

**STANDARD OPERATING PROCEDURES (SOPs)  
FOR SPECIFIC CONSIDERATIONS FOR WORKING IN THE BSL-3 FACILITY**



**BSL3 SOP Version 1.0**

**Updated on November 08, 2023**

**[Revisions to this document will be tracked and documented in subsequent versions]**

**Prepared by Dr Sara Jones, Facility Manager**

**Mr. Sanjai D, Technical Manager**

**Vented by Dr Rajesh Chandramohanadas, Scientist In-Charge**

**Approved by RGCB Institutional Biosafety Committee on March 9, 2023**

**Certified by DBT-Review Committee on Genetic Manipulation (RCGM) on July 04, 2023**

---

**CONTENTS****1.0 GENERAL**

- 1.1. INTRODUCTION
- 1.2. GENERAL REGULATIONS
  - 1.2.1. DOCUMENTATION
  - 1.2.2. GENERAL INSTRUCTIONS
  - 1.2.3. GENERAL LAB PRACTICES
- 1.3. AUTHORIZATION
- 1.4. PENALTY
- 1.5. BSL3 TRAINING
- 1.6. HEALTH MONITORING

**2.0. SOP FOR ENTERING THE BSL-3 FACILITY**

- 2.1. ENTERING DONNING ANTEROOM
  - 2.1.1. BEFORE ENTERING
  - 2.1.2. ENTERING PROCEDURE (Inside the Anteroom)
  - 2.1.3. PPE DONNING
  - 2.1.4. PREPARATION OF BIOLOGICAL SAFETY CABINET (BSC)
    - 2.1.4.1. BSC SHUTDOWN PROCEDURES
  - 2.1.5. CENTRIFUGATION OF INFECTIOUS LIQUIDS
  - 2.1.6. INVENTORY OF PATHOGEN STOCKS

**3.0. SOP FOR EXITING THE BSL-3 FACILITY**

- 3.1. DOFFING PPE AND FINAL EXIT PROCEDURES

**4.0. TRANSPORTING SAMPLES**

- 4.1. MOVEMENT FROM THE BSL3
- 4.2. RECEIVING SAMPLES/PATHOGENS

**5.0. SOP FOR SPILL RESPONSE IN BSL-3**

- 5.1. BIOHAZARD SPILLS INSIDE THE BSC
- 5.2. BIOHAZARD SPILL OUTSIDE THE BSC
  - 5.2.1. IMMEDIATE SPILL CONTROL
  - 5.2.2. DECONTAMINATION OF SPILL:
  - 5.2.3. AFTER A SPILL

**6.0. SOP FOR DECONTAMINATION PROCEDURE**

- 6.1. AUTOCLAVING THE INFECTIOUS SAMPLE
  - 6.1.1. TREATMENT OF WASTE PRIOR TO AUTOCLAVING
  - 6.1.2. BIOMEDICAL WASTE DISPOSAL

**7.0. SOP FOR RISK MANAGEMENT****8.0. MEDICAL EMERGENCY PROCEDURES**

- 8.1. PERSONAL INJURY

**9.0. FIRE EMERGENCY PROCEDURES**

- 9.1. BSL-3 INCIDENT REPORT
- 9.2. BIO-SECURITY
- 9.3. ANIMAL EXPERIMENTS

**10. RECORDS MAINTAINANCE****11.0 VALIDATION**

## **1.0 GENERAL**

### **1.1. INTRODUCTION**

The modular Biosafety Level -3 (BSL-3) facilities at the Campus # 2, Akkulam of RGCB is located at the north side 70ft from the main research block. The facility will be used for basic and applied research by RGCB faculty as well as collaborating partners, as a platform for drug and vaccine testing, optimization and development. The facility consists of (i) TWO BSL-3 suits (ii) ONE animal holding room and (iii) ONE animal procedure room. There are appropriate change rooms, an autoclave room, and a plant room, as part of the facility. This lab has special safety and engineering features for maintaining requisite negative pressure environment to ensure unidirectional airflow for ensuring safety of lab personnel and the environment surrounding the lab according to the respective guidelines and recommendations.

Everyone working in the BSL3 laboratory must respect this SOP. Lack of compliance will result in revoked or forbidden access.

