Institutional Biosafety Protocol for IISER-Kolkata

Under the Government of India
Ministry of Science and Technology
Department of Biotechnology

Constitution of Biosafety Committee

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Institutional Biosafety Committee (IBSC) of IISER-K is committed to its mission of:

- Assuring the Institute community, property and environment are protected against exposures which could result in injury, illness or loss.
- Assuring compliance with established state and local regulations/guidelines for all aspects of workplace safety and health.
- Developing and implementing appropriate programs and safeguards to ensure these institutional goals are met.

A. LABORATORY SAFETY POLICY

ASSIGNMENT OF RESPONSIBILITIES

Policy

IISER-Kolkata has developed a safety program that ensures a safe workplace, acknowledges the possibility of accidents, and realizes the loss prevention benefits of safety management.

The laboratory safety program includes requirements and recommendations related to the staff, equipment, operation, and maintenance of all activities and facilities. The program is designed to produce a safe working environment and to eliminate or reduce hazards to employees, students, visitors, and the environment.

Program Management

The Director of the IISER-K has delegated the implementation of the safety program to the Institutional BIosafety Committee (IBSC). The IBSC is established to address health and safety concerns specific to university activities and facilities. This committee reports directly to the Director.

Authority

The Chair of IBSC and/or his (her) staff have authority from Institute administration to take prompt corrective action whenever unsafe conditions might exist or when unsafe acts by employees, staff, visitors, or students are observed or reported; inspect all areas of the Institute to determine safety hazards and recommend corrective actions; audit procedures and job tasks, evaluate risk probability, and recommend corrective actions; evaluate departmental safety efforts and practices. Reports of these activities are to be provided to administration.

Principal Responsibilities of the Chair of IBSC

- Develop written policies and procedures designed to enhance safety within Institute buildings to the maximum degree possible.
- Coordinate and cooperate in the development of individual department safety practices and procedures.

- Establish an accident reporting system that includes mechanisms for investigating and evaluating all accidents reported and for documenting the review of all such reports as well as actions taken.
- Work with the Departments of Facilities Services and Facilities Design and Construction to ensure that project design and maintenance operations include safety provisions consistent with safety regulations.
- Work with the Offices of Infection Control and Radiation Safety through a mutual exchange of information.
- Provide safety-related information to be used in the orientation and continuing education of all employees and students.
- Conduct periodic inspections of each department.
- Establish methods of measuring results of the safety program at specifically defined intervals.
- Keep current with applicable local and state safety regulations.
- Keep current with information from major safety-oriented agencies, both governmental and nongovernmental.
- Develop a reference library of pertinent documents and publications dealing with all facets of laboratory safety. Copies of all applicable building and safety codes and standards are to be included.

Principal Responsibilities of Department Heads and Managers

Initial

1. Institutional review of physical facilities, activities, personnel, and procedures in their respective areas for the purpose of:
    - Identifying the appropriate subdivisions, if any, in their department for efficient administration of the safety program.
    - Designating an individual as Safety Observer to expedite communication for safety in the department.
    - Reassessing operations to ensure that any actual or potential safety hazards are identified and reported to the Department of Environmental Health and Safety.
2. Familiarize themselves with Institutional policies and regulatory requirements concerning safety.
3. Develop and maintain procedures for continuous attention to safety matters through departmental meetings.

Ongoing

1. Provide safe and healthful working conditions for all employees; conform to safety standards as issued; make available safety devices and personal protective equipment whenever their use is warranted.

2. Be certain that personnel and their supervisors:
    - Ensure that provisions for safety are incorporated into every operation and procedure.
    - Accept as part of their responsibility the safety, not only of the employees reporting directly to them, but also of those persons whose duties from time to time place them in proximity to the areas, operation, equipment, etc. under their jurisdiction.
    - Ensure cooperation with the periodic safety inspections performed by the representatives of the Department of Environmental Health and Safety.
    - Take prompt corrective action(s) whenever unsafe conditions and acts are noted.
    - See that all injuries are properly treated and reported.
    - Investigate and find the cause of all accidents, even if they result in minor injuries, and make reports as required.
    - Give personal support to all safety activities and safety procedures brought to the attention of the staff.
    - Support educational programs developed on safety.
    - Impart to each employee the understanding that violation of established safety rules will not be tolerated.
    - Instill safety awareness in each employee through personal and periodic safety contacts and by conducting group safety meetings when warranted.
    - Post notices to keep employees informed of their rights and duties, including provisions of applicable standards.

Laboratory Supervisor - The laboratory supervisors are responsible for the training of employees in safe practices, for correcting work errors and conditions that may result in personal injury, including exposure to carcinogenic chemicals, and for developing a positive attitude toward safety in laboratory operations. The laboratory safety supervisor must notify the Department of Environmental Health and Safety immediately of the occurrence of any accident which results in an exposure to personnel or the environment.

Chemical Hygiene Officer- Each laboratory (or group of laboratories) is required to have an individual who is familiar with all aspects of the laboratory's operations. This individual is responsible for ensuring the necessary safety training on the hazards generally present in the laboratory as well as during individual experiments. The CHO is usually the Principal Investigator or one of his/her designees who works closely with the Environmental Health Officer of the Department of Environmental Health and Safety on laboratory safety related issues.

Laboratory Worker - Each laboratory worker is responsible for complying with oral and written safety rules, regulations, and procedures required for the task assigned, for his/her own protection as well as that of fellow workers, the public, and the environment. Each is responsible for reporting to his/her immediate supervisor all facts pertaining to every accident resulting in personal injury or exposure to hazardous and carcinogenic chemicals and also any action that could result in such incidents.

