Laboratory Safety Manual

National Centre for Biological Sciences (NCBS)

Institute for Stem Cell Biology and Regenerative Medicine (inStem)

Centre for Cellular and Molecular Platforms (C-CAMP)

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Preamble

This laboratory safety manual is compiled to be used as a binding document for all personnel working in laboratories to ensure safe work conduct and practices on the NCBS/inStem/CCAMP campus.

Procedures and Rules within this Manual are formulated for three reasons:

1.) To avoid health risks and accidents for our personnel.

2.) To be in a position to act appropriately in case of emergencies.

3.) To minimize the environmental burden and risks caused by our work.

This manual cannot cover all circumstances where safety procedures must be applied; it is intended to set up a framework of how NCBS/inStem/CCAMP personnel/students should work in scientific laboratories. The avoidance of safety risks for the personnel at the institutional level requires knowledge of possible hazards in our environment; chemicals, biological agents or radioactivity. In this regard students and personnel of NCBS/inStem/CCAMP are required to familiarize themselves with safe practices for applied laboratory operations.

This manual will be amended as various situations might arise and come to our attention.

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1.0 - GENERAL SAFETY

People who work in scientific laboratories are exposed to various hazards. Most workplaces have hazards that are well recognized (those of ordinary fire, for example) with well-defined actions to control the situation. Laboratories, however, involve a greater variety of possible hazards and some of these hazards need precautions not ordinarily encountered. An introduction to safe practices for a variety of widely used laboratory procedures is listed below.

1.1 - GENERAL SAFETY AND OPERATIONAL RULES

1. No running or jumping in a laboratory is permitted. Stored items or equipment shall not block access to the fire extinguisher(s), safety equipment, or other emergency items. Stairways, hallways, passageways/aisles and access to emergency equipment and/or exits must be kept dry and unobstructed; i.e., no storage, no equipment, phone or other wiring. No combustible material such as paper, wooden boxes, pallets, etc., shall be stored under stairwells or in hallways. Hallways shall be kept free of boxes and materials so that exits and normal paths of travel are not blocked.

2. Eating or drinking within laboratories is not permitted. In all laboratories specific office areas may be designated for food in coordination with the Safety Committee. They must be physically separated from any laboratory operations. In the specified office areas no consumables, reagents or any tools should be shared with work areas.

3. No food or beverage may be stored in the cold rooms/Laboratory refrigerators and freezers.

4. Working core hours at NCBS/InStem/CCAMP are 8am – 8pm (Mon.-Sat.). No employee shall work alone in a laboratory or chemical storage area outside the core working hours. Permission for students and staff to work outside of the core time has to be granted by the PI in charge in writing.

5. Animals, except those that are the subject of experimentation (approved by the Animal Experimentation Committee) are to be excluded from all laboratory areas.

6. Clothing worn in the laboratory should offer protection from splashes and spills, should be easily removable in case of an accident. Nonflammable, nonporous aprons offer the most satisfactory and the least expensive protection. Lab jackets or coats should have snap fasteners rather than buttons so that they can be readily removed. These coats are to be fastened closed while working and removed prior to exit from the laboratory. It is highly recommended that no sandals or open-toed shoes shall be worn by laboratory personnel in the laboratory. Laboratory clothing should be kept clean and replaced when necessary. In procedure performed in biosafety level 2 and chemical operations it is required that lab coats, gloves, closed shoes and safety glasses are worn.

7. Mouth pipetting is never allowed.

A. Electrical

The typical laboratory requires a large quantity of electrical power. This increases the likelihood of electrically-related problems and hazards. One must address both the electrical shock hazard to the facility occupants and the fire hazard potential. The following recommendations are basic to a sound electrical safety program in the laboratory.

1. All electrical equipment shall be properly grounded.

2. Sufficient room for work must be present in the area of breaker boxes. All the circuit breakers and the fuses shall be labeled to indicate whether they are in the "on" or "off" position, and what appliance or room area is served. Fuses must be properly rated.

3. Equipment, appliance and extension cords (junction boxes) must be in good condition and must be routinely dusted..

4. Extension cords shall not be used as a substitute for permanent wiring.

5. Electrical cords or other lines shall not be suspended unsupported across rooms or passageways. Do not route cords over metal objects such as emergency showers, overhead pipes or frames, metal racks, etc. Do not run cords through holes in walls, ceilings, doorways or windows. Do not place under carpet, rugs, or heavy objects. Do not place cords on pathways or other areas where repeated abuse can cause deterioration of insulation.

6. Multi-outlet plugs shall not be used unless they have a built-in circuit breaker. This causes overloading on electrical wiring, which will cause damage and possible overheating.

7. Most of the portable multiple outlets are rated at 15 amps. Employees shall check when all connections are made to determine that the total input average will never exceed 15 amps. (The amperage on electrical equipment is usually stamped on the manufacturer's plate).

8. All building electrical repairs, splices, and wiring shall be performed by the Electrical Department.

B. Vacuum Operations

In an evacuated vacuum system, the higher pressure is on the outside, rather than the inside, so that a break causes an implosion rather than an explosion. The resulting hazards consist of flying glass, spattered chemicals, and possibly fire.

1. When working with a vacuum be aware of implosion hazards. Apply vacuum only to glassware specifically designed for this purpose, i.e., heavy wall filter flasks, desiccators, etc.

2. Never evacuate scratched, cracked, or etched glassware. Always check for stars or cracks before use.

3. Vacuum glassware which has been cooled to liquid nitrogen temperature or below should be annealed prior to reuse under vacuum.

4. Rotary evaporator condensers, receiving flasks, and traps should be taped or kept behind safety shields when under a vacuum.

5. When a vacuum is supplied by a compressor or vacuum pump to distill volatile solvents, a cold trap should be used to contain solvent vapors. Cold traps should be of sufficient size and low enough temperature to collect all condensable vapors present in a vacuum system. If such a trap is not used, the pump or compression exhaust must be vented to the outside using explosion proof methods.

