Standard Operating Procedures for virus infection experiments and operation of Viral BSL3.

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20/01/2021

Review & Approval

The SOPs will be reviewed and revised annually or earlier as required.

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1.0 GENERAL

1.1 DEFINITIONS

A. The Viral Biosafety level three facility (VBSL3) is a containment facility located in the Centre for Infectious Disease Research (CIDR) Building, IISc Campus; Bangalore, for carrying out research activities involving BSL-3 class virus pathogens (SARS-CoV-2 and others). It consists of one Virus BSL3 labs and one animals housing, infection-cum-necropsy room. There are appropriate change rooms, an autoclave 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 according to WHO, CDC, DBT and ICMR recommendations.

1.2 GENERAL REGULATIONS

1.2.1 DOCUMENTATION

Documentation required to be completed and submitted to Facility in-charge before starting the work inside the virus BSL3:

1. Approval of the proposed research project by ICVR (Institutional Committee on Virus Research).

2. Provisional/Final clearance of proposed research project by IISc Institutional Biosafety Committee (IBSC).

3. Provisional/Final Approval of experimental protocol by the IISc, Institutional Animal Ethics Committee (IAEC), when animal usage is proposed.

4. Final Approval of experimental protocol by the IHEC (Institute Human Ethical Committee), when human origin samples to be used.

5. Certificate of completion of IISc Radiation Safety Training (if applicable).

6. BSL3 training form and medical test request signed by the Supervisor of the proposed worker.

7. Details of the Debit head to cover the user charges.

8. Medical Certificate of the proposed worker indicating SARS-CoV2 IgG/IgM and RT-PCR assay test results. (RT-PCR result must be negative.)

9. Certificate of successful completion of BSL3 theoretical and practical training of the proposed worker.

10. Self-declaration of the proposed worker on their voluntary involvement in BSL3 project, considering all the risks involved.

11. Medical insurance copy of the proposed worker.

12. Any applicable updates and additions. (other information will be updated after annual BSL-3 committee review of SOP'S)

1.2.2. GENERAL INSTRUCTIONS

A. Only authorized personnel, *i.e*., those who have gone through required training and submitted requisite documentation can enter virus BSL3.

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

C. From one research group no more than 2 users will be authorized to use the facility. Priority will be given to experienced personnel, when available.

D. At a given time no more than 4 users should be using the facility.

E. Piggyback entry of unauthorized personnel in the Viral BSL3 is strictly prohibited.

F. It is advised to limit the work during working hours on weekdays and avoid working on the weekends and holidays, as much as possible.

G. Every research group is responsible for its own supplies, which include:

- Tyvek Coveralls, Tyvek sleeves, shoe covers, head covers, surgical mask, N95 mask and gloves, surgical gowns and surgical scrubs, hand sleeves, laboratory shoes.
- Pipettes and Filter tips
- Tubes, culture flasks, culture plates, etc.
- Media, PBS, etc.
- Any other supplies as needed and recommended by facility in charge.

H. Common consumables/supplies such as CO2 cylinders, wiping tissues, ethanol, Lysol 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 are 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 virus 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 and passed the examination.
2) Been advised of potential hazards
3) Read and understood this manual
4) Filed requisite documentation (see Documentation)
5) Have been tested against SARS-CoV-2 and vaccinated for SARS-CoV-2 vaccine (when available).

1.4 SANCTIONS

A. It is expected that all users of virus BSL-3 will report safety violations to the virus 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.**

2.1. VIRAL BSL3 TRAINING AND EXAMINATION

- All prospective users must undergo BSL3 orientation and 1-week supervised training with approved users.
- Before commencing the training, they will have to sign a declaration of their readiness to work in the viral BSL3.
- At the end of training users will have to take a readiness questionnaire and pass with 80% marks to be certified for working independently in the facility.

- After training, and passing written exam, prospective user will be evaluated by practical examination with hands on test by facility in-charge/ designated user.

SUBJECTS OF TRAINNING

- Guidelines about Biosecurity
- Introduction of biosafety labs according to risk groups
- Donning and Doffing of PPE
- Entry and exit Procedures for BSL-3
- Operation of Bio-safety cabinets
- Proper use of Centrifuges
- Procedure of pipetting, centrifugation to minimize aerosol formation.
- Procedure of liquid / solid biohazard waste disposal
- Bio risk management
- Infectious Spill management
- Medical Emergency response
- Fire emergency response
- Safe handling of sharps objects
- Working with animal models in A-BSL3.
- Use of fixative/ chemicals to kill the pathogens.
- Handling and transport of BSL-3 class pathogens
- Operation of wall mounted autoclave.
- Sanitization/ disinfection / fumigation/sterilization of lab and laboratory materials
- Emergency preparedness

2.2. HEALTH MONITORING

- Before commencing the training, all prospective users must undergo SARS-Co-V2 IgG/IgM rapid antibody test at the IISc health centre to record the status of prior exposure. The serum collected for this exercise to be stored in Viral BSL3 facility for future records.

- All users must also undergo RT-PCR test of the nasal swab/ throat swab sample which should be negative for COVID-19.

- Routine workers (cleaning staff, facility monitor, regular users) may be asked to undergo periodic RT-PCR test for COVID-19 every 4 weeks.

- All approved users must record their Body temperature before entering the facility.

- All users must inform Facility in-charge in case they develop symptoms of COVID-19 and test positive for the infection by ICMR approved tests. As pandemic is still ongoing, this is critical since users may get exposed to the virus in their community.