### **1.2 GENERAL REGULATIONS**

#### **1.2.1 DOCUMENTATION**

All the required documentation needs to be submitted to Facility in-charge before starting the work inside the BSL3:

- a) Approval of proposed research project by RGCB Institutional Biosafety Committee (IBSC)
- b) Approval of experimental protocol by the RGCB Institutional Animal Ethics Committee (IAEC), when animal usage is proposed.
- c) Approval of experimental protocol by the RGCB IHEC (Institute Human Ethical Committee), when samples of human origin are used
- d) Certificate of completion of RGCB Radiation Safety Training (if applicable)
- e) BSL3 training form and medical test request signed by the Supervisor of the proposed researcher/staff
- f) Details of the funding to cover the user charges
- g) Medical Certificate of the proposed researcher indicating test results if available for the pathogen used (Indicative of a negative Result)
- h) Certificate of successful completion of BSL3 theoretical and practical training of the proposed researcher/staff
- i) Self-declaration of the proposed researcher/staff on their voluntary involvement in BSL3 project, considering all the risks involved.
- j) Any applicable updates and additions if required will be informed or documented after annual BSL-3 committee meetings.

#### **1.2.2. GENERAL INSTRUCTIONS**

- a) Only authorized personnel, i.e., those who have undergone required training and submitted requisite documentation can enter the BSL3 facility

- b) Any person entering the BSL3 must follow the procedures outlined in this document as well as any other RGCB regulations that may apply
- c) From each lab, no more than 2 users will be authorized to use the facility. Priority will be given to experienced personnel, when available
- d) At any given time, not more than 4 users will be allowed to use the facility
- e) Piggyback entry of unauthorized personnel in the BSL3 is strictly prohibited
- f) It is advised to limit the work during regular working hours on weekdays (8.30 AM- 5.30 PM). Working in the BSL-3 on the weekends and holidays is not recommended as much as possible
- g) Every research group is responsible for the supply of consumables for carrying out experiments. Any additional supplies required will be recommended by the facility in charge.
- h) Common consumables/supplies stocked & restocked according to agreements with the individual research groups will be communicated to the parties involved.

### 1.2.3 GENERAL LAB PRACTICES

- a) Researchers need to be aware of the premises and practices of the BSL3 Lab
- b) Proper planning of procedures before entering the lab is mandatory
- c) Understanding the proper care and use of equipment within BSL-3 is mandatory
- d) It is recommended that distractions must be avoided as much as possible
- e) No work with infectious materials is conducted in open vessels on the open bench
- f) Eating, drinking, smoking, wearing contact lenses, and using cosmetics inside the laboratory is prohibited
- g) Persons who wear contact lenses in laboratories should also wear other goggles or a face shield.
- h) Mouth pipetting is strictly prohibited, and mechanical pipetting devices are used.
- i) Policies for the safe handling of sharps and broken utensils are instituted as per RGCB biosafety guidelines and carefully follow the procedure of IMAGE Kerala to dispose of sharps and avoid the use of glassware inside the BSL lab. The use of high-quality plastics inside the lab is encouraged
- j) Mobile phones are not allowed inside the BSL3 lab.

## 1.2. AUTHORIZATION

Entry to the BSL-3 facility will be restricted to individuals who have the following:

- Completed the required training module
- Aware of the potential hazards of the work being carried out in the BSL-3 lab
- Have read and understood the BSL-3 manual
- Have completed all the required documentation as per the SOP
- Have been tested negative against the pathogen proposed to work on
- Have been immunized for Hepatitis B, Tetanus toxoid, Rabies(for animal work)

#### 1.4. PENALTY

- I. It is expected that all users of BSL-3 will report safety violations to the BSL3 In-charge immediately
- II. Anyone who does not follow the regulations described herein is liable to be sanctioned as follows:
  - **First Violation:** Written warning
  - **Second Violation:** Access to BSL3 suspended for ONE MONTH
  - **Third Violation:** Access to BSL3 blocked for LIFETIME.

#### 1.5. BSL3 TRAINING

- All proposed users must undergo RGCB BSL3 orientation and 1-week supervised training with designated approved staffs.
- Before commencing the training, they will have to sign a declaration of their readiness to work in the BSL3 facility.
- At the end of training users should pass the examination as well as the practical examination with hands on test by the facility in-charge to be certified for working in the facility.