6. After completion of an operation in which a cold trap has been used, the system should be vented. This venting is important because volatile substances that have been collected in the trap may vaporize when the coolant has evaporated and cause a pressure buildup that could blow the apparatus apart.

7. After vacuum distillations, the vessel must be cooled to room temperature before it is vented.

8. All desiccators under vacuum should be completely enclosed in a shield or wrapped with friction tape in a grid pattern that leaves the contents visible and at the same time guards against flying glass should the vessel collapse. Various plastic (e.g., polycarbonate) desiccators now on the market reduce the implosion hazard and may be preferable.

C. Handling Glassware

1. Glass breakage is a common cause of injuries in laboratories. Only glass in good condition should be used.

2. Clean all glassware before sending for repair. Glassware that has been in contact with infectious agents shall be disinfected before disposal or repair.

3. Protect hands with leather gloves when inserting glass tubing. Hold elbows close to the body to limit movement when handling tubing.

4. Use glassware of the proper size. Allow at least 20% free space. Grasp a three-neck flask by the middle neck, not a side neck.

5. Conventional laboratory glassware must never be pressurized or used with vacuum.

2.0 - Safe Handling of Chemicals

General Guidelines.

Working with potentially harmful chemicals is an everyday occurrence in a laboratory. Employees are requested to inform themselves about toxicological information and procedures for handling and storage of chemicals used. For most commercially available substances, detailed instructions are available in the Material Safety Data Sheet (MSDS). Facilities for collection and storage are provided within the NCBS/InStem/CCAMP campus. A hardcopy of MSDS for each lab is required.

A. Gathering General Information on Chemicals

The Material Safety Data Sheet (MSDS) describes properties, reactivities, potential chemical hazards, and safe handling procedures for commercially available chemicals you are working with. These sheets must be archived in a specified folder; all lab personnel must know where it is kept. This MSDS log must be updated at regular intervals. Information that is contained in the Material Safety Data Sheets is also required by law to be conveyed to employees on a chemical-by-chemical basis.

MSDSs are generally written for chemicals that are used in the industrial setting therefore some of the information provided on the MSDS may not be applicable to laboratory usage. The use of chemicals in a laboratory is generally in a more controlled environment than in the industrial setting and much smaller quantities of the chemical are being used at any one time. Nevertheless, a great deal of information on hazards associated with laboratory chemicals can be obtained by reading the MSDS.

Familiarize yourself with the pictograms and Hazard codes widely used to mark risks.

Figure source: http://www.sigmaaldrich.com/sigma-aldrich/help/help-welcome/risk-and-safety-statements/risk-and-safety.html#pictograms

Risk and Safety Phrases are widely used and should be known by any user when handling chemicals in the course of laboratory procedures. Further information upon chemical stability can be obtained from **Fluka Brand F Code.**

B. Handling and Transportation of Chemicals

Many laboratory accidents occur by carrying chemicals from one place to another or transferring them from one container to another. The chemicals used in a laboratory are often corrosive, toxic or flammable and any accident involving these has the potential for personal injury. Therefore, it is good practice to assume that **all** chemicals are potentially hazardous.

1. When large bottles of acids, solvents, or other liquids are transported within the laboratory without a cart, only one bottle should be carried at a time. The bottle should be carried with both hands, one on the neck of the bottle and the other underneath. Do not hook a finger through the glass ring on top of the bottle, allowing it to dangle while being transported. Never carry or attempt to pick up a bottle by the cap.

2. Large quantities of concentrated mineral acids, e.g., sulfuric, nitric and hydrochloric acids, shall be kept in specific storage rooms or cabinets for corrosive substances.

C. Chemical Storage

Proper storage of chemicals is necessary to maximize employee safety with regard to chemical compatibility, spill control, fire/explosion control, to provide security, identification, and provide a "user friendly" system with respect to point-of-use.

1. All containers used for storage (even short term) shall be labeled.

2. Quantities greater than one litre of highly flammable liquids (Methanol, Chloroform) should be stored in specified metal cabinets. Quantities smaller than or equal to one litre of chemicals may be held at individual work stations but only one of the same kind. Chemicals should be stored as close as feasible to the point of use in order to maximize efficiency and minimize transport distance.

3. Out-of-date chemicals shall be disposed of on a periodic basis to reduce overall hazard potential and minimize inventory tracking and updating. Contact Laboratory Support Office.

4. Reduce large inventory of chemicals in the laboratory.
5. **Never** pipette by mouth. **Always** use a bulb to pipette.

6.. Chemical containers that have been emptied have to be cleaned off remaining residues by triple rinsing with water or other suitable solvent and air-dried before disposal. Contact Laboratory kitchen (labkitchen@ncbs.res.in) for disposal of empty containers.

2.1 CHEMICAL SPILLS

Any chemical is a possible threat to your personal health and your colleagues. In case of accident causing the release of hazardous chemicals a calm and determined action is required to prevent an escalation of the emergency situation.

Thus, for any individual incident, isolation of the spill and/or securing the area is best prior to or simultaneously with contacting concerned personnel. This should be done according to all available information on the chemical nature of the spill. Under all circumstances, a laboratory coat, safety glasses, and gloves should be used for self-protection.

A. Spill Kits may be obtained from Stores

1. Always send for help first and report the necessary

information. a) Where did the incident happen?

b) How many people are injured?

c) What is the chemical?

d) Is there an intermediate risk of fire, explosion, intoxication or suffocation known?

2. If the spill presents an immediate danger, leave the spill site and warn others, control entry to the spill site, and report to reception.

3. Remove contaminated clothing. Flush skin/eyes with water at least 15 to 30 minutes; use soap for intermediate and final cleaning of skin areas.

4. Protect yourself, then remove injured person(s) to fresh air, if safe to do so.

5. Notify nearby persons and evacuate as necessary. Prevent entry, as necessary, by posting a guard in a safe area and/or shutting doors.

6. If flammable vapors are involved, do not operate electrical switches unless to turn off motorized equipment. Try to turn off or remove heat sources, where safe to do so. TURN OFF ELECTRIC AT THE MAINS, NOT AT SWITCHES INSIDE THE LABORATORY.

