

Translational Health Science and Technology Institute

Biosafety Guidelines

PURPOSE

The purpose of this document is to ensure a safe working environment for all activities and for compliance with all applicable regulations concerning the use of biological agents, biological toxins, select agents, and recombinant DNA in the laboratory. The precautions and guidelines in this Biosafety Manual are compatible with current knowledge and DBT regulations.

SCOPE

These Biosafety Guidelines applies to all laboratory employees working in laboratories that use biological agents, biological toxins and recombinant DNA.

It should be maintained as a working document, reviewed periodically, updated as necessary, and readily accessible to all personnel working in a lab or area with infectious and/or recombinant agents - preferably kept in the work area.

The lab Biosafety Manual should also be available to safety, and emergency response personnel in case of an incident, accident, or an emergency in a specific lab area.

PROCEDURES GOVERNING THE USE OF BIOHAZARDOUS AGENTS

This Biosafety Manual provides a guide to common practices related to working with biological materials.

Biohazardous agents, or "biohazards", are infectious agents or hazardous biological materials that present a risk or potential risk to the health of humans, animals or the environment. The risk can be direct through infection or indirect through damage to the environment. Biological materials that investigators may not consider to be biohazardous may still be regulated under DBT guidelines as biohazardous materials.

Investigators must obtain approval from the Institutional Biosafety Committee (IBSC) of the following biological materials prior to the procurement of the materials necessary to initiate the project:

- recombinant DNA in organisms,
- creation of transgenic plants or animals,
- human and other primate-derived substances (blood, body fluids, or tissues),
- organisms and viruses infectious to humans, animals or plants (e.g. parasites, viruses, bacteria, fungi, prions, rickettsia);
- biologically active agents (i.e. toxins, allergens, venoms) that may cause disease in other living organisms or cause significant impact to the environment or community.

Investigators must register projects with the IBSC by completing specific registration form. All registration documents for use of biologicals should be stored with the Principal Investigator (PI) as well as with the IBSC.

SUMMARY OF BIOSAFETY LEVELS

Assignment of Biosafety Levels (BL) - It is the responsibility of the PI to initially assign the BL to his/her protocol. This level may be changed either upward or downward during review by the IBSC. All parties involved in assigning BLs will follow the standard levels of 1, 2 or 3 as outlined in DBT's *Guidelines for Research Involving Recombinant DNA Molecules*. These are summarized below.

- | | |
|---------|--|
| Class 1 | Organisms not known to cause disease in healthy adults. |
| Class 2 | Associated with human disease, infection transmitted through autoinoculation, ingestion, body fluid exposure and mucous membrane exposure. |
| Class 3 | Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences. |
| Class 4 | Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission. |

Mandatory Submission of Protocol Application - PI's proposing research/academic activities involving microorganisms (including exempt and non-exempt rDNA activities), blood or other potentially infectious body fluids, carcinogens, mutagens or teratogens and biological toxins must submit their protocols to IBSC for review and approval action prior to initiation of the activity.

PROTOCOL REVIEW AND APPROVAL

rDNA Activities - All protocols involving rDNA activities must follow the requirement of DBT *Guidelines for Research Involving Recombinant DNA Molecules* and all supplements published thereafter. Areas to address when rDNA protocols are being prepared for submittal to IBSC are:

- rDNA Insert
 - Synthetic and associated sequence(s)
 - Potential protein product
- Vector
 - Carrier used to introduce rDNA into the host system that facilitates replication
 - Plasmids, organelles, viruses
- Host
 - Organism in which the rDNA replicates
 - Bacteria, yeast, plant, animal cells
- Containment - Several containment methods are described below:
 - Limit infectivity of vector or vehicle for specific hosts
 - Limit dissemination and survivability of host and/or vector in the environment
 - Specifically designed equipment and facilities used to physically contain microbes
 - Limit access to facilities
- Good Laboratory Practices:
 - Specifically designed practices and procedures used to physically contain microbes
 - Mechanisms for inactivation and disposal of microbes

EMERGENCY PROCEDURES

PI should maintain a document accessible to all lab personnel, with a list of procedures to be followed in case of an accident such as a spill, injection, ingestion, aerosolization, splash, etc. The document should have the following information:

- Agent specific actions
- Known first aid procedures
- Effective disinfectants / neutralizing agents (include location in lab)
- Known symptoms associated with exposures to this agent(s)

EMERGENCY NOTIFICATION

- Call Principal Investigator
- Call Security, emergency medical personnel, etc.