B. GENERAL LABORATORY SAFETY

General Procedures

Personnel Practices:

1. PROTECTIVE CLOTHING - Protective clothing, such as a fully fastened laboratory coat must be worn. Gloves which are appropriate to the specific situation must be used when handling chemicals and potentially infectious items. Disposable gloves shall be discarded after each use, immediately after known contact with a chemical carcinogen, and upon becoming visibly wet with a chemical. **Shorts or short clothing without a lab coat are not acceptable laboratory work attire. Sandals and open-toed shoes are also not acceptable.**
2. EYE PROTECTION - Devices to provide appropriate eye protection must be worn in any laboratory work area. These include safety glasses, goggles or face shields, whichever is most appropriate for the operation being performed. Eye washes are required in all laboratories where any chemicals are used.
3. HEARING PROTECTION - Ear muffs or earplugs must be worn whenever ultrasonicators and other excessively noisy laboratory equipment are in use.
4. PARAFFIN WAX - In histology tissue processing areas, paraffin fragments frequently fall on the floor. The resultant slippery conditions should be prevented whenever possible. The use of heavy matting on the floor and holding trays for histology equipment which involves the use of paraffin are strongly recommended.
5. PIPETTING - Mechanical pipetting aids shall be used for all pipetting procedures. **Mouth pipetting is prohibited under all circumstances.**
6. AUTOCLAVES - Open autoclave doors slowly, standing to one side in order to prevent a burn from any residual steam. Wear heat resistant gloves when removing hot items. **Do not attempt to open an autoclave which still contains pressure in the chamber.**
7. EATING, DRINKING, AND SMOKING - Eating, drinking, smoking, chewing of gum or tobacco, application of cosmetics, or storage of food in laboratory areas are prohibited. Food must not be stored in laboratory refrigerators nor can it be heated in laboratory microwaves.
8. PERSONAL HYGIENE - All personnel must wash their hands immediately after completion of any procedures which biological or chemical materials have been used. Personnel must wash or shower areas of their body which have been in direct contact with either.
9. HOUSEKEEPING - Maintain good housekeeping habits. Do not allow aisles to get cluttered with chairs, stools, boxes, etc. Aisles must be kept free of obstacles at all times.

10. SAFETY EQUIPMENT - Learn the location and use of fire extinguishers, fire blankets, water hoses, fire alarms, safety showers, and eyewashes.
11. FUME HOODS - Avoid inhaling chemical vapors and gases. Use fume hoods whenever possible. Do not store materials in fume hoods. Keep hoods clear and clean.
12. UTILITIES - Set up experiments so that it is not necessary to reach through the assembly to turn water, gas or electricity on or off.
13. Do not heat a closed system.
14. Use boiling chips when heating liquids to the boiling point.

Glassware

1. Since glass breaks easily, guard against casual handling of glassware. In order to cut glass tubing safely, wear eye protection. Scratch the glass with a triangular file or glass knife. Wrap a towel around the tubing or wear heavy gloves. Place thumbnails against tubing directly opposite scratch and press while pulling hands apart. Always fire polish tubing ends before using. For tubing with an outside diameter of a centimeter or more, use a cutting wheel or hot wire cutter.
2. Inserting glass tubing into stoppers is easier if it is lubricated with glycerol or water. Wear heavy gloves and use a slow twisting motion.
3. For vacuum traps, use only heavy-walled suction flasks. Wrap adhesive tape around the flask in a crisscross fashion to prevent flying glass in the event of an implosion.
4. When picking up broken glassware, use a brush and dustpan. Fine pieces should be picked up using wet cotton held with tongs. Discard all chipped and broken glassware into a separate, specially marked container. Wear eye protection.
5. Broken glass, pipettes and capillary pipettes must be discarded into a separate, specially marked container used for glass disposal. These disposal boxes are available from the Materiel Management Warehouse.

Centrifuges

The Department of Environmental Health and Safety / Institutional Biosafety Committee arranges for periodic rotor inspections by the major rotor manufacturers. These inspections are conducted at various sites on campus and are always announced well in advance. You are encouraged to participate in this program by offering your rotors for visual "scope analysis" which identifies microscopic cracks in a rotor. These cracks can cause a rotor to break apart at high speeds, creating a hazardous condition. Keep detailed records of operation for high speed centrifuges and rotors. Do not exceed the maximum speed rating for the rotor.

1. Carefully inspect the condition of the centrifuge tubes prior to ultracentrifugation.
2. Stop the centrifuge immediately if an unusual noise or vibration begins.
3. Keep the lid closed during the entire operation.

4. Always properly balance the materials you are centrifuging.
5. If using nitrocellulose tubes:
   a. Do not use if discolored or flexible.
   b. Storage at 4o C extends shelf life.
   c. Use only in swinging bucket heads.
   d. Do not autoclave; they could explode.
6. When centrifuging biological materials, follow the guidelines noted in Section P, Biological Safety.

Chemicals Handling

Nearly all chemicals are poisonous to the human body to some degree. Flammable liquids, exothermic reactions, unstable materials, toxic, and corrosive materials play a large part in causing injuries. Accidents and the resultant injuries in laboratories can be severe. The following suggestions will aid in reducing the possibility of chemically related accidents.

1. Always ensure the proper labeling of containers with contents, concentration, manufacturer, handling precautions, and expiration date (especially in the case of unstable compounds).
2. "Second skin" type safety coating should be used whenever possible for all bottles containing hazardous materials. These coatings are impact resistant and made from a high tear thermo plastic, providing an added level of safety in the event that a bottle is dropped and broken. The coating works to contain the liquid until it can be transferred to another bottle. This will dramatically reduce spills occurring from bottle breakage.
3. Never test chemicals by taste or odor. If in doubt, do not use an unlabeled chemical.
4. Always remember that acids are poured into water, not vice versa.
5. Large mercury spills should be handled by calling the Department of Environmental Health and Safety/ IBC. Mercury spill should be available from Environmental Health and Safety/IBC.
6. When flammable liquids are to be stirred, use air-driven agitators, not electric motor-driven units. Use a heating mantle or steam bath instead of an electric heating unit to heat flammable liquids. Concentrations of ethanol above 40% must be stored in safety cans or in an approved flammable storage cabinet.