7. Do not touch the spill without protection, gloves etc..

8. Where the spill does not present immediate personal danger, try to control the spread or volume of the spill. This could mean shutting a door, moving nearby equipment to prevent further contamination, repositioning an over-turned container or one that has a hole in the bottom or side, creating a dike by putting an absorbent around a spill or opening the sashes on the fume hoods to facilitate removal of vapors.

9. Never assume gases or vapors do not exist or are harmless because of lack of smell.

10. Increase ventilation by opening closed fume hood sashes to the 12 inch or full open position. Exterior doors may be opened to ventilate non-toxic vapors.

11. Use absorbents to collect substances. Reduce vapor concentrations by covering the surface of a liquid spill with absorbent. Control enlargement of the spill area by diking with absorbent.

B. Spilled Liquids

1. Confine or contain the spill to a small area. Do not let it spread.

2. For small quantities of inorganic acids or bases, use a neutralizing agent or an absorbent mixture (e.g., soda ash or diatomaceous earth). For small quantities of other materials, absorb the spill with a nonreactive material (such as vermiculite, clay, dry sand, or towels).

3. For larger amounts of inorganic acids and bases, flush with large amounts of water (provided, the water will not cause additional damage refer to MSDS for this information). Flooding is not recommended in storerooms where violent spattering may cause additional hazards or in areas where water-reactive chemicals may be present.

4. Mop up the spill, wringing out the mop in a sink or a pail equipped with rollers.

5.Carefully pick up and clean any cartons or bottles that have been splashed or immersed.

6. If needed, vacuum the area with a HEPA filtered vacuum cleaner designed and approved for the material involved.

7. If the spilled material is extremely volatile, let it evaporate and be exhausted by the laboratory hood (provided that the hood is authorized for use with the spilled chemical).

C. Spilled Solids

Generally, sweep spilled solids of low toxicity into a dust pan and place them into a container suitable for that chemical. Additional precautions such as the use of a vacuum cleaner equipped with a HEPA filter may be necessary when cleaning up spills of more highly toxic solids.

1. Dispose of residues according to safe disposal procedures. Remembering that personal protective equipment, brooms, dust pans, and other items may require special disposal procedures.

2. Report the chemical spill in writing as required above to laboratory support office.

2.2 Guidelines for Mercury Handling and Spill Clean Up

A. Health Effects

Mercury vapors are odorless, colorless, and tasteless. Mercury poisoning from exposure by chronic inhalation can cause emotional disturbances, unsteadiness, inflammation of the mouth and gums, general fatigue, memory loss, and headaches.

B. Storage and Handling

Because of the health effects of mercury, the extremely difficult and time-consuming procedures required to properly clean spills, every effort should be taken to prevent accidents involving mercury. Always store mercury in unbreakable containers and store in a well-ventilated area. When breakage of instruments or apparatus containing mercury is a possibility, the equipment should be placed in an enameled or plastic tray or pan that can be cleaned

easily and is large enough to contain the mercury. Transfers of mercury from one container to another should be carried out in a hood, over a tray or pan to confine any spills. If at all possible, the use of mercury thermometers should be avoided. If a mercury thermometer is required, many are now available with a Teflon® coating that will prevent shattering. Always wash hands after handling mercury to prevent skin absorption or irritation.

Always wear nitrile gloves while handling mercury. If mercury has been spilled on the floor, the workers involved in cleanup and decontamination should wear plastic shoe covers. **.** Spill Kit for Mercury is available from the Laboratory Manager. Southern Labs has mercury spill kit located with the chemical spill kits near the fume hoods on the first floor.

2.3 Guidelines for Ethidium Bromide (EtBr) Waste Management & Disposal

Ethidium bromide *(3,8 diamino-5-ethyl-6-phenyl phenanthridinium bromide, dromilac, CAS #1239-45-8)*, is a compound used in many laboratories. Ethidium bromide is available as a dark red, crystalline, non-volatile solid and is moderately soluble in water. Since it fluoresces readily with a reddish-brown color when exposed to ultraviolet (UV) light and with increased brightness when bound to double stranded-DNA and single-stranded RNA, it is commonly used in gel electrophoresis applications for visualization of these molecules. For these applications, liquid ethidium bromide solutions are incorporated into the electrophoresis gel as a dye for the DNA, RNA, or other molecules to be visualized.

Ethidium bromide is mutagenic and moderately toxic and must be handled with care. The powder form is considered an irritant to the upper respiratory tract, eyes, and skin. Preparation of stock solutions and any operations capable of generating ethidium bromide dust or aerosols should be conducted in a fume hood to prevent inhalation. Nitrile gloves, a lab coat, and eye protection must be worn at all times.

When working with ethidium bromide, minimize the potential for spills. Where practical, purchase pre-mixed stock solutions from chemical manufacturers in lieu of preparing solutions. If solutions of ethidium bromide must be prepared, perform this process in a fume hood. Perform all processes that generate ethidium bromide dusts or mists inside the fume hood to minimize inhalation exposures. Prevent accidents by transporting small quantities of ethidium bromide in secondary containment.

***Do Not Use sodium** hypochlorite (bleach) to treat ethidium bromide. Bleach treatment can produce mutagenic products and leave behind up to 20% of the original ethidium bromide.

Ethidium Bromide Waste Disposal

Ethidium bromide waste should **NOT** be poured down the drain or thrown in the trash, unless the waste has been deactivated or filtered. The following are the recommended disposal procedures for ethidium bromide.
NOTE - Ethidium bromide DOES NOT go in red bags or red containers, should not be labeled with a biosafety symbol, and especially should not be treated in an autoclave.

A. Electrophoresis Gels, Contaminated Gloves and EtBr contaminated Solids
Handle ethidium bromide gels, contaminated gloves just as you would chemical hazardous waste. Use sealable, disposable plastic baggies to store ethidium bromide gel waste. Minimize free flowing liquids in these bags when they are brought for disposal.

A bin is kept for gloves and gels contaminated with EtBr
1) at the Centrifuge Room in the first floor near the Gel documentation system.
2) at the Common equipment room ground floor next to the Gel documentation system.