TRAINING

Records of appropriate training (technical, safety, procedural, etc.) of lab personnel will be maintained. PI should ensure enrollment of new lab personnel to biosafety training and notify the IBSC on December 1st of each year about training status of all lab personnel.

VACCINATION

The Biosafety Officer will assess requirement of vaccination for students and staff working in various laboratories depending on the micro-organism used and level of exposure. A regular regime will be followed with documentation of baseline serum values and subsequent values for different antibodies as decided by the Biosafety Officer.

INSTITUTIONAL NOTIFICATION REQUIREMENT

It is the responsibility of the PI to make appropriate notifications in the wake of an occupational exposure, laboratory accident, loss of potential containment, etc. The IBSC should be notified of incidents or adverse events involving biological agents or toxins.

ROLE OF IBSC IN APPROVAL

The role of IBSC in research, large-scale experiments/ production/field release and import and shipment shall be as under:

Research activities related to rDNA technology: IBSC has to review all recombinant research carried out by an organisation depending upon the category of experiments, IBSC can simply note the information provided by PI, give permission before start of the experiments or forward it to RCGM for approval as per the Recombinant DNA Safety Guidelines, 1990 of DBT.

- Category I experiments involving self cloning, using strains and also inter species cloning belonging to organism in the same exchanger group etc. are exempt for the purpose of intimation and approval.
- Category II experiments falling under containment levels II, III and IV, large scale use of recombinants made of self cloning in systems belonging to exempt category etc. require prior intimation to IBSC.
- Category III experiments involving toxin gene cloning, cloning of genes for vaccine production, use of infectious animals and plant viruses, self fusion experiments, field testing and release of GMOs etc. require review and approval of IBSC before commencement.

Different levels of containment have been prescribed for different categories of rDNA experiments in the Guidelines. IBSC should allow genetic engineering activity on classified organisms only at places where such work can be performed as per Guidelines and containment facilities.

Provision of suitable safe storage facility of donor, vectors, recipients and other materials involved in experimental work should be made and may be subject to inspection for accountability on biosafety.

ROLE OF PRINCIPAL INVESTIGATOR

All recombinant research projects carried out by an organisation involving the use of GMOs/LMOs or rDNA materials should have a Principal Investigator (PI). It is the duty of the PI to apprise the IBSC about the nature of the experiments being carried out. Depending upon the risk category, the PI has to inform the IBSC, seek permission of IBSC before starting the experiments or seek permission of the RCGM through its IBSC.

Based on the nature of the GMOs/LMOs and rDNA materials, the PI determines the proper containment level for the project and, in accordance with the guidelines, develops the necessary experimental protocols. This information is then submitted to IBSC for review. The responsibilities of PI are summarized below:

- To make an initial determination of the required levels of physical and biological containment in accordance with the stipulated guidelines.
- To submit the initial research protocol and any subsequent changes (such as changes in the source of DNA or host vector system) to the IBSC for review and approval.
- To ensure that no work is initiated until the research project has been approved by the IBSC and has met all requirements of DBT guidelines.
- To communicate with IBSC throughout the conduct of the project.
- To ensure safe conduct of the rDNA experiments in his laboratory.
- To make available the protocols that describe the potential biohazards and the precautions to be taken to all laboratory staff.
- To instruct laboratory staff about the practices and techniques required to ensure safety, and the procedures for dealing with accidents including the reasons and provisions for any precautionary medical practices advised or requested (e.g. vaccinations or serum collection).
- To supervise the performance of the laboratory staff to ensure that the required safety practices and techniques are employed.
- To undertake corrective measures promptly for any work errors and conditions that may result in the release of recombinant DNA materials.