Collection for Chemical Waste

Check with the Department of Environmental Health and Safety for proper disposal of **chemicals. The** department should operate a weekly chemical collection service for chemical waste. For this service, simply call --------- and leave a message. In general,

well-diluted acids and bases (between pH 6 and 9) can be flushed directly down the drain with plenty of water. For more specific information regarding chemical waste disposal, consult Section -------, Hazardous (Chemical) Materials.

Incompatible Chemicals

Separate storage areas should be provided for "incompatible chemicals," which may react and create a hazardous condition. For example, some oxidizing acids (nitric, sulfuric, perchloric), when stored together with flammable solvents can create a fire if the bottles are broken, allowing contact of the two materials. Do not store chemicals by alphabetical listing. Instead store by acids, bases, salts, flammables, etc.

Cryogenic Liquids

1. Store liquid nitrogen, liquid helium, dry ice, and any other liquefied gases in well-ventilated areas. **Do not store these materials in walk-in cold rooms** as these are not ventilated. The sublimation of dry ice, for example, will reduce the percentage of available oxygen, posing a threat to those who enter.
2. Liquid nitrogen is commonly used for long storage of small biological samples. Generally the sample containers are small ampules which are lowered into the liquid nitrogen. Improper sealing of the ampules can cause an explosion upon removal from liquid nitrogen temperatures. To prevent this, always test the ampules for tight-sealing by placing in a dye solution for two minutes prior to freezing.
3. When removing a sample container from the liquid nitrogen, wear safety goggles, lab coat, and insulated gloves. Quickly place the ampules in a beaker of warm water inside a Styrofoam ice bucket, and cover immediately. All these precautions can be obviated by the use of the plastic vials available and designed specifically for cryogenic use.

Needles and Syringes

Needle punctures are one of the most frequent laboratory related injuries. Incidents involve laboratory workers, maintenance and custodial personnel. Discarding of needles in wastebaskets is prohibited. A specially marked box, impervious plastic container (sharps container) is to be used for disposal of all needles and syringes.

It is strongly recommended that cannulas be used, if a sharp needle is not needed. Needles must never be left lying about without the plastic guards in place. Needles must never be recapped unless under certain conditions, an approved needle-capping device can be used. Destroying needles by clipping or "shearing" is prohibited because the potential for hazardous aerosols exists.

C. BIOLOGICAL SAFETY

The most important element of containment of infectious materials is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or infected materials must be aware of potential hazards and be trained and proficient in the practices and techniques required for safely handling such material.

Procedures for Biohazard Control

Each laboratory supervisor must develop or adopt safety and operational procedures to identify the hazards that will, or may likely, be encountered. They must also specify practices and procedures designed to minimize or eliminate identified risks, as well as the procedures to be used in the event of an accidental exposure. Personnel must be required to read and follow the established practices and procedures and must be advised of any special hazards present in the laboratory.

Institutional Biosafety Committee

The Institutional Biosafety Committee (IBC) exists to ensure that research involving recombinant DNA and pathogenic agents is assigned the appropriate Risk Group (biosafety level) and that all work is conducted in accordance with Ministry of Human resources guidelines. Additionally, the IBC ensures that research protocols are carried out in a manner which complies with the Occupational Health and Safety Administration's (OSHA) Blood borne Pathogens Standard.

Members of the IBC will be appointed annually by the Director of the Institute in accordance with ---------- guidelines. . Each member serves for a three year term.

The day to day functions of the IBC are carried out by three subcommittees: the Recombinant DNA Subcommittee, the Traditional Pathogen Subcommittee and the Institutional Review Board/IBC Subcommittee. These subcommittees meet monthly to review research protocols. Their actions are ratified by the IBC at quarterly meetings. **External member of the committee?? Whether he or she should meet quarterly or bi annually??**

<u>OSHA Blood borne Pathogens Standard</u>

Any laboratory which works with potentially infectious material (i.e. blood, tissue, viruses, etc.) must comply with the Occupational Safety and Health Administration's *Blood borne Pathogens Standard.* Under this law, the employer is required to ensure that the laboratory employee is trained on appropriate methods to control accidental exposure to potentially infectious agents.

Employers must communicate the potential hazards, provide employees with appropriate personal protective equipment (gloves, eye protection, lab coats etc.) and ensure these are used whenever the potential exists for accidental exposure.

Additionally, each employee who is covered by the standard must also be offered a Hepatitis B vaccination by the employer at no cost to the employee. Employees may refuse the vaccination. If they choose not to receive the vaccine, they must sign a declination form provided by the employer. Additional vaccinations are available to employees who come into contact with other infectious agents such as rabies, vaccinia virus, etc. All vaccinations should be available to employees at no charge.

Routes of Transmission

Exposure and potential infection from biological material can occur by one or multiple forms of direct contact between the laboratory worker and an organism. For these reasons, workers must always be on guard against the following types of exposure and must take adequate measures to reduce the risk to the following types of contact.

Respiratory Route Infection

A variety of agents infect by the respiratory route. Aerosol generation and dissemination can be reduced by the following:

1. Properly operating laminar-flow biological safety cabinets for protection against immediately generated aerosols.
2. Thorough decontamination of work surfaces before and after work following spills of biohazardous material. This method is particularly effective in preventing secondary aerosols generated by agents resistant to drying.
3. Use of absorbent materials on immediate work surfaces, to contain splashes and drips.

Infection by Ingestion

A variety of organisms used in the laboratory are enteric pathogens which use ingestion as the primary route of infection (intestinal parasites, Salmonella, agents of infectious hepatitis, polio virus, and enteropathogenic E. coli strains). Infection by these organisms generally occurs in the following ways:

1. Direct ingestion of the culture by mouth pipetting.
2. "Hand to mouth" infection whereby infectious materials are indirectly transferred by the hand to the oral cavity. Activities such as smoking, eating, and drinking are therefore prohibited in laboratories. Frequent hand washing with germicidal soap between activities is strongly recommended.

