Practices and Procedures for the BSL3 Facility at IISc

Centre for Infectious Disease Research (CIDR)
IISc, Bangalore-12

Contents

1.0 GENERAL

1.1 DEFINITIONS

A. The Biosafety level three facility (BSL3) is a containment facility located in the Centre for Infectious Disease Research (CIDR) Building, IISc Campus; Bangalore, for carrying out associated research activities involving airborne pathogens such as Mycobacterium tuberculosis (*Mtb*) and other human pathogens including HIV and Salmonella. It consists of three BSL3 labs and one infection-cum-necropsy room. There are appropriate change rooms, an autoclave room, a media preparation room, and a plant room. This lab has special safety and engineering features for maintaining requisite negative pressure environment to ensure unidirectional airflow, and for ensuring safety of lab personnel and the environment surrounding the lab.

B. BSL3-IISc is a central facility accessible to all IISc researchers.

C. CIDR committee: Centre for Infectious Disease Research Committee comprising of Profs. Dipankar Nandi, Vijaya Satchidanandam, Sandhya S. Visweswariah, Saumitra Das, Annapurna Vyakarnam, Amit Singh, and Arun Nagraj will oversee the BSL3 facility operation, rules/regulations and administrative issues.

1.2 GENERAL REGULATIONS

1.2.1 DOCUMENTATION

A. Documentation of all persons conducting research in the BSL3 must be filed with Dr. Amit Singh, Assistant Professor, Microbiology and Cell Biology Department (MCBL), IISc, Bangalore-12.

B. Documentation of all persons approved to work in the BSL3 facility will be available to the authorized persons for review. Please contact Dr. Amit Singh for this purpose.

C. Any person conducting research in BSL3 acknowledges on the BSL3 Training Form that he/she has undergone requisite training, the procedures described in this document were read and understood and any questions were answered to his/her satisfaction.

D. All experiments to be conducted in the BSL3, together with details of methods and work plan should be submitted to the BSL3 in-charge for a review at least two weeks prior to the proposed start date.

E. All experiments to be conducted in the BSL3 should have approval of the IISc Institutional Biosafety Committee (IBSC).

F. Documentation required to be completed before starting work inside the BSL3:

    1) BSL3 Training Form, signed by the worker, and the supervisor responsible for the work.

2) Certificate of completion of IISc BSL3 laboratory safety training.
3) Documentation of Hepatitis B Vaccination, BCG vaccination / Mantoux testing.
4) Approval of experimental protocol by the CIDR Committee.
5) Approval of experimental protocol by the Institutional Biosafety Committee (IBSC).
6) Approval of experimental protocol by the IISc, Institutional Animal Ethics Committee (IAEC).
7) Certificate of completion of IISc Radiation Safety Training (if applicable).
8) Any applicable updates and additions

1.2.2. GENERAL INSTRUCTIONS

A. Any person entering the BSL3 must follow the procedures outlined in this document as well as any other IISc regulations that may apply.

B. The procedures described herein must be followed any time human or animal tissue is used. All blood and blood products, all tissues derived from humans or animals, must be considered potentially infectious, and handled with requisite safety procedures.

C. Only authorized personnel, *i.e*. only those who have gone through required training and submitted requisite documentation are allowed to enter BSL3.

D. Any other person may enter the BSL3 only under the supervision of an authorized and approved worker.

E. Any research group is responsible for its own supplies. This includes:

- Tyvek Coveralls, Tyvek sleeves, shoe covers and gloves
- Pipettes and tips
- Tubes, culture flasks, culture plates, etc.
- Media, PBS, etc.
- Any other supplies needed

F. Common consumables/supplies such as CO_2 cylinders, wiping tissues, ethanol, Lizol and other disinfectants, bleach, waste collection autoclavable bags, etc. are expected to be restocked according to agreements with the individual research groups.

1.2.3 GENERAL LAB PRACTICES

A. The use of sharp instruments, metal instruments, needles, glass pipettes, glass Pasteur pipettes is not allowed in the culture lab; however, in the necropsy room, and animal quarters, such necessary instruments may be used as required with proper safety precautions.

