Management and Handling) Rules 1998).

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References

- Indian Standard (IS) 12035-1986 Code of Safety in Microbiological Laboratories.
- Biosafety guidelines, Dept of Biotechnology, Govt. of India.
- Centers for Disease Control and Prevention (CDC) biosafety guidelines (US).
- Biosafety Manual, World Health Organisation.
- Occupational Safety and Health Administration (United States)