Needle sticks, Punctures, Contact with Non-intact Skin

1. Contact can be avoided by limiting the use of needles and syringes and by using nonbreakable containers whenever possible.
2. Workers' hands must never come into direct contact with infectious agents. Therefore, gloves should be worn and *discarded appropriately before handling other equipment or objects.*

Exposure to Mucous Membranes

1. All manipulations capable of generating a splash or spray must be conducted within a biological safety cabinet with the sash and seat properly adjusted to afford protection of the eyes.
2. Manipulations which may create splashes and that cannot be conducted within a biological safety cabinet (e.g. disposal of disinfected liquid waste to the sanitary sewer) must be performed while wearing eye protection and a mask (to protect the nose and mouth) or a full face shield. Work can also be conducted behind an acrylic splash shield; however, this must be adequately sized to provide ample protection against eye, nose and mouth exposure.

Operations and Equipment

Hand washing

One of the most effective methods of protection against accidental exposure to potentially infectious agents is executed every time an individual washes his/her hands. Hand contamination with transient microbes (some of which can be pathogenic) can easily occur during manipulation of specimens, equipment and supplies as well as from contact with work surfaces. For these reasons, it is important that all laboratory personnel wash their hands:

a. whenever they come on duty

b. when leaving the laboratory for whatever reason

c. when hands are obviously soiled

d. before and after completion of a task in a biological safety cabinet, even if gloves are worn

e. upon completion of tasks

Standard hand washing protocol:

1. *Turn on the faucets and wet the hands using warm water.*
2. *Dispense antiseptic soap compound into a cupped hand.*
3. *Spread the soap around both hands and between the fingers. If needed, add a small amount of water to facilitate spreading and lathering.*

4. *Wash the hands for approximately 10 seconds. Vigorously rub both sides of the hands beginning a few inches above the wrists and moving downward between the fingers, around and under the fingernails.*
5. *Rinse thoroughly under warm water beginning with the area above the wrists and continuing downward past the fingers.*

If the sinks are foot, knee or elbow operated, turn off the water. If not, leave the water running, dry the hands with paper towels and then use the towel (as a barrier to your clean hand) to turn off the faucet.

Pipetting

Mouth pipetting is prohibited in all situations. Use one of the mechanical aids that are commercially available. Delivery must be accomplished with the tip of the pipette resting against the container, allowing the fluid to flow down the surface thereby minimizing aerosols. In addition, the following practices must be observed:

1. No infectious mixture should be prepared by bubbling air through the liquid with the pipette.
2. No infectious material should be forcibly discharged from a pipette.
3. Placing a disinfectant soaked towel over the immediate work surface is useful in minimizing aerosolization from accidental splashing.

Use of Syringes and Needles

- To reduce the risk of accidental injection, aerosol production or spills, the following practices must be observed:
- Do not use a syringe and needle as a substitute for a pipette in making dilutions of hazardous or infectious fluids. Syringe-type pipettes with blunt-ended delivery are preferable.
- Reusable or disposable syringes used with biohazardous materials should be of the LEUR-LOK or equivalent type to assure that the needle cannot separate during use.
- Used disposable needles must not be bent, sheared, broken, recapped, or removed from disposable syringes.
- Disposable needles and syringes must be disposed as a single unit into puncture-resistant leak-proof "sharps" containers. Full containers must be sealed and placed into the red bag waste stream for incineration. Refer to waste disposal guidelines, **Appendix** --------.
- Syringes not associated with needles or which have not come into contact with biohazardous material must also be disposed into the red bag waste stream for incineration. Syringes and needles must never be discarded into the regular waste stream.
- Never discard syringes and needles into pans containing pipettes or other glassware which must be separated from syringes and needles.

Use of Centrifuges and Shakers

To reduce the opportunity for aerosol production of biological material when using centrifuges and shakers, the following practices must be observed.

1. All tubes must be capped.
2. Biohazardous agents must be centrifuged in an enclosed centrifuge with sealed rotor. Safety cups and rotors with covers and O-rings are both effective at minimizing aerosol production.
3. Decanting from centrifuge tubes must be performed in a biological safety cabinet.
4. When mixing broth cultures utilizing a Vortex or similar mixer, avoid wetting the plug or cap.
5. As an additional safety measure, centrifuges and shakers are not permitted in corridor areas and must be housed within laboratory or common equipment spaces.

Note: Items for centrifugation should always be balanced to avoid vibration, which can result in failure of the unit as well as considerable aerosolization.

Opening Culture Plates, Tubes, Bottles, and Ampules

Aerosols are produced when contaminated plugs or screw caps are removed from tubes and bottles. Employing good, sterile technique when opening tubes, bottles and culture plates will minimize the potential for aerosolizing the culture.

Opening ampules is also potentially hazardous after the seal has been broken because air rushing in causes the dry contents to be dispersed.

1. After scoring the ampule with a file, wrap it in cotton that has been wet with disinfectant. Wear gloves.
2. If a disinfectant may damage the culture, use a biological safety cabinet and the following procedure:

After scoring the ampule with a file, apply a hot, glass rod to the mark. The glass will crack, allowing air to enter the ampule and equalize the pressure. After a few seconds, wrap the ampule in a few layers of tissue, and break it along the crack. The tissues and ampule neck must be discarded appropriately.

Employing good, sterile techniques when opening tubes, bottles and culture plates will minimize the potential for aerosolizing the culture. Also, it is recommended that a culture plate be open so that the lid is between you and the culture medium.

Blenders, Ultrasonic Disintegrators, Grinders, Mortars and Pestles, and Homogenizers

Blenders, disintegrators, grinders and homogenizers release considerable aerosols during their operation.

1. Blending, grinding, and homogenizing must be performed within a biological safety cabinet.
2. Disinfectant-soaked absorbent material can be placed over the blender during operation to further reduce the production of aerosols.