PERSONS RESPONSIBLE FOR COMPLIANCE

The responsibility for biosafety in THSTI rests with the Head of the organisation and PI, who obtains, possesses or uses GMOs/LMOs/rDNA materials. IBSC provides guidance to ensure compliance. Any possession and/ or use of these materials at the organisation must be conducted with appropriate safeguards against environmental release.

Head of the Organisation

The head of the organisation is responsible for compliance with Rules, 1989 and other related regulations regarding rDNA research. The head maintains ultimate responsibility for the safe conduct of activities involving GMOs/LMOs/rDNA research.

Principal Investigator (PI)

The PI is required to comply with the appropriate research guidelines and all applicable laws related to biosafety and is accountable to the Head and IBSC .

Laboratory Personnel (Technician, Technologist, Student, Post-doctorate)

Laboratory personnel must:

- i. Follow all safety guidelines and establish good laboratory practices. They must work within the assigned biological safety containment level and use personal protective equipment as recommended by the PI.
- ii. Immediately notify the PI or Biological Safety Officer (BSO) of any health condition that may be due to their work in the laboratory or any health condition that may be compromised prior to the initiation of a research project (i.e. pregnancy, immunosuppression).
- iii. Follow all practices and procedures as provided by the PI and BSO, and ensure strict compliance with all required biosafety regulations and guidelines.
- iv. Report problems, procedural mistakes, spills, etc. to the PI, and if necessary to the BSO, as soon as they occur.
- v. Report to the PI, BSO or IBSC on non-compliance of biosafety guidelines or policies.

ADDRESSING NONCOMPLIANCE

The IBSC can address non-compliance to the Rules, 1989 or to the organisation's policies and procedures and any other relevant legal requirements.

Non-compliance can result in the IBSC taking one or more of the following actions:

- i. Suspension of the use of GMOs/LMOs/rDNA materials.
- ii. Cessation of the approval for use of the GMOs/LMOs/rDNA materials.
- iii. Confiscation and/or destruction of the GMOs/LMOs/rDNA materials.
- iv. Any other action necessary to protect the public and/or the organisation, including suspending the relevant research activity.
- v. Reporting to the RCGM.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

- Laboratory garments, e.g. lab coats, scrubs, and gowns, are long-sleeved and used to prevent contamination of the skin and street clothes. If splashes may occur, the garment must be fluid-resistant. If required, lab coats should be provided for visitors, maintenance and service workers.

- Gloves must be worn when working with biohazards. Temperature resistant gloves must be worn when handling hot material or dry ice. If personnel develop or have latex allergies, then nitrile gloves should be used in the lab with biohazards instead of latex gloves. Gloves should overlap the sleeve of the lab garment. Double-gloving adds further protection and is recommended in some circumstances, e.g. for BSL-3 laboratories, or if a spill or splash may occur.
- Face protection, e.g. goggles or safety glasses with side shields in combination with masks, or face shields, or other splatter guards are required for anticipated splashes or sprays of infectious material.

SAFE WORK PRACTICES

- Proper work practices protect you and others from exposure to infectious materials, reduce the possibility of cross-contamination, and improve the quality of the work performed.
- Label all equipment used to store infectious materials with a biohazard warning label.
- Keep an uncluttered work space.
- Plan work procedures with safety in mind.
- Remove PPE and wash hands when leaving the lab.
- Don't eat, drink, smoke, apply cosmetics, and handle contact lenses in the lab.
- Don't mouth pipette.
- Decontaminate work surfaces at the end of an experiment and after a spill occurs.
- Decontaminate reusable PPE as soon as possible after it has been contaminated. Lab coats can be spot treated with 10% bleach or autoclaved before laundering. Never take lab coats home.
- Protect house vacuum lines and vacuum pumps by using a hydrophobic HEPA filter installed between the collection flask and vacuum source.
- Change gloves often and as soon as possible when visibly contaminated.
- Minimize aerosol production by working carefully.
- Perform procedures that may result in aerosols or splashes in a BSC.
- Use aerosol-proof rotors or safety cups when centrifuging and load and unload them in a BSC.