#### 1.6. HEALTH MONITORING

- Before commencing the training, all users must undergo the required test at the RGCB designated testing centre to record the status of any prior exposure to pathogenic infections relevant to their study. Baseline serum samples of the users will be collected and stored in the BSL3 facility for future records. Additional serum specimen may be periodically collected, depending on the agents handled, or the duration of experiment in the laboratory, as and when required.
- Routine workers (cleaning staff, facility monitor, regular users) may be asked to undergo periodic test every 4 weeks (or at a reasonable interval, decided by the BSL2 Management committee).
- All users must inform facility in-charge in case they develop any unexplained symptoms or discomfort or health issues.

#### 2.0. SOP FOR ENTERING THE BSL-3 FACILITY

- All research personnel requesting access to the BSL3 facility must have approval from their principal investigator
- All research personnel approved for use of BSL3 must receive
  - ✓ Appropriate training
  - ✓ Approval by RGCB IBSC committee
  - ✓ Approval to get training and access to the lab by the BSL3 In-Charge
- Restricted access to the BSL3 is ensured by Biometric system
- All users in the BSL3 labs must be working in pairs

#### 2.1. ENTERING DONNING ANTEROOM

### 2.1.1 BEFORE ENTERING

- Check through the front door glass panel to ensure that the inner door is closed
- Use finger access biometric reader to enter the BSL3 laboratory

**NOTE:** Each user will be given entry access as per the authorization SOP. No piggyback entries of unauthorized personnel will be allowed.

### 2.1.2 ENTERING PROCEDURE (Inside the Anteroom)

- Sign in using the login book. Indicate the following: Name, Department/Laboratory, Time and Date of entry, Duration of booking, Name of the Pathogen used, Pressure of the anteroom at entry
- Monitor the Body temperature with Digital thermometer and record in the login register. Do not enter the facility if the body temperature recorded is higher than 38°C
- All animals, supplies 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, if any: 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 staff in charge will spray the outer surface of the boxes with disinfectant and transport to the appropriate location inside the bio-containment facility.

### 2.1.3 PPE DONNING

- Remove watch, jewellery, mobile phone, wallet, keys and contents of the pockets
- Perform hand hygiene, use alcohol-based hand rub
- Allow hands to dry before moving to next step
- Inspect PPE items prior to putting them on
- Put on inner gloves with long cuff
- Put on surgical face mask
- Put boot covers
- Put on cover all /gown
- Make sure that the cuffs of the inner gloves are tucked under sleeves of the cover all
- Zip-up cover all
- Make thumb holes at the border of the cuff and sleeves
- Put on N95 respirator; cup the respirator in your hand with the nose piece at the fingertips
- Put on outer gloves- ensure the cuffs are pulled over the sleeves of the cover all
- Put on face shield / goggles, goggles should be inside the cover all hood

**NOTE:** Make sure that all parts of the skin are covered.

#### 2.1.4. PREPARATION OF BIOLOGICAL SAFETY CABINET (BSC)

- The BSC blowers should be operated at least five minutes before beginning work to purge any air-borne particulates inside the BSC. Check the display to confirm airflow, prior to initiating work.
- The work surface, the interior walls, and the interior surface of the window should be wiped with 70% ethanol (EtOH)
- Place necessary safety and waste management items inside the BSC before beginning work to minimize any disruption of airflow
- Place appropriate autoclavable biohazard bag for solid waste collection, polypropylene sterilizing tray containing approved disinfectant, and a spray bottle containing approved disinfectant
- Place all necessary materials inside the BSC before beginning the work to minimize disruption of airflow
- Decontaminate the surfaces of all materials and containers with 70% EtOH prior to placement inside the BSC
- All materials should be placed as far back inside the BSC as practical
- Place the polypropylene sterilizing bin near the BSC
- Use only filtered tips/pipettes for handling infectious liquids
- Avoid rapid operation of the micro pipettes/pipette-aid
- For vortexing, close the sample container tightly, spray with disinfectant before and after vortexing.