B. EtBr Liquid disposal
Intermediate storage and collection of liquid EtBr waste is the responsibility of the individual laboratory**. A log book must be maintained listing: concentration, volume, date and the person who generated it.** The liquid EtBr waste should be collected in an appropriately labeled 2- or 5- liter glass reagent bottle.
A Bind-ET Ethidium Bromide Removal System (Elchrom Scientific) for final disposal of liquid EtBr waste is located in the Laboratory kitchen first floor(Southern Laboratory) Disposal of the decontamination filters is done by the laboratory kitchen personnel. **A log-book entry has to be generated for every disposal entering LAB-number, volume, concentration and date.**

C. Sharps Contaminated with Ethidium Bromide

If contaminated blades or sharps need to be discarded they should be carefully rinsed with water into the intermediate liquid waste container. **No disposal of sharps or blades is permitted into regular biohazard red bins or blue glass bins**. We recommend reusing the labeled blades specifically for EtBr work.

D. Ethidium Bromide Alternatives

Consider switching to less-toxic alternatives to ethidium bromide (GelRed ,SYBR SafeTM DNA gel stain, Biotium) to reduce potential hazardous exposures in the lab.

2.4 Guidelines for Bis-Acrylamide

When handling Bis-Acrylamide formulations gloves, eye protection and Lab coat are required.

Dispose remaining acrylamide in original chemical containers by polymerization. For that add 100µl TEMED and 100µl of 10% Ammonium Persulfate (APS) and shake it thoroughly and allow to react over night. Dispose the container in the red biohazard bin.

2.5 Guidelines for Phenol/Chloroform

Handle Phenol or Phenol/Chloroform formulation only with gloves, eye protection and Lab coats. These liquids have to be disposed in dedicated organic solvent containers.

The tubes should be collected separately and allowed to dry in the chemical fume hoods in common equipment rooms in Ground and Second Floor.

2.6 COMPRESSED GAS SAFETY

A. Identification

1. The contents of any compressed gas cylinder shall be clearly identified for easy, quick, and complete determination by any laboratory worker. Such identification should be stenciled or stamped on the cylinder or a label, provided that it cannot be removed from the cylinder. If the labeling on a cylinder becomes unclear or an attached tag is defaced to the point the contents cannot be identified, the cylinder should be marked "contents unknown" and returned directly to the manufacturer. Users are requested to inform themselves through the Material safety data sheets (MSDS) about safe handling of the specific technical gas.

Examples of color code used in India for identification of gas cylinders:

1. Argon : Peacock Blue
2. Nitrogen : French Grey with a black band on the shoulder of the cylinder
3. Helium : Light Brown
4. CO_2 : Black with a silver band on the shoulder of the cylinder
5. Oxygen : Black
6. Zero Air : French Grey

(Ambient air filtered to contain less than 0.1 parts per million (PPM) of total hydrocarbons.)

7. Hydrogen : Signal Red
8. D.A : Maroon

(Dissolved Acetylene)

B. Safe Use of High pressure cylinders

1. Gas cylinders shall be secured at all times to prevent tipping. Cylinders may be attached to a bench top, individually to the wall, placed in a holding cage, or have a non-tip base attached.

2. When new cylinders are received, they should be inspected; insure the proper cap is securely in place and the cylinder is not leaking. Cylinders shall have clear labels indicating the type of gas contained. If the cylinders are acceptable, they shall be stored in a proper location.

3. Standard cylinder-valve outlet connections have been devised to prevent mixing of incompatible gases. Cylinders should be placed with the valve accessible at all times. Cylinder valves should be opened slowly. Main cylinder valves should never be opened all the way. Valves should not be closed with force.

4. Regulators are gas specific and not necessarily interchangeable. Always make sure that the regulator and valve fittings are compatible. Never use oil or grease on the regulator of a cylinder valve.

5. For transportation of high pressures cylinders general precautions are required. To protect the valve during transportation, the cover cap should be screwed on hand tight and remain on until the cylinder is in place and ready for use. When moving large cylinders, they should be strapped to a properly designed wheeled cart to ensure stability. Cylinders should be moved only with dedicated trolleys. Cylinders should never be rolled or dragged.

C. Leaking of compressed gas cylinders

If a leak is suspected, do not use a flame for detection; rather, a flammable-gas leak detector or soapy water should be used. If the leak cannot be remedied by tightening a valve gland or a packing nut, emergency action procedures should be initiated. Laboratory workers should never attempt to repair a leak at the valve threads or safety device; rather, they should consult with the supplier for instructions.

2.7 Safe handling of Cryogenic Liquids (liquid Nitrogen)

In the context of the NCBS/inStem/CCAMP campus this refers largely to liquid nitrogen. Usage of other cryogenic liquids has to be performed under supervision of trained individual and such operations should be reported to PI in charge and the Lab manager (Ranjith,).

The transfer of liquefied gases from one container to another should not be attempted for the first time without the direct supervision and instruction of someone experienced in the operation. In all procedures involving liquid nitrogen cryo-gloves and eye protection (preferably a face shield) should be worn at all times. Gloves should be chosen that are impervious to the fluid being handled and loose enough to be tossed off easily.

Fire/Explosions; Neither liquid nitrogen nor liquid air should be used to cool a flammable mixture.

Adequate ventilation must always be used to avoid suffocation or the possibilities of build up to explosive gas mixtures.

Only appropriate impact-resistant containers must be used that have been designed to withstand the extremely low temperatures for handling and storage of larger quantities of cryogenic liquids. Only dedicated Polyurethane Foam Ice Buckets are permitted for usage with liquid nitrogen. Styrofoam boxes (thermo col) are NOT allowed for handling liquid nitrogen at any time.

2.8 Safe Handling of Dry Ice

Dry ice is solidified carbon dioxide (CO_2). Dry ice sublimates (changes directly from solid to gas), releasing CO_2. Carbon dioxide vapor is substantially heavier than air. In confined, poorly ventilated spaces it can displace air, causing asphyxiation. It is even possible for CO_2 vapor to accumulate in low-lying areas, out-of-doors. Gloves and eye protection (preferably a face shield) should be worn at all times when handling dry ice.