GENERAL GUIDELINES FOR USING BIOSAFETY CABINETS & LAMINAR FLOW HOODS

- If adjustable, the window should be lowered to 8 inches, with a ≥ 75 ft/min face velocity.
- Keep the amount of equipment used or stored in the cabinet to a minimum.
- Before work is started, everything needed for the procedures should be placed in the cabinet, and let cabinet air exhaust for a few minutes.
- Nothing should be placed on or blocking the front or rear grilles.
- Contaminated items should be segregated from clean ones and located so that they never have to pass over clean items.
- Avoid disrupting the air barrier in a safety cabinet by frequent and rapid arm movements and bringing hands in and out of the cabinet.
- Waste containers should be placed inside the cabinet to avoid breaking the air barrier and bringing contaminated items out into the room.
- Do not use a burner inside a Class II BSC because the air currents induced counter to the normal air flow, can cause contamination of the work surface and ignite ethanol and other materials in the cabinet and damage the HEPA filter.

- When working with biohazards, keep absorbent towels and decontaminating solutions in the cabinet and wipe down work surface with ethanol prior to and at the completion of each session, and after any small spills.
- Decontaminate all equipment removed from the cabinet. Pipetting aids and tools that are used repeatedly should remain in the cabinet.
- Decontamination of the entire cabinet (filter, plenums, work surfaces and the fan) is achieved by exposing these areas to a paraformaldehyde vapor. This type of decontamination must be performed only by a trained professional.
 - Should the power to the unit fail during use, stop work with biohazardous agents immediately, seal all cultures securely, and decontaminate the work area with a suitable disinfectant. BSC should be put on UPS for continuous power backup in case of power failure.
 - Horizontal Laminar Flow Hoods provide a very clean environment but must be used only for the manipulation of non-hazardous materials. Since the operator sits in the downstream exhaust from the clean bench, this equipment must never be used for the handling of toxic, infectious, or sensitizing materials, including volatile chemicals, cell culture materials (except plant cell cultures), or drug formulations.

MAINTENANCE OF INCUBATORS

Incubators can become the inadvertent and undesired repository of microorganisms. Although they may present a hazard to laboratory workers, most often they are a source of contamination of laboratory cultures. Besides the moist surfaces, rubber gaskets, the humidity trough if present, and the fan mechanism are areas in which contaminating microorganisms concentrate.

- It is recommended that an anti-microbial agent such as Zepharin chloride be added to the humidity source water (do not use sodium azide).
- Panels, trays and other removable parts should be autoclaved and the gaskets and non-removable parts wiped thoroughly with 70% ethanol every 2 months.

GUIDELINES FOR USE OF CHEMICAL FUME HOOD

General Purpose Hoods

Use this type of hood only for the removal of vapors released or generated by chemical reactions involving:

- mildly toxic materials
- acids (not heated)
- organic solvents
- < 10 milliCurie of radioactive materials

In this type of hood, do *not* use:

- hot perchloric acid
- hot concentrated acids
- highly toxic materials
- unstable chemicals or explosives
- > 10 milliCuries of radioisotopes
- perchloric acid if used routinely

If a CAUTION or DANGER sign is posted, do not work in the hood until the face velocity has been adjusted

- Do not use large pieces of equipment in the hood.
- Close doors and windows when hoods are in operation.
- Avoid foot traffic and rapid arm/body movement.
- Place chemical sources and equipment at least 6 inches behind the face of the hood.
- Do not extend your head inside of the hood while experiments are being performed
- Perform work with the sash height as low as possible (at most 10-12 inches).
- Keep fume hoods and adjacent work areas clean since solid debris can enter the hood's exhaust duct work.
- Protect spark sources from flammable vapors. Permanent electrical receptacles are not permitted in the hood.
- Do not cut holes into the hood or its duct work.
- Do not store chemicals in a fume hood unless storage is the sole use of the hood. Only those chemicals necessary to perform the experiment should be left in the hood.
- Do not use hood for evaporation as a means of chemical disposal.