##### 2.1.4.1. BSC SHUTDOWN PROCEDURES

- Spray the inside of the BSC and wipe down items to be removed from the BSC
- Only remove items from the BSC that have been surface decontaminated
- Prepare the small biohazard waste bag in the BSC for sterilization
- Add a small volume of disinfectant to the small autoclave bag as a steam source
- Seal the biohazard bag with autoclave tape (do not twist the autoclave bag to allow escape of steam)
- Spray the exterior of the red waste bag that is inside the BSC with disinfectant
- Double bag the autoclave waste bag in a new autoclave bag and seal with autoclave tape
- Spray the exterior of the red waste bag that is inside the BSC with disinfectant
- Transfer the autoclave bag from the BSC to the polypropylene sterilizing pan
- Perform a final surface decontamination of the BSC working area, including wipe-down of the BSC work surface, sides and back, and the interior of the glass sash with an approved disinfectant. Allow dwell time of at least 10 minutes
- All equipment should be surface decontaminated with approved disinfectant. Allow dwell time of at least 5 minutes
- Allow sufficient contact time with disinfectant as indicated by the manufacturer's instructions. (Sodium hypochlorite at 0.1% (1000 ppm) for disinfecting surfaces
- Place a new biohazard bag inside the decontaminated BSC.
- Spray outer gloves with disinfectant. Do not touch anything inside the BSC once you remove your outer gloves. Remove your hands from the BSC and don new outer gloves.



- Shutdown the BSC if not to be used by next user within next 30 minutes.

#### **2.1.5. CENTRIFUGATION OF INFECTIOUS LIQUIDS**

- All liquid samples for centrifugation should be contained in tightly closed container/tube inside the BSC
- The exterior of the containers should be wiped with 70% ethanol to sterilize and then placed inside swinging bucket rotors or click on microtube rotors
- Once tubes are placed securely and in a balanced manner, the lid of the centrifuge bucket/rotor should be securely closed with sealed cover, while inside BSC
- The sealed Rotor containing the samples should be wiped with 70% EtOH and taken out of the BSC, secured in the centrifuge, and subjected to centrifugation
- After completion of centrifugation, the sealed rotor/bucket should be brought back to BSC and opened within the BSC
- The centrifuge/rotor buckets should be wiped with 70% EtOH and 2% Lizol once their use is complete
- Same disinfection should be done with the operation buttons and rotor area of the centrifuges after completion of run
- The centrifuge rotors and buckets should be autoclaved periodically (at least once every 2 weeks, more as needed).

#### **2.1.6. INVENTORY OF PATHOGEN STOCKS**

- A complete inventory of pathogens generated during experiments will be maintained
- Number of vials, volume, details, of strains, date of experiment, user name to be recorded
- All the stocks will be stored in -80 and access will be restricted to BSL3 Operator/In-Charge
- New vials will be issued to users on request and usage will be documented

### **3.0. SOP FOR EXITING THE BSL-3 FACILITY**

- Confirm that all virus stocks and/or samples are secured in the ultra-cold -80°C freezer
- Decontaminate all work surfaces with disinfectant including:
  - ✓ Laboratory bench top
  - ✓ Biosafety cabinets
  - ✓ Tabletop centrifuge
  - ✓ Cell culture incubator
  - ✓ Microscope
  - ✓ Handles of Refrigerators and freezer
  - ✓ Allow sufficient contact time with disinfectant.
  - ✓ Outer gloves
- Perform a final check of the culture rooms:
  - ✓ Confirm decontamination of all surfaces and items inside the BSC with disinfectant
  - ✓ Confirm placement of a new small biohazard waste bag inside the BSC
  - ✓ It is now acceptable to touch the door handle to exit the tissue culture suite
- Transfer all biohazardous waste to the autoclave
- All biohazardous waste must be sterilized before final exit from the facility



- All infectious waste should be sterilized with a 60-minute steam sterilization cycle

### 3.1 DOFFING PPE AND FINAL EXIT PROCEDURES

- Outer gloves and sleeves removed in BSC in solid discard cover
- PPE should be taken off in a designated PPE removal area
- Put all PPE in a leak proof container for infectious waste
  - ✓ Disinfect outer gloves (use 1:10 bleach disinfection (sodium hypochlorite) or an alcohol-based hand rub)
  - ✓ Remove boot covers
  - ✓ Touch only the inner surface of boot- (use 1:10 sodium hypochlorite or an alcohol- based hand rub)
  - ✓ Remove outer gloves- be careful, not to contaminate the inner gloves-bird beak's method
  - ✓ Remove goggles –lift back of strap over the head, pull out and away
  - ✓ Avoid touching the front surface of the goggles and sanitized and place in the designated box for reuse
  - ✓ Remove cover all: First remove hood touching outside, do not touch inside the coverall during removing
  - ✓ After removing the cover all from the body, touch only the inside of the cover all when rolling in prior to disposal
  - ✓ Disinfect and change inner gloves.
  - ✓ Hand rub and put new gloves
  - ✓ Remove the n95 respirator, without touching the front side.
  - ✓ Disinfect boot
- Enter the time and sign on the exit log book placed at the preparatory room and exit the BSL-3 facility.