Dry Ice is obtained in blocks and has to be crushed for usage. Use only provided tools, during this procedure eye protection and gloves are a must.

Never store dry ice in glass or other sealed (airtight) containers or coolers. Storage in a sealed container can result in a rupture or explosion of the container from over-pressurization.

A. Disposal of Unused Dry Ice

Allow the dry ice to sublimate or evaporate to the atmosphere in a well-ventilated area where no build-up of carbon dioxide vapor can occur.

Do not dispose of dry ice in sewers, sinks or toilets. The extreme cold will harm sink disposal, toilet parts and

pipes. Do not dispose of dry ice in garbage receptacles or garbage chutes.

2.9 Guidelines for Imaging Stations

When an ultraviolet(UV) light source is used in work with ethidium bromide, added caution is required. As a general rule, avoid exposing unprotected skin and eyes to intense UV sources. Eye protection must be worn if a face shield is not available.

Leave the imaging station in a clean and EtBr-free state.

3.0 BIOLOGICAL SAFETY

Four bio-safety levels are established to regulate laboratory practices, techniques and safety equipment appropriate for handling of biological agents. Biological agents in that context are:

a) Microorganisms
b) Arthropods
c) Toxins of different origin and those produced using rDNA technology
d) Viruses
e) Allergens of different origins and those produced using rDNA technology

At NCBS, the area of biological safety is under the guidance of the BioSafety and Bioethics Committee and the Biological Safety Officer. For Biological Safety Levels I or II, researchers are bound to follow the Committee guidelines and inspections.

Bio-safety Level I:
Practices and safety facilities are appropriate for working with well-defined biological agents. These biological agents are not known to cause disease in healthy adult humans and pose only a minimal potential hazard for laboratory personnel and the environment. *Bacillus subtilis*, *Naegleria gruberi*, infectious canine hepatitis virus and non infectious *E. coli* are representative of those microorganisms meeting these criteria. Many agents not ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, immunodeficient or immunosuppressed individuals.

Bio-safety Level II:
Practices and safety facilities must meet the perquisites to deal with a broad spectrum of indigenous moderate-risk agents. These agents are known to cause diseases but immunization or antibiotic treatment is available. Examples are Hepatitis B virus, the *Salmonellae*, *Toxoplasma* spp. and infectious *E. coli* strains. Primary hazards to personnel working with these agents may include accidental autoinoculation, ingestion, and skin or mucous membrane exposure to infectious materials. Procedures with aerosol potential that may increase the risk of exposure to personnel, must be conducted in primary containment equipment or devices.

Bio-safety Level II+:
The term 'Infectious Material' applies to blood, any other body fluid, and other potentially infectious material like biopsies. All body fluids shall be considered as infectious materials. Research involving Biological Safety Levels II+ shall contact the Biological Safety Committee for appropriate authorization, guidelines and inspections. Such work can only be performed in the Biohazard laboratory in the Ground Floor.

Bio-safety Levels III and IV are not applicable to NCBS/InStem/CCAMP.

3.1. Basic Working Principles in Bio-safety laboratories

The primary principle of biological safety is containment. This refers to a series of safety procedures which have to be conducted to reduce or eliminate human and environmental exposure to potentially harmful biological agents. While working in NCBS/inStem/CCAMP laboratories one might handle specimens, cultures and agents without full knowledge of the biohazard risk; these materials may contain infectious agents. To minimize exposure, observe universal precautions when handling any biological specimen.
While working in any of the above defined bio-safety levels it is required of any personnel at NCBS/inStem/CCAMP to follow the regulations listed below:

A. Wash your hands thoroughly:
1. Before and after working with any biohazard
2. After removing gloves, laboratory coat, and other contaminated protective clothing
3. Before eating, drinking, smoking, or applying cosmetics
4. Before leaving the laboratory area
5. Do not touch your face when handling biological material
6.Never eat, drink, smoke, or apply cosmetics in the work area

B. Clothing Guidelines:

1. Always wear appropriate lab clothes and gloves when working with biological agents.
2. Wear gloves over gown cuffs.
3. Remove gloves by peeling them from the inside out.
4. Never wear contact lenses when dealing with infectious agents.
5. Do not wear potentially contaminated clothing outside the laboratory area.
6. Additional appropriate protective clothing should be selected and worn based upon the task and degree of exposure anticipated.

C. Handling Procedures liquid infectious materials:

1. Use mechanical pipetting device (examples; pipette aid, pipetteman or bulb).
2. Minimize aerosol generation. Decanting culture supernatants, opening of culture and streaking of plates should only be done in Safety cabinets or in a circular area around a burner of 0.5 meter radius. Decanting/Transferring of cultures in common equipment rooms outside of safety cabinets is forbidden.
3. Add disinfectant to water baths for infectious substances.
4. Use only closed tubes for centrifuging procedures. Inspect the tubes before use.
5. Use secondary leak-proof containers when transporting samples, cultures, inoculated petri dishes, and other containers of biohazardous materials within the institute.
6. Avoid using syringes and needles whenever possible. Special care has to be taken when usage of needles is not avoidable:
   - Use a needle-locking or disposable needle unit.
   - Take care not to stick yourself with a used needle.
   - Place used syringes into a pan of disinfectant without removing the needles.
   - Do not place used syringes in pans containing pipettes or other glassware that require sorting.
   - Do not recap used needles.
   - Dispose of needles in an approved sharps container.

D. Work Area:

1. Keep laboratory doors shut when experiments are in progress.
2. Limit access to laboratory areas when experiments involving biohazardous agents are being performed.
3. Ensure that warning signs are posted on laboratory doors. These signs should include the universal biohazard symbol and the approved biosafety level for the laboratory.
4. Transport contaminated materials in leak-proof containers.
5. Keep miscellaneous material (i.e., books, journals, etc.) away from potentially contaminated working areas.
6. Follow a rigorous disinfection plan:
   - Completely decontaminate equipment before having maintenance or repair work done.
   - Decontaminate work surfaces daily and after each spill.
   - Decontaminate all potentially contaminated equipment.
   - Decontamination should only be performed with these disinfection solutions such as 70%Ethanol or 5-10% Sodium hypochlorite (bleach) solutions

- Work of Bio-safety Level II has to be performed at assigned workbenches. Ensure that warning signs are posted accordingly. All procedures generating aerosols have to be performed in safety cabinets. Minimize traffic in the area where Bio-safety Level II work is done.