Water Baths and Warburg Baths

It is recommended that water baths and Warburg baths used to inactivate, incubate or test biohazardous materials, contain a disinfectant such as Clorox (2.9 ml/3.8 L of water) or a phenolic detergent (29 ml/3.8 L of water). Water should be changed at frequent intervals.

Laboratory vacuum lines

When a laboratory vacuum is used to manipulate biohazardous materials, a trap containing a suitable disinfectant must be employed to ensure that the building vacuum lines do not become contaminated. Clorox, added such that the final concentration will equal 10%, is a suitable agent. An inline filter must also be present between the trap and vacuum line. Empty all traps frequently and whenever more than 3/4 full.

Contaminated Glassware (flasks, beakers, reusable pipettes, etc.)

Contaminated glassware and similar materials which will be used again must be disinfected before washing.

Labeling

Storage vessels containing biohazardous agents must be labeled to provide identification of their contents. Equipment used for the manipulation or storage of biohazardous material must be labeled with a biohazard sticker and a description of contents (e.g. human cell lines).

Contaminated Materials

Contaminated materials that are transferred from work sites to decontamination and disposal staging areas shall be properly labeled with the individual's name and transported in a manner that prevents accidental spills.

Containers

Nonbreakable impermeable closed containers must be used during transport of biohazardous material through a building corridor or between buildings.

Personal Protective Equipment (PPE)

Gloves and adequate protective clothing such as a fully fastened laboratory coat must be worn as a minimal form of protection against exposure to biological agents. When additional risks are

present, other types of PPE may be necessary (such as faceshields to protect against splashing, etc.). PPE must not be worn outside the laboratory or to public eating areas.

Refrigerators, Deep Freezers and Dry Ice Chests Used to Store Biological Material

1. Refrigerators, deep freezers, and dry ice chests must be checked, defrosted and disinfected periodically. Remove any samples which may have broken during storage.
2. Equipment containing potentially biohazardous material must be locked at all times when stored outside of the laboratory in a hallway or common equipment area. Placement of this equipment must comply with the requirements of the Institutional corridor storage policy. Such equipment must also be labeled with the name and telephone number of a contact individual, as well as the laboratory room number, in the event of an equipment failure.

Class II Biological Safety Cabinets

General Information

The Class II cabinet, also known as the biological safety cabinet, provides protection of personnel as well as the product. The cabinet has an open front with inward air flow for personal protection. Air flowing downward over the working surface is filtered by a high efficiency particulate air (HEPA) filter for product protection. The cabinet exhaust air is also filtered through a HEPA filter.

Cabinet Usage

The Class II Type A biological safety cabinet is used when working with infectious agents requiring Biosafety Level 2 or 3 containment. It is not for use with volatile or toxic chemicals and radionuclides, since the HEPA filtered cabinet exhaust is discharged into the workspace.

The Class II Type B biological safety cabinet differs from Type A in that it is hard ducted to the exhaust system and has an increased face velocity. These features allow for work with small amounts of toxic chemicals and radionuclides in addition to infectious agents.

Effectiveness

The effectiveness of Class II cabinets in controlling contamination depends on:

- The integrity of the filter
- Filter housing
- The uniformity of air flow
- Proper decontamination methods

Certification/Decontamination

All biological safety cabinets must be certified (to be working correctly) at least once each year or whenever the equipment is relocated.

Decontamination of a biological safety cabinet must be performed prior to moving the equipment. It is also recommended whenever the use of the cabinet changes.

CAUTION: Formaldehyde decontamination procedures should not be attempted by research personnel unless they have received proper instruction.

Consultation

Consultation regarding purchase, installation, testing, certification or decontamination of biological safety cabinets can be arranged with the coordination of Department of Environmental Health and Safety

Posting of Biological Hazard Signs

Purpose

The necessity for establishing policies and procedures for proper identification of hazardous biological agents within the laboratories is to alert support personnel who may enter the area to take precautionary measures and to restrict traffic to potentially hazardous areas.

Responsibility

It is the primary responsibility of the Principal Investigator (PI) to properly identify biohazards. Upon determination that a potential biohazard exists, the PI should notify the Department of Environmental Health and Safety.

Biohazard Warning Sign

To ensure proper identification, a standardized, easily recognized sign is essential.

For the purpose of issuance, the term "biohazard" includes only those infectious agents presenting a risk or potential risk to the well-being of a human.

The warning sign shall be prominently placed so that it can be easily seen and shall be displayed ONLY for the purposes of signifying the presence of actual or potential biohazardous agents.

Procedures

Before research of a biohazardous nature is begun or when it is determined that a biohazard exists:

1. Requests for biohazard signs must be made to the IBC.
2. A review of the required information to appear on the sign will be conducted with the PI before any signs are provided.

Recommended Biosafety Levels for Infectious Agents and Infected Animals

The selection of an appropriate Biosafety level or work with a particular agent or animal study is dependent upon a number of factors. The most important of these include the:

- Virulence, pathogenicity, biological stability, and communicability of the agent
- Nature or function of the laboratory
- Quantity or concentration of the agent
- Endemicity of the agent
- Availability of effective vaccines or therapeutic measures
- Documented or suspected route of transmission of the agent

In general, the biosafety level used for activities involving infectious agents or infected animals must be commensurate with that required for the agent of highest virulence known, or likely to be encountered in the course of the contemplated work. For example, all material of human origin, including cell lines, tissue, and blood, must be considered potentially infectious for hepatitis and HIV and handled under Universal Precautions, which reasonably preclude cutaneous, oral, and parenteral exposure to personnel.

If, in the course of diagnostic or other laboratory examination, there is evidence that the materials being studied contain an agent of higher or lower risk than expected, the biosafety level can be raised or lowered accordingly.

Occasions will arise when it will be necessary to assign a biosafety higher than that recommended in these guidelines. For example, a higher biosafety level may be indicated by the unique nature of the proposed activity (e.g., the need for special containment for experimentally generated aerosols for inhalation studies).