B. Eating, drinking, handling contact lenses and wearing cosmetics are prohibited in the BSL3 Lab.

C. Entire BSL3 is designated as “NO SMOKING” zone.

D. Mouth pipetting is strictly prohibited.

E. Anyone who is found to violate the regulations stated herein, and not complying with safety regulations in action and spirit, will be liable to **SANCTIONS** as described in this manual.

1.3 AUTHORIZATION

A. Entry into the BSL-3 is restricted to individuals who have:

1) Completed the required training
2) Been advised of potential hazards
3) Read and understood this manual
4) Filed requisite documentation (see Documentation)
5) Have been immunized with BCG/ tested with Mantoux
6) Have been immunized against Hepatitis B virus.

B. Dr. Amit Singh, Assistant Professor, MCBL, IISc, is responsible for determining and reviewing requirements for entering the BSL3. He can be reached at:

Tel: 3063

Mobile: +91-9481700318

E-mail: asingh@mcbl.iisc.ernet.in

1.4 SANCTIONS

A. It is expected that all users of BSl3 will report safety violations to the BSL3 In-charge immediately

B. Anyone who does not follow the regulations described herein is liable to be sanctioned as follows:

1. First Violation: Written warning
2. Second Violation: Access to BSL3 suspended for ONE MONTH
3. Third Violation: Access to BSL3 blocked for LIFETIME

2.0 SOP

SOP 2.1: RESPIRATORY PROTECTION FOR WORKERS EXPOSED TO TUBERCULOSIS

General:

- Surgical masks are not respirators and are not certified as they do not protect the user adequately from exposure to TB. Disposable respirators (e.g., N-95s) are commonly used in TB isolation rooms, in transport of TB cases, or in other areas of the health care facility. However, when high risk procedures are conducted, respiratory protection exceeding the CDC (full forms needed) standard performance criteria may be needed. This protection includes full face piece negative pressure respirators, powered air purifying respirators (PAPRs), or positive pressure airline respirators equipped with half mask or full face piece. The **NIOSH (full name?) certified disposable respirators labelled N, R, or P** meet CDC criteria and may be obtained with or without exhalation valves. Disposable respirators will *only be used once* and will be discarded once staff exit BSL3 suite and enter the secondary anteroom.
- Respirators must be stored in convenient, clean, and sanitary location.
- Respirators, that are used routinely, must be inspected during cleaning, and damaged or deteriorated parts must be replaced.

In the BSL3, two types of respirators to be used are:

A. Disposable Particulate Respirators

The NIOSH- certified disposable respirators labelled N, R, or P meet CDC criteria and may be obtained with or without exhalation valves.

Advantages:

1. The respirator is disposable and most models require no cleaning or maintenance.
2. The respirator is lightweight and comfortable to wear.

Disadvantage:

1. The respirator is a negative-pressure device using the suction produced by inhalation to draw air through the filter. The inhalation process, even under the best of circumstances, will allow some contaminated air leak into the face piece.

B. Loose Fitting PAPR

This respirator consists of a hood or helmet, breathing tube, battery-operated blower, and HEPA filters.

It meets CDC guidelines.

Advantages:

1. More protective than a half-mask respirator
2. The respirator is more comfortable because it is loose fitting.
3. Provides a cooling effect in the hood or helmet.
4. The respirator is durable.
5. Breathing resistance is lower.
6. Vision may be better.
7. Can be worn with facial hair as long as facial hair does not interfere with valve or function of the respirator.

Disadvantages:

1. The equipment cannot be used where a sterile field must be maintained because air exits around the hood or helmet.
2. Batteries must be charged and maintained.
3. The respirator must be inspected, cleaned and repaired.
4. Communication may be difficult.
5. A PAPR may be bulky and noisy.

SOP 2.2: ENTERING THE BSL3 FACILITY

Facility Access:

- All research personnel requesting access to the BSL3 must have approval from their principal investigator.
- All research personnel approved for use of BSL3 must receive appropriate training and approval by CIDR Committee.

Personnel:

- Restricted access to the BSL3 is ensured by Biometric system. Access will be granted upon completion of training and approval by the BSL3 supervisor.
- Never work alone. All Personnel working in the BSL3 must be in pairs.