**NOTE:** Proper procedure to wear and dispose of masks:

- Before putting on a mask, clean hands with alcohol-based hand rub
- Cover mouth and nose with mask and make sure there are no gaps between your face and the mask
- Avoid touching the mask while using it; if you do, clean your hands with alcohol-based hand rub
- Replace the mask with a new one as soon as it is damp, do not re-use single-use masks
- To remove the mask: remove it from behind (do not touch the front of mask); discard immediately in a closed bin; clean hands with alcohol-based hand rub.

## 4.0. TRANSPORTING SAMPLES

### 4.1. MOVEMENT FROM THE BSL3

- BSL3 facility approved protocol for inactivation of virulent pathogens, such as heating at 80°C for 2 hours and other appropriate methods will be used
- Commercially available disposable 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

### 4.2. RECEIVING SAMPLES/PATHOGENS

- All sample details should be recorded and must be registered with the RCGM
- All samples should be taken into the BSL-3 facility, in adherence with the entry protocol
- The specimen transport containers should be disinfected properly with 70% alcohol and placed inside the BSC
- After removing the infected material in the BSL3 suite, technical staff are responsible for autoclaving or disposing of specimen transport containers. Re-usable specimen transport containers must have a visual indicator (autoclave tape) to show that they have been sufficiently sterilized.

## 5.0. SOP FOR SPILL RESPONSE IN BSL-3

- All spills of toxic, irritating, or potentially infectious substances must be reported to the facility in-charge and technical person in-charge
- If the spill involves toxic or irritating chemicals or infectious agent exposure to mucous 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 by 2% Lizol for a minimum of 20 minutes contact time
- The location and contents of the spill should be identified as specifically as possible to facilitate effective clean-up
- Spills of virus samples must be decontaminated with suitable disinfectant and clean-up, according to the outlined procedures described below

## 5.1. BIOHAZARD SPILLS INSIDE THE BSC:

**NOTE:** Do not instinctively remove hands from BSC.

- ✓ BSC blower must remain **ON** during and after spill clean-up
- ✓ Wipe gloved hands and sleeves with 70% alcohol. Remove them inside the BSC and don new pair of gloves
- ✓ Immediately disinfect the area using Lizol 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.
- ✓ A spill on a diaper can be handled by soaking the diaper with Lizol, folding diaper carefully, and then discarding them off
- ✓ 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 facility in-charge can determine if filters or blowers should 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

## 5.2. BIOHAZARD SPILL OUTSIDE THE BSC

### 5.2.1. IMMEDIATE SPILL CONTROL

- Inform everyone in the room about the spill, and ask the personnel to evacuate the room immediately
- Hold your breath and leave the area after discarding the outer glove on the floor, 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 them in bag for autoclaving. Wash hands, face; shower if necessary.
- If spill occurs on Tyvek suit, wipe area with copious amounts of 2% Lizol; remove Tyvek before exiting the BSL3 and place inside autoclave barrel.
- Notify BSL3 in charge and facility manager, complete the spill report.

### 5.2.2. DECONTAMINATION OF SPILL

- All spills must be reported to the PI, BSL3 in-charge and the Facility manager
  - Note the volume and/or estimated number of pathogens spilled
  - Depending on the characteristics of the spill, BSL3 in-charge will determine if gaseous decontamination is necessary
- 
- ✓ After one hour, dress in protective clothing - rubber gloves over nitrile gloves and boots, disposable jumpsuit, head covering, and respirator (PAPR)

- ✓ Pour Lizol solution around spill and cover the area with paper towels soaked in Lizol. Let it stand for 30 minutes
- ✓ Use paper towels to wipe up spills, working towards the centre of the spill