It is the responsibility of the Principal Investigator to inform the Institutional Biosafety Committee (IBC) when he or she begins to work or ceases to work with any agents at the BL-2 or BL-3 levels. All information will be recorded on a computer file that will be accessed by others who may be called to respond if an emergency occurs in that laboratory.

Summary of Laboratory Practices for Each Biosafety Level*

Biosafety Level 1 (Risk Group 1)

This level is suitable for work involving agents of no known or of minimal potential hazard to laboratory personnel and the environment. The laboratory is not separated from the general traffic patterns in the building. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

Biosafety Level 2 (Risk Group 2)

This level is similar to Level 1 and suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by scientists competent in this biosafety level, (2) access to the laboratory is limited when work is being conducted and (3) certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment.

Biosafety Level 3 (Risk Group 3)

This level is applicable to clinical, diagnostic teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal protective clothing and devices. The laboratory has special engineering and design features. It is recognized, however, that many existing facilities may not have all the facility safeguards recommended for Biosafety Level 3 (e.g., access zone, sealed penetrations, directional airflow, etc.). In these circumstances, acceptable safety may be achieved for routine or repetitive operation (e.g., diagnostic procedures involving the propagation of an agent for identification, typing and susceptibility testing) in laboratories where facility features satisfy Biosafety Level 2 recommendations provided the recommended "Standard Microbiological Practices," "Special Practices" and "Containment Equipment" for Biosafety Level 3 are rigorously followed. The decision to implement this modification of Biosafety Level 3 recommendations may only be made by the Institutional Biosafety Committee. Entry into a BL-3 facility is restricted to those individuals who have had training and have demonstrated knowledge of BL-3 Standard Operating Procedures and Safety Practices by means of a written exam.

Biosafety Level 4 (Risk Group 4)

This level is reserved for work with dangerous and exotic agents who pose a high individual risk of life-threatening disease. No work may be performed with agents requiring Biosafety Level 4 containment for time being.

- The former classification according to Biological Safety Level (BL) has been replaced with the World Health Organization classification according to Risk Group (RG). The terms are equivalent.

Summary of Classification of Biological Agents According to Risk

(Modified from Biosafety in Microbiological and Biomedical Laboratories, Centers for Disease Control and the National Institutes of Health, 3rd Ed., 1993.)

Biosafety Level 2: Bacterial Agents†

Bacillus anthracis
Bordetella pertussis
Campylobacter - all species
Chlamydia psittaci, C. Pneumoniae, C. trachomatis
Clostridium botulinum, C. tetani
Corynebacterium diptheriae
Leptospira interrogans- all serovars
Legionella pneumophila; other Legionella-like agents
Mycobacteria except *M. bovis* or *M. leprae*
Neisseria gonorrhoeae, N. Meningitidis
Pseudomonas psudomallei
Salmonella - all serotypes
Shigella - all species and all serotypes
Vibrionic enteritis (*Vibrio cholerae, V. parahaemolyticus*)

† Additional primary containment and precautions, such as those described for work at the BL-3 level are recommended for activities with high potential for droplet or aerosol production, for work with antibiotic - resistant strains and for activities involving production quantities or concentrations of infectious materials.

Biosafety Level 2: Fungal Agents

Blastomyces dermatitidis
Cryptococcus neoformans
Sporothrix schenckii

Pathogenic members of the genera *Epidermophyton, Microsporum* and *Trichophyton*

Miscellaneous molds

Cladosporium (Xylohypha) trichoides
Cladosporium bantianum
Penicillium marnefii
Exophiala (Wangiella) dermatitidis
Fonsecaea pedrosoi
Dactylaria gallopava (Ochroconis gallopavum)

Biosafety Level 2: Parasitic Agents

Nematode parasites of humans

Ascaris spp.
Stronglyoides spp.
Hookworms
Enterobius spp.

Protozoal Parasites of Humans

Toxoplasma spp. *Plasmodium* spp.
Trypanosoma spp. *Entamoeba* spp.
Coccidia spp.
Giardia spp.
Leishmania spp.
Sarcocystis spp.
Cryptosporidia spp.

Trematode Parasites of Humans

Schistosoma spp.
Fasciola spp.
Cestode Parasites of Humans
Echinococcus granulosus
Taenia solium (cysticercus cellulosae)
Hymenolepsis nana.

Biosafety Level 2: Viral Agents ††

Hepatitis A, B, C, D, and E virus
Herpes viruses - except Herpesvirus simiae (Monkey B virus) which is BL-4
Influenza virus
Polioviruses
Poxviruses - all types except variola which is restricted
Rabies virus

Mouse hepatitis virus . For animal work it needs a separate contaminant suite.

†† Additional primary containment and personnel precautions, such as those described for Biosafety Level 3, may be indicated for activities with potential for droplet or aerosol production and for activities involving production quantities or concentrations of infectious materials.

Biosafety Level 3: Bacterial Agents

Brucella - all species
Francisella tularensis
Mycobacterium bovis; M. tuberculosis
Yersinia pestis

Biosafety Level 3: Fungal Agents

Coccidioides immitis
Histoplasma capsulatum

Biosafety Level 3: Viral and Rickettsial Agents

Lymphocytic choriomeningitis virus (LCM)
Rickettsiae - all species when used for transmission or animal inoculation experiments
Vesicular Stomatitis Virus
Retroviruses, including Human and Simian Immunodeficiency viruses (HIV and SIV)
Transmissible Spongiform Encephalopathies (Creutzfeldt-Jakob, kuru and related agents)

Arboviruses and Arenaviruses

A complete listing of all arboviruses and arenaviruses assigned to Biosafety Levels 2 and 3 is available from the Department of Environmental Health and Safety at extension 3-6260.

Respiratory Protection Program for *M. tuberculosis*

No ***M. tuberculosis*** experiment will be performed at IISER.

Cell Culture Systems

Cultured cells are a routine source material in many research laboratories. Most cultured cells are known to harbor viruses either adventitiously (in many cases of detectable C-type particles) or deliberately (as in the cases of SV40 transformed rodent and human cell lines or human lymphoid cell lines, which are transformed by Epstein-Barr virus).