E. Safety Equipment (Containment Barriers)

Safety equipment includes a variety of personal protection items and laboratory devices which provide the ability to keep infectious agents in a specified contained area that is easily accessible. The biological safety cabinet is the principal device used to provide containment of infectious aerosols generated by many laboratory procedures. Open-fronted Class I and Class II biological safety cabinets are partial containment cabinets which offer significant levels of protection to laboratory personnel and the environment when used with good microbiological techniques. As part of the individual familiarization with laboratory procedures individual PIs are asked to ensure proper handling of the Safety cabinets.

F. Disposal of liquid cultures:

1. All culture supernatants in conical plastic tube never more than 25ml can be disposed in the red biohazard bin.
Tubes have to be closed but not overly tight.

2. All cultures ≥ 25ml must be put in a glass beaker or Erlenmeyer flask and treated to become 10% with bleach **in a biosafety cabinet**. Examples; 30 ml culture/3-4 ml of straight bleach, 250 ml culture/25-30 ml straight bleach, let stand at least 30 minutes. Dispose off in sink: Turn on the water faucet, pour treated culture in, keep water running for a minute or two after you have finished pouring the culture.

3. Cultures with other hazardous chemicals and/or heavy metals must be disinfected as above and then disposed of according to the method prescribed in the MSDS.

3.2 Biohazard spills

Biological spills outside biological safety cabinets will generate aerosols that can be dispersed in the air throughout the laboratory. These spills can be very serious if they involve microorganisms that require Biosafety Level II and above containment. The Biosafety lab has its own procedures to follow.

General reaction plan for a biological spill:

1. Cordon off the area to stop anyone from spreading the contamination throughout the laboratory.
2. Cleaning procedures should be started in a timely manner by a person from the lab where the spill has occurred. Before starting to clean the spill, Personal Protection Equipment (gloves, face mask, safety goggles, long sleeve lab coat and shoe covers) must be obtained and put on.
3. Disinfect the area, all surfaces using 70% Ethanol in a spray bottle. Any material used to wipe up the spill must be placed in a biohazard bag and decontaminated using an autoclave.

Decontamination should only be performed with these disinfectants: 70%Ethanol or 10% Chlorox
A. Spills on the Body
1. Remove contaminated clothing.
2. Apply disinfection solution. Vigorously wash exposed area with soap and water for one minute.
3. Obtain medical attention.
4. Report the incident to the Laboratory Manager (Ranjith)

B. Bio-safety Level I Organism Spill decontamination procedure
1. Wear disposable gloves.
2. Soak paper towels in disinfectant (70% ethanol or 10% chlorox) and place over spill. Allow a 30-minute contact period on the spill.
3. Place towels in a biohazard bag and decontaminate using an autoclave before disposal.
4. Clean up spill area with fresh towels soaked in disinfectant.

C. Biosafety Level 2 Organism Spill

1.Shut down the air conditioning units for your laboratory or section of the building. (call Sub Station 6425/26)
2. Alert people in immediate area of spill.
3. Put on protective equipment, a laboratory coat with long sleeves, back-fastening gown or jumpsuit, disposable gloves, disposable shoe covers, safety goggles, mask or full-face shield.
4. Cover spill with paper towels or other absorbent materials.
5. Carefully pour a freshly prepared disinfectant around the edges of the spill and then into the spill. Avoid splashing. Allow a 30-minute contact period. We recommend using a spray bottle.
6. After the spill has been absorbed, clean up the spill area with fresh towels soaked in disinfectant.

7. Place towels in a biohazard bag and decontaminate in an autoclave. Please inform the laboratory kitchen to autoclave infected material before being sent for final disposal.

4.0 Specialty Laboratories

4.1 Working with Radioactive Materials

The Radioactive Laboratory (Rad Lab)facility at NCBS has been classified as a Type I radioactive laboratory. The radioactive nuclei that the Radioactive Laboratory is equipped to handle are H-3, S35, P-32, C-14 , Fe-55 and Ca-45.

The process to become a Radioactive Laboratory user is to fill out an application form available with Laboratory manager (Ranjith), pass written test conducted by NCBS Radiological Safety Officer (Dr.Shivaprasad) and go through a rigorous orientation programme under the supervision of the Radioactive Laboratory student representative. One of the most important aspects of the orientation program and tests is that the candidates must be aware of are the specific protocols for disposal of waste materials generated during experiments performed in the Rad Lab. Once cleared by the test and passed through the orientation, the user must obtain badges before starting to use the Radioactive Laboratory. This process may take 1 to 2 months. Initially you will be under the supervision of a senior Rad Lab user before you become an independent user.

Forms may be obtained at this site: http://intranet.ncbs.res.in/node/139

4.2 Laser Laboratory

A special training process must be undertaken to use lasers in the laboratory. Please contact your PI for appropriate instruction. Safety glasses have to be worn at all times within Laser Laboratories.