ENTERING FROM CLEAN AREA AND DONNING A NEW TYVEK SUIT IN THE PRIMARY ANTEROOM:

- All personnel will enter the BSL3 through the anteroom. Don a Tyvek jumpsuit, hair-cap (or more commonly known as “bonnet”), one pair of colored nitrile gloves (pull over the sleeves of the Tyvek suite and thoroughly tape around the palms), shoe covers, and face mask in the primary anteroom.
- Respirator to be used in BSL3; see SOP 1.
- Research staff may choose to wear street clothes under Tyvek jumpsuit.
- Upon entering the BSL3 suite, immediately don a second layer of latex gloves and sleeves. These outer gloves and sleeves must be discarded into medical waste upon exit from BSL3 suite (See SOP 3: Exiting the BSL3)

ENTERING FROM CLEAN AREA AND DONNING A USED TYVEK SUIT IN THE SECONDARY ANTEROOM:

- Tyvek jumpsuit can be re-used ONLY if suit is not soiled or damaged AND if personnel participate in the activity that limits contact with the organism (e.g., taking picture of plates, inspecting density of liquid cultures, performing general duties etc.).
- When entering the main anteroom, personnel will take a pair of nitrile gloves, hair cap, shoe covers, face mask,N-95 and don the used Tyvek suit as described above. Used Tyvek suit will be clearly labelled by the personnel with a permanent marker and kept in the wooden almirah in the main anteroom.

Equipment:

- All animals, supplies, equipment, etc. must enter the bio-containment facility through the anteroom.

- All receipt of suppliers, equipment (including investigator equipment), etc., must be scheduled in advance with BSL3 in-charge. All materials must be sprayed with disinfectant in the main anteroom/ hallway before entering the BSL3.

Scheduled deliveries:

- On the expected receipt day, BSL3 technical staff will leave a clean cart in main anteroom. Supplies are to be stacked on the cart. Only the BSL3 in-charge will spray the outer surface of the boxes with disinfectant and transport to the appropriate location inside the bio-containment facility.

SOP 2.3: EXITING THE BSL3 FACILITY

Procedures:

- The following exit procedures are intended to protect other research personnel, public, and the environment from accidental release of infectious material.

Animal BSL3 room

- A double set of gloves (outer and inner) must be worn at all times in the bio-containment areas because of possible contamination of doors, keypads and walls with experimental pathogens.
- Remove the outer pair of gloves and discard in the animal room waste receptacle immediately prior to exiting the animal room.

Exiting the BSL3 labs

- Remove the “outer” pair of gloves, and sleeves and discard them in medical waste barrel near biosafety cabinet(BSC);
- Spray the “inner” gloves with Lizol (next to medical waste barrel)/hand sanitizer before exiting;
- Visually inspect the BSL3 lab one final time, and proceed to the anteroom area.
- Remove the shoes covers, hair cover and discard into appropriate biohazard receptacles. Remove Tyvek jumpsuit and N-95 carefully and store in the labelled bags. Discard (if necessary) into the appropriate biohazard receptacle. Protective garments must be replaced if they become soiled or damaged or if personnel participate in activities that expose them to large quantities of bacilli for long periods (e.g., scraping colonies from plates).
- Avoid contact between the contaminated outer surface of the jumpsuit and your street clothes.
- Exit the anteroom and use hand-sanitizer to clean the hands.

Equipment

All equipment either must be decontaminated by autoclave or approved surface decontamination before exiting (see appropriate SOP 9, 17). Delicate equipment

that cannot be autoclaved may be decontaminated by another method after advance approval by CIDR committee.

SOP 2.4: PREPARING THE BIOSAFETY CABINET (BSC)

Pre-preparation:

- Turn blower motor on and allow to run for three minutes to fully purge contaminated air from the inside of the BSC.
- Cover the inside surface of the BSC with absorbent pad; do not obstruct the front and back BSC grills.
- Position a plastic transparent jar (with blue rag) filled with Lizol, spray bottle with Lizol and plastic container with screw cap (e.g., "used" Corning roller bottle) filled 1/3 with Lizol in the corner.
- Tape a medium size autoclave bag on the left or right inside wall of the BSC.
- Make sure all supplies and equipment needed to conduct the experiment, including the appropriate number of pipettes, tips and other consumable plastics, are ready inside the BSC BEFORE bringing in live mycobacteria.