CLEAN UP RESPONSIBILITIES

- Each area of the Institute should have an appropriately trained person who is responsible for the clean up of blood or potentially infectious materials.
- Employees and students who have been through the biosafety training are designated to clean up blood or potentially infectious material spills that occur in their work areas.
- Blood or potentially infectious material spills which occur inside buildings, in an area outside of a trained person's work or research area, shall be cleaned up by the designated housekeeping person (trained employee) who is on duty at that time in consultation with housekeeping in charge.
- Blood or potentially infectious material spills which occur outside of a building, but on institute property shall be cleaned up by a trained person in consultation with housekeeping in charge.
- Blood or potentially infectious materials that are on a person's body shall only be cleaned up by a trained first responder, who is trained. Employees who are not trained in blood-borne pathogens protection are not expected to perform CPR or First Aid on accident victims or injured employees.
- Only approved cleaning products may be used to clean up blood or potentially infectious materials. Specific products and instruction will be provided for housekeeping personnel and other designated spill clean up employees during training provided by IBSC.

DEVELOPING A GENERAL LABORATORY SPILL KIT

Listed below are the materials necessary to develop a fairly inexpensive general spill kit for a laboratory. This kit should be able to handle < 1 litre of most acids, bases, and solvents found in a typical laboratory. Do not attempt to clean up a spill of greater volume.

Materials:

Large plastic box to hold the contents of the kit (3-5 gallons)

Plastic dust pan and brush (non-sparking)

Chemical safety goggles and face shield

Appropriate chemical resistant gloves (ex. neoprene)

3-5 waste disposal bags that can be sealed/closed

4-5 absorbent pads

Note: brown paper towels and other combustible tissue may ignite when brought into contact with certain chemicals

pH paper

Bleach (if biohazards are present)

Absorbent paper towel for liquid spills

Sodium bicarbonate for acids

Citric acid for bases

“Powersorb Universal Absorbent Pads” (3M) or other “universal” pads

Specific Hazardous Chemicals:

This general chemical spill kit is not meant for use with mercury, hydrofluoric acid, sodium metal, cytotoxic drugs, and numerous other chemicals. It is the responsibility of all laboratory personnel to evaluate a potential spill and develop spill response procedures for the specific hazards present. This kit can be modified to meet the needs of your laboratory. Refer to the MSDSs for the chemicals handled in the lab to identify if special spill materials are needed.

POST-EXPOSURE PROPHYLAXIS GUIDELINES

In case of accidental exposure to infectious material eg., infected body fluid, pathogenic strains etc., prophylaxis measures should be initiated as follows:

- Immediate repeated washing with soap, water, antiseptic solution (where applicable)
- Report to respective PI and the Biosafety Officer
- Initiation of post-exposure counseling and testing and treatment (as applicable)

WASTE DISPOSAL GUIDELINES

Definition of infectious waste includes but is not limited to:

- Cultures and stocks of infectious agents and associated biologicals including cultures from medical and pathological laboratories; cultures and stocks of infectious agents from research and industrial laboratories, waste from the production of biologicals; discarded live and attenuated vaccines and culture dishes and devices used to transfer, inoculate, and mix cultures.
- Pathological wastes which includes body tissues and their containers.
- Human or animal blood and blood products including liquid waste, human and animal blood, products of blood, items saturated or dripping with human or animal blood; items that were saturated and dripping that are now caked with dried human or animal blood; serum, plasma, and other blood components and their containers which were used or intended for use either in patient care, testing, laboratory analysis, research, or the development of pharmaceuticals. Intravenous bags are also included in this category.

- Used and unused sharps that have been used in animal or human patient care, treatment or medical research, or industrial laboratories; including hypodermic needles, syringes (with or without the attached needle), Pasteur pipettes, scalpel blades, blood vials, needles with attached tubing, and culture dishes (regardless of presence of infectious agents). Also included are other types of broken or unbroken glassware that were in contact with infectious agents such as used slides and cover slips.
- Contaminated animal carcasses including whole contaminated animal carcasses, body parts, and bedding of animals that were known to have been exposed to infectious agents during research (including research in veterinary hospitals), production of biologicals or testing of pharmaceuticals.
- Other waste that has been intermingled with infectious waste including any material (i.e., paper products, plastic products, and disposables) that has at any time been in contact with or believed to have been in contact with any infectious agent.