Laboratory Waste Management

SL No.	BIN Type	Waste Type
1. 	**RED Bin** Biohazard – Laboratory waste	Tips, tubes, Gloves, SDS PAGE gel, serological pipette, TC Flask, Plastic pertridish , tubes 15ml and 50ml etc..
2. 	**BLUE BIN** Broken Glass ware	Disinfected/Treated broken Glass wares, Any glass equipment containers, pipettes
3. 	**Halogenated Solvent Waste** White Canister, 5 Ltrs	Chloroform, Dichloromethane, Carbontetrachloride, TetraChloroethylene, Perchloroethylene …
4. 	**Non-Halogenated Solvent waste** White Canister, 5 Ltrs	Acetone, Acetonitrile, Diethylether, Ethanol, Hexane, THF, Methanol, Toluene, Methyl tert-butyl ether (MTBE), Dimethyl sulfoxide (DMSO), Dimethyl formamide(DMF) …
5. 	**White round Container**	Syringes and needles
6. 	White square Container	Sharps, slides, coverslips, blades etc…
7. 	**LARGE GREY BIN** Ethidium Bromide (Imaging Stations)	Ethidium Bromide Gel, gloves, tubes and tips which contaminated with EtBr
8. 	**Liquid Ethidium Bromide Waste** (Ground Floor Kitchen)	Buffers containing Ethidium Bromide
9. 	**Paper Recycle**	Waste papers
10. 	**GREEN BIN** (Near Lab 7 and Lab 1)	Books, papers, catalogues…
11. 	**CYAN BIN** E-waste (Ground Floor Kitchen)	Battery, CDs, pendrive, cartridge,floppy etc..
12. 	**SMALL GREY BIN** Phenol/Chloroform Solid Waste (Ground / Second Floor Fume Hoods)	Eppendorf tubes, falcon tubes, tips contaminated with phenol
13. 	New Bins For Fly Labs For Broken Bottles and Vials With Media	Broken fly vials with media in them

5.0 Waste Disposal System at NCBS/inStem/CCAMP

The above table (on page 15) indicates the bin and items which may be put in them.

5.1 Waste Pick up Schedule

Please see the schedule below. If you notice your lab bin is full before the schedule, you may kindly inform laboratory kitchen staff for collection. They can be reached at Extn: 6670/6144 or labkitchen@ncbs.res.in

Southern Labs has daily pick-up

Labs	Waste pick up Day	Time
Basement labs	Monday and Thursday	10 am - 12 noon
Ground floor Labs	Tuesday and Friday	10 am – 12 noon
First Floor Labs	Wednesday/ Saturday	10am – 12 noon
Second Floor Labs	Monday and Thursday	10am - 12 noon
Third Floor Labs	Tuesday and Friday	10am – 12 noon
Hood Area (Common Rooms)	Everyday	10am – 12 noon
Southern Labs	Daily	2-3:30pm

SOPs- Handling, Segregation and Disposal of Waste /Scrap/Debris

Maintenance of a clean & green campus needs to be accorded top priority in view of the large number of personnel working and visiting our campus. All should endeavor to preserve the cleanliness and aesthetics of the campus. As large numbers of works are taken up by the Engineering/other supporting services, due importance is to be given to the aspect of proper handling, segregation and disposal of waste materials. This will also help in proper house keeping and present a clean & healthy campus conducive for research-related activities apart from enhancing the tidiness of surrounding areas. Towards achievement of the above goal, the following guidelines are issued for compliance by all concerned:-

- All contractors undertaking works at NCBS are to be briefed prior to commencement of work to collect and dispose of waste/debris etc at designated places where bins are provided.
- However, for small quantity of debris, weekly debris clearance will be organized by respective agencies undertaking work for which a separate item will be created in SOQ of each contract and will be operated as "per trip basis".
- Debris is to be segregated at designated point and disposed off suitably. Debris is not to be accumulated and left in the campus. Final bill clearance should be subject to disposal of all waste by the contractor.
- Useful items or any reusable material are to be segregated and stored properly at designated points or storage area.
- Contractors are not to leave any leftover materials in the campus on completion of works.
- All Engineers are required to take surprise rounds of work sites and ensure proper and prompt disposal of waste/debris etc. Specific instructions to be recorded in the site register.

- A suitable clause has to be incorporated in all contracts for the contractor to comply with waste disposal procedure and the same needs to be linked up to the stage payments.

- Whenever works are going on for a longer duration separate enclosure with barricade to be created for interim accumulation of waste to avoid dumping of the same haphazardly and spoiling the aesthetic look of the campus.
- All unused/unserviceable items are to be handed over to store section periodically for disposal in normal course as per procedure in vogue.
- All section in-charges holding stores are also required to follow proper waste disposal procedure and avoid cramping up of store space with unwanted materials.
- "Repair, Reuse and Recycle" norms are to be followed strictly to reduce environmental damage due to dumping of waste materials and to reduce wastage.
- The site order book must be maintained for each work in which respective engineer will record instructions relating to day-to-day work and signed by both Engineer and Contractor. This will also cover waste handling/disposal aspect.
- Engineers who record the work done by contractor will be directly responsible for strict compliance of above guidelines and to levy penalty to the offending party as per contract terms.
- Packing materials (wooden/cardboard/plastic) are not to be left on the corridors/side of the pathways. They are to be handed over to stores-section for proper disposal.
- Personnel attending works in the lab area are to be specifically instructed to collect/remove debris generated in the process of work and maintain cleanliness of lab area.

6.0 EMERGENCY RESPONSE
6.1 FIRES

Fires are a common emergency in a laboratory.

ACTION TO BE TAKEN IN CASE OF FIRE

- ON SEEING FIRE, DO ALERT OTHERS BY SHOUTING **"FIRE, FIRE, FIRE"** REPEATEDLY. BREAK GLASS OF MANUAL CALL POINT (MCP) AND ACTUATE ALARM.

- INFORM RECEPTION **(Tel 6666)**, SECURITY OFFICER **(Tel 6022)** AND ADJOINING LABS. ALERT OCCUPANTS USING PUBLIC ADDRESS SYSTEM.

- STAY CALM –DO NOT GIVE RISE TO PANIC.

- JUDGE THE TYPE OF FIRE – USE RIGHT TYPE OF FIRE EXTINGUISHERS.

- TYPE OF FIRE FOR WHICH EXTINGUISHER TO BE USED IS MARKED ON THE EXTINGUISHER ITSELF, VIZ

  A-SOLID FIRE **C**-ELECTRIC FIRE **K-**KITCHEN FIRE

  B-LIQUID/GASEOUS FIRE **D**-METAL FIRE

Participate and learn about these fire extinguishers by our security team's periodic demonstrations

For small fires/fires to a body, use the fire blanket.