Handling the organism in the BSC:

- **RULE 1: *BEFORE* hands enter the BSC, they must be double gloved.**
- Once you open a mycobacterial culture inside the BSC – the BSC is DIRTY!
- **RULE 2: *BEFORE* hands are taken out of the BSC – DIRTY hands have to be cleaned with the Lizol rag and gloves discarded in the BSC autoclave bags.**
- Slowly take hands out of the BSC WITHOUT touching anything (DIRTY) inside the BSC.
- Foil covered plates, vials, centrifuge tubes and other items can be removed from the BSC by first wiping off (with Lizol) the surface in the BSC, then wipe off the item and place it on the clean surface. The investigator should remove the DIRTY gloves (see above) and then wear a clean pair of gloves, and then remove the item without touching anything else inside the BSC.

Discarding BSC waste and cleaning up:

- Discard plates, flasks with Lizol-treated liquid culture, and plastics in separate bags, do not seal bags.

- After use, pipette tips will be rinsed (by up and down pipetting once) with Lizol from the plastic screw cap waste container, and discarded in the pipette tips discard box inside the BSC.
- Remove diapers/absorbant pad and discard in autoclave bags inside BSC.
- Clean thoroughly ALL surfaces in BSC with Lizol rag.
- Wipe waste bags and change outer gloves.
- Bring trash container/drum (containing autoclave bag ~100ml dH_2O) close to the BSC.
- Enter hands in BSC, wipe gloves with the Lizol rag, then remove bags slowly, and carefully place in bottom of trash drum (without pressing down on the waste bag!).
- Close big autoclave bags loosely with rubber band- the waste bag is now double bagged.
- **RULE 3: All infectious solid/liquid waste must be double bagged or must have two (strong) layers of protection.**
- Date and label barrel. Move barrel to the entrance/exit door. BSL3/autoclave technicians are responsible for autoclaving waste .
- Wipe down the surfaces of the BSC with 70% ethanol (to prevent corrosion). Switch on the UV light.

SOP 2.5: HANDLING CULTURES OF MYCOBACTERIA INSIDE THE BSL3 FACILITY

Aseptic guidelines:

- Only disposable loops, conical falcons, cuvettes and spreaders are to be used.
- Items which would be needed to be removed from BSL3 facility, like flasks, should be autoclavable.
- Lizol/Microkleen and 70% ethanol are used to disinfect surfaces and used items.
- All items removed from BSC should be disinfected as above.
- Use only minimum items necessary in BSC to avoid cluttering which interferes with the airflow. Only pipette aid, a set of micropipettes and marker should be left in the BSC.
- BSC should be wiped first with Lizol/Microkleen and then with 70% ethanol before and after every use. Switch on UV light after use for surface decontamination.

Inocula:

- Enter BSL3 only after wearing the required clothing.
- All manipulation of cultures will be done in BSC.
- Infectious materials should be properly prepared and transported in leak proof secure containers.
- The outside of the transport container must be sprayed with Lizol/Microkleen before leaving the laboratory where it is prepared.
- Inocula should not be prepared or transported in syringes with needles.

Centrifugation:

- Centrifuge buckets should have O-ring sealed aerosol free safety caps. The O-rings will be inspected at least once a month and replaced when necessary. The sample to be centrifuged is transported to and from the BSC and centrifuge inside the sealed bucket. Caps are removed from the buckets only inside the BSC. Buckets and caps will be wiped down carefully after use with Lizol/Microkleen, inside and outside and removed from the BSC according to SOP 4.
- Supernatants will be poured or pipetted into a deep container with disinfectant (e.g, a recycled filter bottle or Roller bottle).
- All pipetting is done with a pipette-aid, which is left in the BSC at all times.

- When possible, resuspend cell pellets by flicking tube with finger or running tube along rack. Since mixing creates aerosols, vortexing will be kept to a minimum, and always performed on tightly capped culture tubes using an autoclave bagged/sealed vortex set on the lowest speed possible to provide adequate mixing.
- After mixing, let cultures stand for a minute or so before opening. Mixing by pipetting will be done sparingly.