Long term culture of cells may enhance the risk of rescuing an oncogenic agent, whereas an autonomous infectious virus is more likely to be released upon short-term manipulation (two to three weeks) of freshly isolated cells.

It is therefore prudent to adopt Universal Precautions for the handling of cultured cells. All cell manipulations should be performed in a biological safety cabinet using BL-2 practices and procedures, including the use of personal protective equipment such as a buttoned lab coat and gloves.

Shipment of Diagnostic Specimens and Infectious Substances*

- Adopted with modification from the International Air Transport Association (IATA) publication "Dangerous Goods Regulations, 37th Edition", Effective January 1996.

Definitions:

Infectious Substances: Substances containing viable microorganisms including a bacterium, virus, rickettsia, parasite, fungus or a recombinant, hybrid or mutant that are known or reasonably believed to cause disease in humans.

Diagnostic Specimens: Any human or animal material including, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluids, being shipped for purposes of diagnosis, but excluding live infected animals.

Shipper's Responsibility

Investigators must be aware that all biological products, both diagnostic and infectious, are subject to specific shipping regulations. The Shipper must determine whether a substance is infectious and therefore classified as dangerous goods, or whether it can be classified as a diagnostic specimen. The Shipper is responsible for properly packaging, labeling and documenting all shipments. The Shipper's responsibility does not end when the Carrier accepts the package. The Shipper's responsibility ends when the package arrives at its destination in good condition.

Consultation

> Consultation regarding the classification, packaging, marking, labeling, and documentation of biological shipments can be arranged with IBC.

Emergency Response

Biological Spills Inside a Biological Safety Cabinet

1. Leave the cabinet operating in order to contain aerosols.

2. Initiate cleanup as soon as possible with a suitable disinfectant such as 10% Clorox.
3. Items within the cabinet should be wiped carefully, with disinfectant.
4. Allow the cabinet to run at least 10 minute after cleanup before activity is resumed.

Biohazard Spills Outside of a Biological Safety Cabinet

The following procedure should be followed in the event of a spill of a Biosafety level 1 or 2 agent outside of a biological safety cabinet:

1. Notify others of the spill.
2. Remove any contaminated clothing and wash any affected body parts with a disinfectant soap.
3. Wearing personal protective equipment, cover the spill with paper towels, and add a suitable disinfectant such as 10% Clorox. Allow at least twenty minutes of contact time.
4. Remove toweling, and wipe entire area with 10% Clorox.
5. Dispose of all cleanup materials as biohazard waste.

The following procedure should be followed in the event of a biohazard spill of highly infectious material, such as a Biosafety Level 3 agent:

1. Notify others in the room that a spill has occurred.
2. Remove contaminated protective garments (including shoes) and leave the room.
3. Wash any affected body parts with disinfectant soap.
4. Notify the Emergency Response Team by calling.

Decontamination and Cleanup

After the above immediate actions are accomplished, decontamination and cleanup will be directed by the laboratory supervisor and the Emergency Response Team as follows:

1. Before reentering the affected area, wait a minimum of 30 minutes to permit settling and reduction of airborne particles.
2. Personnel involved in the cleanup should put on disposable Tyvek gowns (tied in back), head and foot coverings, a mask, eye protection, and medium to heavy weight rubber gloves.
3. Cover the spill with paper toweling, and then gently pour a suitable disinfectant (10% Clorox) onto the site. Allow at least 20 minutes of contact time.
4. Using a disposable dustpan and squeegee, transfer all materials from the spill area to a biohazard waste container.
5. Wash and mop the spill area and adjacent areas with disinfectant-detergent solution.
6. Gas sterilizes equipment that requires decontamination but cannot be subjected to liquids or heat. This will be arranged by the Department of Environmental Health and Safety.
7. Before leaving the immediate area, the decontamination team should remove shoe covers and wipe shoes on pads soaked with disinfectant solution. All personal protective equipment must be disposed of as biohazard waste. Personnel should then shower using a germicidal soap.

8. The laboratory supervisor should assure that all waste, equipment, and clothing is properly decontaminated or disinfected and disposed of as biohazard waste.

Biohazardous Waste

The following categories are considered potentially infectious by definition must be packaged as red bag waste items. Red bags must be of adequate strength to resist punctures. These bags containing the waste material must then be placed into an appropriately labeled box (which is resistant to moisture) prior to transport off site for incineration. Approved bags and boxes can be obtained by contacting the Custodial or Environmental Services supervisor for your building.

Biohazardous Waste Definitions

Waste material which meets any of the following definitions must be disposed in a responsible safe manner.

Sharps Waste

All needles, syringes (with or without the attached needle), Pasteur pipettes, scalpel blades, blood vials, needles with attached tubing, culture dishes, suture needles, slides, cover slips and other broken or unbroken glass or plasticware.

Microbiological Waste

All materials containing or in contact with cultures of microbiological organisms and all patient specimens sent for microbiological culture or items contaminated by patient specimens.

Biological Materials Waste

All discarded vaccines, immunoglobulin, plasma, albumin, blood or tissue fractionation products, enzyme preparations, etc.
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Animal Pathogen Contaminated Waste

All bedding and other materials contaminated with blood, excreta or secretions of animals infected with transmissible human or animal pathogens.

Decontamination, Sterilization, Disinfection

All biohazardous waste must be rendered noninfectious prior to final disposal. While Biosafety Level-3 and the higher risk BL-2 agents must be autoclaved prior to further processing, most infectious material can be disposed safely through proper handling and packaging directly for incineration.

Each generator of biohazardous waste has an obligation to handle and dispose their material in a manner which affords protection from leakage and injury or exposure to service personnel handling their waste material.