All biological material (microbial culture, used/unused plates and media, broth/solid media, tissue culture waste, blood and other biological sample from human, animal body part or tissue, pathogenic strains, plasticware and glassware used for biological material) should be autoclaved on site, labelled and discarded for disposal. This waste disposal approach should be strictly followed.

CATEGORIES OF BIO-MEDICAL WASTE

As per Section 6, 8 and 25 of the Environment (Protection) Act, 1986 of the Central Government

Category No.	Type of Waste	Examples	Treatment & Disposal
1	Human anatomical waste	human tissues, organs, body parts	Incineration @/deep burial*
2	Animal waste	animal tissues, organs, body parts carcasses, bleeding parts, fluid, blood and experimental animals used in research, waste generated by veterinary hospitals colleges, discharge from hospitals, animal houses	Incineration @/deep burial*
3	Microbiology & Biotechnology waste	wastes from laboratory cultures, stocks or specimens of micro-organisms live or attenuated vaccines, human and animal cell culture used in research and infectious agents from research laboratories, wastes from production of biologicals, toxins, dishes and devices used for transfer of cultures	local autoclaving/micro-waving/incineration @
4	Sharps contaminated	needles, syringes, scalpels, blades,	disinfection (chemical

	with biological waste	glass, etc. that may cause puncture and cuts. This includes both used and unused sharps	treatment @ /autoclaving /micro- waving and mutilation/shredding ##
5	Discarded medicine & cytotoxic drugs	wastes comprising of outdated, contaminated and discarded medicines	incineration@/ destruction and drugs disposal in secured landfills
6	Solid waste contaminated with biological sample	Items contaminated with blood, and body fluids including cotton, dressings, soiled plaster casts, lines, beddings, other material contaminated with blood	Incineration @ /autoclaving / microwaving
7	Solid waste	wastes generated from disposable items other than the waste sharps such as tubings, catheters, intravenous sets etc	treatment@@ /autoclaving /microwaving and mutilation/shredding ##
8	Liquid waste	waste generated from laboratory and washing, cleaning, house-keeping and disinfecting activities	disinfection by chemical treatment@@ and discharge into drains
9	Incineration ash	ash from incineration of any bio-medical waste	disposal in municipal landfill
10	Chemical waste	chemicals used in production of biologicals, chemicals used in chemical disinfection, as insecticides, etc.	chemical treatment@@ and discharge into drains for fluids and secured landfill for solids

@@ Chemicals treatment using at least 1% hypochlorite solution or any other equivalent chemical reagent. It must be ensured that chemical treatment ensures disinfection.

Multilation/shredding must be such so as to prevent unauthorised reuse.

@ There will be no chemical pretreatment before incineration. Chlorinated plastics shall not be incinerated.

* Deep burial shall be an option available only in towns with population less than five lakhs and in rural areas.

COLOUR CODING AND TYPE OF CONTAINER FOR DISPOSAL OF BIO-MEDICAL WASTES

Colour Coding	Type of Container - Waste Category	Treatment options
Yellow	Plastic bag Cat. 1, Cat. 2, and Cat. 3, Cat. 6.	Incineration/deep burial
Red	Disinfected container/plastic bag Cat. 3, Cat. 6, Cat.7.	Autoclaving/Microwaving/ Chemical Treatment
Blue/White translucent	Plastic bag/puncture proof Container Cat. 4, Cat. 7.	Autoclaving/Microwaving/ Chemical Treatment and destruction/shredding
Black	Plastic bag Cat. 5 and Cat. 9 and Cat. 10. (solid)	Disposal in secured landfill

1. Colour coding of waste categories with multiple treatment options as defined in Schedule I, shall be selected depending on treatment option chosen, which shall be as specified in Schedule I.
2. Waste collection bags for waste types needing incineration shall not be made of chlorinated plastics.
3. Categories 8 and 10 (liquid) do not require containers/bags.
4. Category 3 if disinfected locally need not be put in containers/bags.