SOP 2.6: ELECTROPORATION OF MYCOBACTERIA

Electroporation of mycobacteria inside the BSC:

- Prepare electrocompetent cells according to lab protocol.
- Set up the BioRad electroporation device so that the cuvette chamber is inside the BSC and the power source outside on a cart.
- Date and label 15/50ml falcon that will be used later.
- With one hand inside the BSC, add DNA and mycobacterial cells to the 2mm cuvette, place in cuvette holder, close the lid and press with other (*outside clean*) hand the pulse button.
- Observe the time constant, and if OK, quickly add the 7H9/OADC media to the cuvette, mix and transfer with one hand to falcon.
- If the sample "arc", quickly cover chamber with a Lizol soaked rag and leave for 5 min. Discard cuvette in liquid discard container.
- Close the falcons, seal with parafilm and remove from BSC according to SOP 4.
- Place falcons inside incubator on slow shaker.

SOP 2.7: TRANSPORTING MYCOBACTERIAL CULTURES IN BSL3

For large volumes (25ml - 200ml in 490cm^2 Corning roller bottles, flasks):

- All cultures must be labelled with name and contents. *Unlabelled items will be appropriately discarded.*
- Wipe roller bottle/flask with Lizol inside BSC.
- Cover roller bottle/flask with absorbent gauze, large enough to absorb all liquid if spill should occur.
- Label and date.
- Remove item according to SOP#4 from hood and transport in plastic tray to incubator.
- All roller bottles will be put inside metal canisters and placed on the roller bottle apparatus in the 37ºC incubator.

For small volumes (<25ml in conical flasks [250ml] and 50ml falcons):

- Place flasks with liquid cultures inside a plastic container.
- Wipe outer surface of the flask with Lizol.
- Remove from BSC according to SOP#4.
- Place in incubator on slow shaker.

General:

- Bacteria on plates must be wrapped in foil or placed in metal canisters.
- Incubators are to be kept clean at all times, with unused cultures disinfected and discarded as soon as possible. All personnel should use their designated area of incubator.

SOP 2.8: TRANSPORTING SAMPLES FROM THE BSL3

BSL3 facility approved methods of inactivation of virulent mycobacteria:

- Heating at 80ºC for 2 hours effectively inactivates up to 0.5ml volumes of *Mtb*.
- Treatment with phenol and phenol/chloroform (1:1 ratio) and Trizol (contains 50% phenol) should be carried on for at least 15 min.
- Treatment with Lizol at a 1:10 final dilution for at least 15 min.
- For OD_{600} readings: treat liquid cultures with 1 volume of phosphate-buffered formalin in falcon (or equivalent) tube, wipe with Lizol, double bag and remove from BSC, leave for 30 min in anteroom, remove from BSL3.
- For removal of containers from BSC or BSL3, appropriate SOP 4 must be followed.

Others:

- Disposable commercially available specimen transport containers are preferred.
- Unfixed tissue, including blood samples, must be securely packaged into an appropriate container in the procedure room.
- The primary container is placed in a secondary container or bag displaying the universal biohazard symbol.
- Sufficient gauze to completely absorb all leakage from the primary container is also placed in the secondary container, which is then securely sealed.
- The container is then brought to the anteroom where it is sprayed with disinfectant before exiting the facility.

Receiving samples/strains:

- After removing the infected material in the BSL3 suite, the technical staff is responsible for autoclaving or disposing of specimen transport containers.
- Reusable specimen transport containers must have a visual indicator (autoclave tape) to show that they have been sterilized.

SOP 2.9: HAZARDOUS OR INFECTIOUS SPILLS

General:

- All spills of toxic, irritating, or potentially infectious substances must be reported to the facility supervisor. If the spill involves toxic or irritating chemicals or infectious agent exposure to mucus membranes, immediately flush the affected body part with copious amounts of fresh water from the nearest shower-room.
- To minimize further contamination, the area around the spill should be properly disinfected.
- The location and contents of the spill should be identified as specifically as possible to facilitate effective clean-up.
- Spills of mycobacteria must be decontaminated with Lizol and clean-up according to the outlined procedures described below.