1. Each individual working with biohazardous material or contaminated items is responsible for their decontamination, disinfection, and appropriate preparation prior to disposal or reuse.
2. All laboratories, in which work with biohazardous materials is carried out, must have labeled, leak-proof, covered containers for temporary holding of infectious materials awaiting disinfection or disposal.
3. When autoclaving:
    - Test tape or another suitable indicator must be used on each load placed in the autoclave. This will aid in determining which items have been sterilized.
    - Waste bags must be marked with the room number from which the waste originated.
    - Bagged waste must be placed into a containment pan prior to autoclaving. The purpose of the containment pan is to prevent release of material in the event that the bag loses its integrity during the cycle. This prevents waste from building up within drainage pipes, allowing for proper function of the autoclave.
    - only approved autoclavable bags are to be used. Red bags are reserved for incineration, and are not acceptable for autoclaving.
    - autoclaves may only be operated by trained individuals. Operators must never attempt to open an autoclave door while the chamber contains any pressure. Doing so may result in severe burns, forceful release of the autoclave door and injury, as well as damage to the unit.
    - log sheets must be available at each autoclave to record the name of the user, time of run, and amount being autoclaved.
4. All floors, laboratory benches, and other surfaces in areas where biohazardous materials are handled must be disinfected upon completion of operations involving plating, pipetting, centrifugation and similar procedures.
5. Floors should be mopped with disinfectant. Avoidance of dry sweeping and dusting will reduce the formation of aerosols. If sweeping is necessary, a push broom and floor sweeping compounds should be used. Waxing and buffing should be done only after mopping.
6. Floor drains must be flooded with water periodically in order to fill traps and prevent the backflow of sewer gases.

Specific Disinfection and Sterilization Methods

Wet Heat

1. 1. The destruction of all forms of microorganisms is most readily accomplished by wet heat or autoclaving (saturated steam under pressure).
    - Higher pressures give higher internal temperatures.
    - Appropriate biological indicators should be used in containers or between densely packed materials to determine the effectiveness of the decontamination cycle.
2. Other critical factors which ensure the effectiveness of the autoclaving (besides saturated steam and proper temperature) are the removal of air from the chamber and its contents and adequate exposure time as related to the "soil" load on contaminated items.
    - Heavily "soiled" items, especially if the "soil" is of proteinaceous nature, should not be flash autoclaved because that "soil" may briefly protect the microorganism from the lethal effects of the wet heat.
    - Autoclave times are directly proportional to the volume of materials to be autoclaved. Twenty min. at 121oC is adequate for the smallest loads. When volumes in excess of 500 ml are autoclaved, times must be increased. Consult the autoclave manufacturers' handbook for your unit.
    - It should also be noted that overloading or underloading of an autoclave also reduces the efficiency of decontamination.

Suggested Temperatures and Exposure Times from NIH Biohazards Guidelines

Laundry - 121oC (250oF), 30 min.
Trash - 121oC (250oF), 1 hr.
Glassware - 121oC (250oF), 1 hr. or 160o 320oF) dry heat, 4 hr.
Liquids - 121oC (250oF), each gallon, 1 hr.
Small Animals - 121oC (250oF), 8 hr.

CAUTION!

Never autoclave hazardous chemicals! Doing so can create hazardous conditions. Very few chemicals are considered acceptable to autoclave. If you have questions about autoclaving chemicals, contact IBC .

Dry heat

1. 1. The use of dry heat for the disinfection or sterilization of biohazardous materials and contaminated items is less efficient than autoclaving and requires a longer exposure time with higher temperatures.
    - a) It may be possible to disinfect "soiled" materials by exposing them to 160oC (320oF) for four hours.
    - b) If items are heat sensitive, a temperature of 120oC (248oF) must be used, and exposure time necessary for disinfection or sterilization is usually greater than 24 hours.
    - c) The use of biological indicators (Bacillus subtilis spores) is also necessary with dry heat to determine the effectiveness of the sterilization cycle, and to determine

the most effective temperature and/or exposure time for sterilization of materials or equipment.

CAUTION!

Dry heat at high temperatures and for long durations should be used to sterilize oils and anhydrous materials such as powders.

Ethylene Oxide

Ethylene oxide (EtO) gas is lethal for all known microorganisms. This is true whether EtO is used undiluted or with CO2, or other diluents. Some of the process variables which affect the microbiocidal rate are as follows:

1. Temperature affects the penetration of EtO through microbial cellular components and wrapping and/or packaging materials. The microbiocidal activity of EtO increases with the increase in temperature. Generally, temperatures between 38oC and 54oC (100oF and 130oF) are employed in the EtO sterilization process.
2. Microbiocidal activity is increased as the concentration of EtO is increased, up to about 1,000 micrograms per liter of EtO. For practical sterilization, gas concentrations of 500 to 1,000 micrograms per liter at approximately 49oC to 60oC to (120oF to 140oF) are recommended.
3. Moisture is required for the microbiocidal activity of EtO and appears to be related to the moisture content of the exposed microorganism. This is especially true for the moisture content of the bacterial cell wall. A relative humidity of 30 to 60% is frequently employed in EtO chambers to ensure the proper moisture conditions.
4. The exposure time depends on the above noted variables. Since these variables will not be the same in different commercially available EtO chambers, exposure times recommended by the manufacturers should be followed.

PRECAUTIONS FOR USE OF ETHYLENE OXIDE

1. The use of EtO to sterilize heavily "soiled" items has not been adequately documented. Thus, if "soiled", heat sensitive items are sterilized with EtO, subsequent treatment with a chemical disinfectant is recommended.

    - All items except those made of glass and metal should be aerated prior to handling or contact with human skin because EtO which has been absorbed by PVC, rubber, etc., can cause burns or skin irritation unless first removed by aeration. The elimination of harmful EtO residues from the most challenging materials (PVC) can be achieved as follows:
        - Storage at room temperature for seven days.

        - Mechanical aeration at elevated temperature (60oC) in an aeration cabinet for eight hours.
2. Mixtures of EtO and air are explosive. However, commercially available mixtures of EtO and CO2 are not explosive.
3. Use the manufacturer's recommended exposure time for EtO.