### 5.2.3. AFTER A SPILL

- ✓ Medical surveillance may be required for potentially affected workers
- ✓ All the required details will be documented in the facility Incidence Report Form

## 6.0. SOP FOR DECONTAMINATION PROCEDURE

### 6.1. AUTOCLAVING THE INFECTIOUS SAMPLE

- Each researcher/staff is responsible for correctly bagging and labelling his/her own biological waste arising from experiment
- The BSL 3 technical staff 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 staff 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 bins
- Discard bins will be stored in the autoclave room, sealed after being filled, and subsequently removed from the BSL3 for disposal

#### 6.1.1. TREATMENT OF WASTE PRIOR TO AUTOCLAVING

- All biological waste will be treated as biohazardous as described below
- All waste must be double bagged, labelled, marked with autoclave tape, and placed in covered plastic barrel for autoclaving
- 2% Sodium hypochlorite will be added to the autoclave bag before disposing infectious material bags kept in the BSC. Pipette/tips will be rinsed with 2% Sodium hypochlorite by immersing in liquid waste autoclavable container and then placed in an autoclave bag
- The autoclave bag must be inspected for leakage, wiped with Bleach/Lizol, and placed inside a second bag prior to placing in the discard bin
- All waste bags are to be closed with a autoclave indicative tape and the outside surface of the bag needs to be wiped with Bleach/Lizol. Thus, every waste bag is double bagged
- It is of utmost importance that the waste bags are not overfilled, as the sterilization will not be complete
- Chemical waste must be contained in separate plastic bottles and properly labelled
- Concentrated Bleach/Lizol must be added to the alcohol bottle for a final dilution factor of 50. 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 effective sterilization.

#### 6.1.2. BIOMEDICAL WASTE DISPOSAL

- Solid Waste: (such as gloves, tissue papers, tube, tips, flasks and plates)

- ✓ Disinfection with chemical treatment and double discard bags packing inside the biosafety cabinets
- ✓ Autoclaving with autoclave tape indicator and labelled as BSL-3 waste.

**NOTE:** Waste disposal to be done by IMAGE Pvt, Ltd. Kerala

- Liquid Waste: (such as cultures, culture medium, serum, blood etc. )

Disinfection by chemical treatment in autoclavable bottle inside the biosafety cabinets, packing the bottle with discard bag, labelling, autoclaving and discharge into drains connected to the effluent treatment plant.

- Animal Waste:

- ✓ Double discard bag packing
- ✓ Autoclaving with autoclave tape indicator and labelled as BSL-3 waste.

**NOTE:** Waste disposal to be done by IMAGE Pvt, Ltd. Kerala

Waste Sharp:

- ✓ Packing in puncture resistant container inside the biosafety cabinets, (use of needle cutter and electrical needle blunter) disinfection with chemical treatment, autoclaving.

**NOTE:** Waste disposal to be done by IMAGE Pvt, Ltd. Kerala

## 7.0. SOP FOR RISK MANAGEMENT

### **BIO RISK- 1: Spill of infectious materials outside a BSC**

- ✓ Every person should immediately vacate the affected laboratory area
- ✓ The laboratory in-charge should be informed of the incident immediately, and staff must be prevented from re-entering the laboratory for least one hour to allow aerosol to be removed through the laboratory's ventilation system and allow time for heavier particles to settle.
- ✓ Sign should be posted indicating that entry is forbidden during the clean-up procedure. Appropriate protective clothing and respiratory protection must be worn.
- ✓ Use the absorbent material to cover the spill. Pour the disinfectant on the spill and allow the sufficient contact time to minimize the aerosol formation.
- ✓ Recover any sharp with forceps, collect the absorbent material.
- ✓ Place waste in leak-proof biohazard bags, ensure safe final management of waste disposal.

**BIO RISK- 2 : Spill of infectious materials inside a BSC**

- ✓ When a spill of infectious materials occurs within a BSC, a clean-up procedure should begin immediately, and the cabinet should continue to operate.

**BIO RISK- 3 : Breakage of tubes inside sealed buckets/ centrifuge machine**

- ✓ Always use sealed centrifuge buckets, and load and unload them in a BSC. If breakage occurs during centrifugation, broken tubes must be discarded in puncture-resistant container and disposed off immediately.
- ✓ Decontaminate the centrifuge buckets by soaking them in a suitable disinfectant/ buckets, then it may be decontaminated by autoclaving.