Biohazard spills inside the BSC:

- DO NOT INSTINCTIVELY REMOVE HANDS FROM BSC!
- BSC blower must remain ON during and after spill clean-up.
- Wipe gloved hands and sleeves with Lizol. Remove inside BSC and don new pair of gloves.
- Immediately disinfect the area by placing dry towels on spill to absorb liquid. Then soak these towels with Lizol, working from the outside towards the centre of spill area. Disinfectant should be Lizol. A spill on a diaper can be handled by soaking the diaper with Lizol, folding diaper carefully, and then discarding.
- Clean all affected areas within BSC with Lizol. Do not spray the upper diffuser (ceiling) of the BSC as damage to the BSC may result.
- The BSL3 supervisor can determine if filters or blowers need to be decontaminated, or if the BSC needs to undergo complete decontamination.
- Leave UV light ON in BSC for a minimum of 30 minutes after decontamination is complete.

- The BSC must not be used while UV light is ON.

Biohazard spill outside the BSC:

1. Immediate spill control:

    - Inform everyone in the room about the spill. Everyone present in the room must evacuate immediately.
    - Avoid breathing and leave the area, evacuate personnel in the affected areas and close the door.
    - Post a notice stating the emergency and preventing entrance into affected areas (DO NOT ENTER – CLEAN-UP IN PROGRESS). No one may enter the room prior to decontamination for at least one hour.
    - Remove contaminated clothing and place in bag for autoclaving. Wash hands, face; shower if necessary.
    - If spill occurs on Tyvek suit, wipe area with copious amounts of Lizol; remove Tyvek before exiting the BSL3 and place inside autoclave barrel.
    - Notify senior BSL3 supervisor and complete spill report.

2. Decontamination of spill:

    - All spills must be reported to the PI and BSL3 supervisor. Note volume and/or estimated number of bacilli spilled. Depending on the characteristics of the spill (volume, number of bacilli, etc.) BSL3 supervisor will determine if gaseous decontamination is necessary.

        a) After one hour, dress in protective clothing - rubber gloves over nitrile gloves and boots, disposable jumpsuit, head covering, and respirator (PAPR).

        b) Pour Lizol solution around spill and cover the area with paper towels soaked in Lizol. Let it stand for 30 minutes.

        c) Use paper towels to wipe up spills, working towards the centre of the spill.

d) Swab area around spill, including floor, walls, counters, using paper towels soaked in Lizol.

e) Dispose of all contaminated material used in cleanup in autoclave bags, and autoclave immediately.

3. <u>After a spill:</u>

- Medical surveillance may be required for potentially affected workers.

SOP 2.10: Autoclaving the infected samples

General:

- Each worker is responsible for correctly bagging and labelling his/her own waste.
- A BSL3 technician will be responsible for transporting and autoclaving the waste. Waste will be autoclaved once or twice per day, depending on use and will be done at specified times. All workers must cooperate with the BSL3 technician to ensure proper and safe disposal of waste.
- As the cycle is complete, autoclaved waste must be removed from the autoclave and disposed off in the discard barrels. Discard barrels will be stored in the anteroom, sealed after being filled and removed from the BSL3 for disposal.

Treatment of waste prior to autoclaving:

- All waste will be treated as biohazardous as described below.
- All waste must double bagged, labelled, marked with autoclave tape, and placed in covered plastic barrel for autoclaving.
- Lizol will be added to infectious material before disposal in autoclave bags.
- All waste is put into an autoclave bag. The bag is closed with a rubber band and the outside surface of the bag is wiped with Lizol. The wiped bag is removed from the BSC and placed inside a second bag (in plastic barrel). Thus, every waste bag is double-bagged.
- Approximately 100ml of water should be pre-placed in the bottom of the second bag to increase steam inside the bag during autoclaving. It is of utmost importance that the waste bags not be overfilled, as the sterilization will not be complete.
- Pipette tips will be rinsed with Lizol (see SOP 8) once (in screw cap liquid waste container) and then placed in an autoclave bag inside pipette tips discard bag. The discard bag must be inspected for leakage, wiped with Lizol and placed inside a second bag (in plastic barrel) as above.

Chemical waste (alcohol and phenol/chloroform) must be contained in separate plastic bottles and properly labelled.

- Concentrated Lizol must be added to the alcohol bottle for a final dilution factor of 50.
    - Both bottles will be placed in a specially designated area. Once full, the bottles will be carefully wiped with Lizol and removed from the BSL3 for proper disposal.