ACTION TO BE TAKEN IN CASE OF FIRE

- IN CASE OF ELECTRIC FIRE, **SWITCH OFF** POWER SUPPLY AND AC UNITS. INFORM SUBSTATION **(Tel 6425)**.
- DO NOT FIGHT FIRE IF IT IS TOO LARGE TO CONTROL AND ATMOSPHERE IS TOXIC. CALL FOR THE HELP. COVER FACE/NOSE WITH WET CLOTH TO AVOID INHALING OF TOXIC GAS.
- EVACUATE THE PREMISES ENSURING ALL DOORS AND WINDOWS ARE **CLOSED**.
- DO **NOT** USE ELEVATORS FOR EVACUATION. USE ONLY BUILDING STAIR CASE. WALK BRISKLY DO NOT RUN TO AVOID STAMPEDE AND DO NOT STOP TO PICK UP BELONGINGS, GIVE WAY TO STAFF ATTENDING FIRE.
- DOUSE THE FIRE AT THE BOTTOM AND THEN MIDDLE LEVELS; DO **NOT** USE WATER ON ELECTRIC FIRE.
- IN CASE OF MAJOR FIRE, ON HEARING THE FIRE ALARM ALL THE PERSONNEL IN THE AFFECTED AREA SHOULD EVACUATE THE BUILDING IMMEDIATELY. NCBS NORTHERN LAB WILL ASSEMBLE AT GROUND FLOOR. PAVE WAY FOR EASY APPROACH TO INCIDENT SPOT BY FIRE FIGHTING PERSONNEL. SOUTHERN LAB PERSONNEL WILL MEET AT THE CRICKET NET ON THE LAWN
- Contact our emergency extension 6666/ RECEPTION/SECURITY will CALL FOR AMBULANCE ALONG WITH MEDICAL ATTENDENT FOR FIRST AID AND EVACUATION OF INJURED TO HOSPITAL.
- IN CASE OF THICK SMOKE FILLED ROOM, DO NOT WALK; INSTEAD CRAWL WITH WET CLOTH COVERING NOSE AND MOUTH.

6.2 ACCIDENT REPORTING

ALL injuries shall be reported to Laboratory Support Office using the form at this site http://intranet.ncbs.res.in/node/144. Minor injuries many times are not reported because they are perceived to be embarrassing or that "careless actions" lead to the accident. However, minor injuries can sometimes lead to more serious complications that only become evident at a later time. In addition, all minor accidents should be investigated by safety and management personnel. Taking corrective action as a result of a minor accident may keep a major incident from happening. Without knowledge of all minor accidents, the desirable investigation is circumvented.

Employees should understand that the purpose of reporting and documenting accidents is not to affix blame, but instead to determine the cause of the accident so that similar incidents may be prevented in the future.

6.2.1 ACCIDENT REPORT FORM

Ref No:

INCIDENT / ACCIDENT REPORT FORM

Use this form to report all accidents

PART I

When did it happen? Date: Time:

Where?

PART II

What happened and how it was happened:

PART III

About the injured person (if no one injured go to IV):

Injured person's full name:

Address/ Contact information:

Age: Male/Femal

If the injured person was employed by someone else at the time of the accident what is the name and address of his / her employer:

PART IV

Any witnesses to the accident:

PART V

Recommendations to prevent similar accident.

Describe any action, which you recommend to prevent similar accident in the future:

Details about the person who complete this form:

Name:

Contact Information:

(Department, Telephone No and E-mail ID):

Note: Kindly forward form to Laboratory Manager, NCBS

Ranjith P.P. ranjith@ncbs.res.in

6.3 Emergency Contact Information

In case of medical, fire and major non-containable accidents it is required that you first inform the RECEPTION (6666/6001/6002/6019). The reception acts as an information hub to contact all concerned personnel.

Medical centre Dr. Vishwanath N Patil Dr.Tehzib	Extn : 6450/6446	M: 9845032410 M: 8904158500
Reception / Ambulance / Police / Fire	Extn: 6666 or (6001/002/019)	
Shri. Shaju Varghese (Administrative Officer – Services)	Extn: 6437	M: 9448753980
Baptist Hospital	23330321/323	
Columbia Asia Hospital	41791000	
Ranjith P.P., Laboratory Manager	Extn: 6065	M :98447-35338
Bhoomika,Laboratory Manager	Extn: 6065	M: 9535231225
Prem Chand Gautam, (instrumentation)	Extn: 6066	M: 9243112864
Shri. Suresh Kumar (Electrical)	Extn: 6430	M: 9243206827
H M Basavaraja (Civil Engineer)	Extn: 6352	M : 9880630029
Dr. Shivaprasad (Rad. Safety)	Extn: 6511	M: 9740618525
Dr. Vatsala (Laboratory safety)	Extn: 6101	M: 9844721581
Dr. Mohan Scientific Officer- Animal House	Extn: 6432	M: 9900307607

6.4 Committees

Radiological Safety Committee:

Dr. Shivaprasad(Radiological Safety Officer)

Ashwin Nair (Student In-charge)

Mr. Ranjith (Co-ordinator)

Chemical Safety committee

Dr. Vatsala Thirumalai (Faculty In-charge, Laboratory Safety)

Mr. Ranjith

Ms. Bhoomika

Institutional BioSafety and Bioethics Committee:

NCBS:
Prof. M. M. Panicker (Chairman)
Prof. S.K. Shankar (NIMHANS)
Prof. Anuranjan Anand (JNCASR)
Dr. Vatsala Thirumalai
Prof. Sumantra Chattarji
Prof. Utpal Nath, IISc, External expert
Prof. Sudhir Krishna
Dr. Shachi Gosavi

inStem:
1. Dr Colin Jamora, inStem (Chair)
2. Prof. Anuranjan Anand JNCASR, DBT Nominee
3. Prof. S.K. Shankar NIMHANS ,External expert/Biosafety Officer
4. Dr. Akash Gulyani, inStem,
5. Prof. M.M. Panicker, NCBS
6. Prof. Sumantra Chattarji inStem/NCBS
7. Prof. Utpal Nath, IISc, External expert
8. Dr. Praveen Vemula