**BIO RISK- 4 : Safe handling of sharps**

- ✓ After use, needle should not be recapped, use needle cutter or electrical needle blunter.
- ✓ After the use, disposable syringes, needles, scalpel blades, and other sharp items should be placed in puncture –resistant containers for disposal.

**BIO RISK-5 : Other important guidelines**

- ✓ Emergency medical treatment of exposed and injured persons
- ✓ Medical surveillance of persons exposed to an incident.
- ✓ Clinical management of persons exposed to an incident.
- ✓ Epidemiological investigation

**8.0. MEDICAL EMERGENCY PROCEDURES****8.1. PERSONAL INJURY:**

- **Injury or exposure to hazardous chemicals or infectious agents**
  - ✓ Report the incident immediately to PI, BSL3 in charge and the Facility Manager
  - ✓ Seek medical attention at RGCB- designated health centre/health officer in-charge
  - ✓ All injuries must also be reported to the principal investigator (PI) and BSL3 in-charge.
- **Needle sticks or cuts involving potential infectious agent:**
  - ✓ Allow wound to bleed, leave BSL3 suits and wash the hand in washroom
  - ✓ Wash the affected area with soap and water
  - ✓ Wash the affected area with Betadine
  - ✓ As soon as possible, contact RGCB -designated health centre/health officer in-charge for advice
  - ✓ Symptoms associated with infectious agent in use at the BSL3 facility must be reported to BSL3 In-charge and PI.
  - ✓ A completed set of on-the-Job Incident report form to be submitted.

**NOTE:** 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
- In case of complete incapacitation of BSL3 researcher/staff, a co-worker in BSL3/ BSL3 monitor should immediately phone the PI's Lab and report the incident
- If incident happens over the weekend, call BSL3 In-charge and RGCB Health Officer
- The BSL3 in-charge will contact RGCB designated health centre for appropriate emergency advice and response
- If injury permits, remove gloves, shoe covers, sleeves of injured person and move to the area just outside BSL3 lab.
- If injury does not permit removal of injured from the BSL3, stay with the person.

## 9.0. FIRE EMERGENCY PROCEDURES

- The fire alarm and extinguisher are located inside the BSL 3 suits, entry & exit areas, autoclave rooms, UPS room, effluent treatment room and the plant room
- Emergency telephone information is located at the telephone area

### **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 away 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 Firefighting services, 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.

### **9.1.BSL-3 INCIDENT REPORT**

In the event of an exposure or injury requiring medical care, please seek care immediately prior to completing the incident report form. Students can visit the physician on campus or their personal physician after-hours. Staff and faculty should seek medical attention from their personal physicians.

### **9.2. BIOSECURITY**

- Access to the laboratory facility and biological materials should be limited and controlled.
- The control and tracking of biological stocks and other sensitive material should be monitored by an inventory or material management process.
- Transport of infectious biological materials are controlled, tracked and documented as the potential risks.
- Laboratory should be monitored 24x7 hour by CCTV.

### **9.3. ANIMAL EXPERIMENTS**

- For animal experiments, investigators have to submit a detailed work plan with time points and copy of RGCB IAEC / IBSC approval certificate to the BSL-3 in-charge, after confirming the availability of space in the BSL-3. After confirmation by BSL-3 in charge/BSL-3 committee, the booking of usage by investigators will be done.
- Animal work will be done on the basis of first come first work basis, strictly in accordance with the RGCB IAEC guidelines.

### **10.0. RECORDS MAINTAINANCE**

- Training and refresher training to be documented; records to be kept on file.
- Inventory of stocks of pathogens, toxins, and other regulated infectious material in long-term storage to be maintained, including location and risk group.
- Provision for detection of a missing sample in a timely manner.
- Map and physical specifications of BSL-3.
- Records of regular inspections of the containment zone and corrective actions to be kept on file.
- Records of regular inspections of the containment zone and corrective actions to be kept on file.
- A record of all individuals entering and exiting the containment zone to be maintained and kept on file.
- Records of routine decontamination and it's verification
- Records of incidents and it's response

### **11.0 VALIDATION**

- Facilities will be revalidated annually.
- BSCs will be revalidated 6 monthly.