Autoclave Procedures: Only the BSL3 technicians are allowed to operate the autoclave. They will adhere to the maximum rated capacity of each load to assure sterilization.

- Use the designated cycle (121^{o}C–30 min) for contaminated equipments, bedding and animal carcases. Use the indicator autoclave tape for every bag.
- Autoclaved equipment and cages may be delivered to the procedure room to be washed and handled as standard, non-hazardous equipment.

SOP 2.11: TISSUES CULTURING IN THE BSL3

General:

- Microtiter plates and Lab-Tek slides will be used.
- Only infected cells are to be incubated in BSL3, and only authorized individuals are allowed access to the CO_2 incubator.
- Plates are to be carried from BSC to incubator in tightly sealed Nalgene boxes to contain accidental spilling.
- All plates must be clearly labelled with user's name and infectious agent.

SOP 2.12: EQUIPMENT FAILURES

Motor malfunction (Intact rotor):

If it is obvious that a spill inside has occurred in the rotor cup:

- Turn speed to 0 rpm; continue to run to remove any potential aerosol.
- Leave room, evacuate other personnel, and notify BSL3 supervisor and PI.
- Wait 30 minutes before re-entering suite.
- Examine for cracks or leaks. If none present, remove rotor from centrifuge and proceed to BSC.
- Load BSC with supplies to complete decontamination of rotor and contents.
- Open rotor inside BSC and commence decontamination using procedure outlined in SOP#9 (spill inside BSC).
- If leaks or cracks in rotor are present, BSL3 supervisor will assist in determining appropriate decontamination procedures, which may include gaseous decontamination of the suite.

Rotor Malfunction (Ruptured rotor):

- Treat as a spill outside the BSC and follow procedures outlined in SOP #9 (Spill outside the BSC).

BSC Malfunction:

- Red warning light and/or alarm signifies insufficient airflow
- Terminate work; notify all other workers to leave the room.
- Cover and contain all infectious materials; turn off vacuum.
- Leave room, warn others not to enter.
- Notify BSL3 supervisor so that decontamination procedures can begin.

SOP 2.13: MEDICAL EMERGENCY PROCEDURES

Personal injury:

i. Injury or exposure to hazardous chemicals or infectious agents:

Report incident immediately to PI and BSL3 supervisor. Seek medical attention at IISc health centre emergency room after hours. All injuries must also be reported to the principal investigator (PI) and BSL3 supervisor.

ii. Needle sticks or cuts involving potential infectious agent:

a. Wash affected area with soap and water.
b. Allow wound to bleed, leave BSL3 and wash hand in washroom.
c. Wash affected area with Betadine.
d. Immediately or as soon as possible, contact the IISc health centre.

iii. Symptoms associated with tuberculosis or any other infectious agent in use at the BSL3 facility must be reported to BSL3 Supervisor and PI. Complete on-the-Job Incident report form (see below).

iv. How to treat a life-threatening medical emergency inside the BSL3 laboratory.
Keeping calm is extremely important when treating someone during an emergency.
Warning sign of medical emergency include:

- Difficulty in breathing
- Chest or upper abdominal pain
- Fainting or sudden dizziness
- Bleeding that won't stop
- Severe or persistent vomiting
- Coughing up or vomiting blood

a. "Buddy" should immediately phone the PI's Lab and report the incident.
b. If incident happens over the weekend, call BSL3 Supervisor and IISc Health centre/Ambulance at 2227/2234.
c. The BSL3 supervisor will contact CMO, IISc Health Centre for appropriate emergency response.
1. If injury permits, remove gloves, shoe covers, sleeves of injured person and move to "grey" area just outside BSL3 lab.
2. If injury does not permit removal of injured from the BSL3, stay with the person.

SOP 2.14: FIRE EMERGENCY PROCEDURES

General:

- The fire alarm and extinguisher is located inside the incubator room, lobby, plant room and BSL3 labs.
- Emergency telephone information is located at the telephone.
- In **Handling the fire extinguisher- P.A.S.S- pull aim squeeze sweep:**
- Place the extinguisher on the floor. Hold it by the tank (pressure on the handle could pinch the pin). Pull the pin straight out.
- Stand 10 feet back from the fire. Aim at the base of the fire.
- Squeeze the lever on the fire extinguisher. Sweep from side, moving in slowly until the fire is out.

If you detect FIRE or SMOKE, do this at once:

- **STAY CALM** and use common sense.

Close the door to CONFINE the fire and smoke (e.g., switch off and close BSC, close cubical doors, remove other potential flammable material from source of fire).

- **ACTIVATE THE FIRE ALARM**. A small red box located on the wall in the incubator room.

Follow the instruction on the alarm.

- **REPORT THE FIRE**. Call Fire fighting services at 101/22971544/22971550, identify yourself and tell the dispatcher the exact location of the fire or smoke and what is burning.
- **EVACUATE**. Exit BSL3 according to SOP. Evacuation of staff will be carried out in a timely and orderly manner and will occur as follows:
    - All building occupants should proceed to the nearest exit, move away from the building and assemble in allocation predetermined by your instructor. This will provide a quick and easy way to account for all personnel. It is also important that the fire department have clear and unobstructed access to the building.
    - Do not return to the building unless informed to do so by the fire department, police or the Safety Officer.

SOP 2.15: TRANSPORTING CRYO SAMPLES BETWEEN BSL3 AND -80°C FREEZER

General:

- The -80°C freezer is located in BSL3 suite.
- All infectious materials that require storage at -80°C must be removed from the BSC (according to the SOP#3, 4 and/or 7 as appropriate) and the BSL3 laboratory (see below).
- This SOP will be followed primarily to obtain cryovials of mycobacterium cells stored at -80°C.

Procedure:

- Discard outer gloves and exit BSC.
- Put on new set of outer gloves.
- Quickly remove cryobox from -80°C freezer, place in plastic transport container.
- Secure container with rubber band(s).
- Access and place cryobox in the BSC, clean with Lizol, remove rubber band and lid; carefully remove vial and place in tube rack.
- Close box (Use 1-2 strong rubber bands) and follow SOP #3 for removal of item from BSC.

3.0 Annexure I

3.1 BSL3 request form

Name of Worker: _____

Position: _____

PI and Department: _____

Title of the Project:

Summary of Work to be carried out in BSL3:

PLEASE ATTACH A SEPARATE SHEET GIVING OUTLINE OF THE PROPOSED WORK AND TECHNIQUES TO BE USED

Undertakings

- I understand that working inside the BSL3 involves a level of risk that is greater than in a normal lab environment.
- I have read and properly understood the following documents:
  - Practices and Procedures for the BSL3 facility at IISc.
- I have been provided the requisite training for working in the BSL3.
- I shall abide by the rules and regulations outlined in the above mentioned documents, failing which I will be liable for sanctions outlined in these documents.
- I understand that I may have to undergo periodic medical screening before, during and after working in the BSL3 environment.
- I shall personally be responsible for my own health; IISc or its any employee will not be held responsible for any illness I may develop. I have adequate medical insurance cover to take care of my all possible medical needs.

Signature of Worker

Place:

Date:

Signature and Name of Project PI

Place:

Date:

Signature of BSL3 In-charge

Date:

3.2 SUMMARY SHEET

Date: _____

Name of Worker: _____ _____

(Signature)

Details of Medical Insurance: Policy No._____

Insurance Provider:

1. BCG vaccination Scar Present: ___Yes ___No

2. Mantoux Test Date: _____Result: ___Positive (……x……mm) ___Negative

2.1 If Mantoux Negative, Fresh BCG Vaccination Date: _____

3. HBV Vaccination Dates: I……II…….. III…….

4. BSL3 Practices and Procedures Training on: ……

5. Training given by ……..

6. Training updates ……. ……. ……. …….

7. Record of General Health Screening:

Date	Mantoux	Blood	Urine	Remarks

8. IBSC Approval for project: _____ Yes _____ Date

9. IAEC Approval for Project: _____Yes _____ Date

Remarks:

4.0 REFERENCES

- National Research Council. Guide for the Care and Use of Laboratory Animals. Washington: National Academic Press, 1996.

- National Research Council. Occupational Health and Safety in the Care and Use of Laboratory Animals. Washington: National Academic Press, 1997.

- US department of Health and Human Services. Biosafety in Microbiology and Biomedical Laboratories. Washington: US Government Printing Office, 1999.