2.3. ENTERING THE VIRAL BSL-3 FACILITY

Before Entering the Donning Anteroom

- Check through the front door glass pane to ensure that the inner door is closed.
- Tap your proximity ID card on the biometric card reader.
- Activation of the card reader allows entry into the anteroom.
- Keep the access card inside your pocket or in the ante room and do not expose inside BSL3 working area.

NOTE: Each Research group will be issued 1 access card to be shared between their lab members. No piggyback entries of unauthorized personnel will be allowed.

Entering Procedure (Inside the Anteroom)

- Sign in using the login book. Indicate: your name, use of facility, use of SARS-CoV-2, pressure of the anteroom at entry, and time of entry.
- Monitor your Body temperature with Digital thermometer and record in the login register. Do not enter the facility if Temp is higher than 38°C.

2.4. PPE Donning

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

Make sure that all parts of the skin are covered.

2.5. PREPARATION OF BIOLOGICAL SAFETY CABINET (BSC)

1. The BSC blowers should be operated at least five minutes before beginning work to purge any air-borne particulates inside the BSC.
Note: Check the display to confirm airflow prior to initiating work.

2. The work surface, the interior walls, and the interior surface of the window should be wiped with 70% ethanol (EtOH).

3. Place necessary safety and waste management items inside the BSC before beginning work to minimize disruption of airflow.
a. One small autoclavable biohazard bag for solid waste collection
b. One polypropylene pipet/instrument sterilizing tray containing approved disinfectant, and
c. One spray bottle containing approved disinfectant.

4. Place all necessary materials inside the BSC before beginning work to minimize disruption of airflow.
a. Note: Surface decontaminate all materials and containers with 70% EtOH prior to placement inside the BSC.

5. All materials should be placed as far back inside the BSC as practical.

6. Place the polypropylene sterilizing bin near the BSC.

Note: Working instructions to minimize aerosol generation.

- 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.

BSC Shutdown Procedures:

1. Spray the inside of the BSC and wipe down items to be removed from the BSC.
Note: only remove items from the BSC that have been surface decontaminated.

2. 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.

3. 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.

4. All equipment should be surface decontaminated with approved disinfectant. Allow dwell time of at least 5 minutes.

5. Allow sufficient contact time with disinfectant as indicated by the manufacturer's instructions. (Sodium hypochlorite at 0.1% (1000 ppm) for disinfecting surfaces and 0.5% (5000 ppm) for disinfection of blood or bodily fluids spills in health-care facilities.)

6. Place a small new biohazard bag inside the decontaminated BSC.

10. Perform glove hygiene:
a. Spray outer gloves with disinfectant.
b. Do not touch anything inside the BSC once you remove your outer gloves.
c. Remove your hands from the BSC and don new outer gloves.

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

2.6 CENTRIFUGATION OF INFECTIOUS LIQUIDS

- All liquid samples to be subjected to 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 Biosafe swinging bucket rotors or click on Microtube rotors.

- Once tubes are placed securely and in 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 tubed should be wiped with 70% EtOH and taken out of BSC, secured in the Centrifuge machine, and subjected to required centrifugation.

- After completion of centrifugation the sealed rotor/bucket should be brought back to BSC and opened inside only.

- The Biosafe centrifuge/rotor buckets should be wiped with 70% EtOH and 2% Lizol once their use for the session is complete.

- Same disinfection should be done with the operation buttons and rotor area of the centrifuges after completion of the session.

- The biosafe centrifuge rotors and buckets should be autoclaved periodically (at least once every 2 weeks, more as needed).

Inventory of SARS-Co-V2 Stocks

- A detailed inventory of SARS-Co-V2 virus stocks generated during experiments will be maintained.
- Number of vials, volume, strains details, date to be recorded.
- SARS-Co-V2 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.

2.6. EXITING THE BSL-3 FACILITY

1. Confirm that all virus stocks and/or samples are secured in the ultra-cold -80°C freezer.
2. Decontaminate all work surfaces with disinfectant including:
   - Laboratory bench top,
   - Tabletop centrifuge,
   - Cell culture incubator,
   - Microscope
   - Handles of Refrigerators and freezer
   - Allow sufficient contact time with disinfectant.
   - Outer gloves.

3. Perform a final check of the tissue culture room:
   - Confirm decontamination of all surfaces and items inside the BSC (e.g., pipettors, tube racks, plates, etc.) 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.

4. Transfer all biohazardous waste to the steam sterilizing autoclave.
   - All biohazardous waste must be sterilized before final exit from the facility.
   - Sterilize the infectious waste with a 60-minute steam sterilization cycle.

2.7. DOFFING PPE AND FINAL EXIT PROCEDURES

- Outer gloves and sleeves removed in BSC in solid discard.
- PPE should be taken off in a designated PPE removal area.

- Put all PPE in a leak proof bag 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 bleach disinfection (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 it 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
- put New gloves
- Remove the n95 respirator, without touching the front side.
- Disinfect boot.

Note: Correct way to wear and dispose of masks

- Before putting on a mask, clean hands with alcohol-based hand rub or soap and water.
- 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 or soap and water.
- Replace the mask with a new one as soon as it is damp and 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 or soap and water.

2.8. IMPORTANT INSTRUCTIONS

1. Once the experiment completes with SARS CoV-2, all the essential PPEs such as TYVEK Suites, N95 Masks, Face shields, double gloves, should be discarded in biohazard bags.

2. No point of reusing any PPE's.

3. ICMR recommended disinfectant 2% Lizol solution is always used for disinfecting the surfaces, floors and primary spill management.

4. The Spills of virus cultures on floors will be managed by 1% Sodium hypochlorite solution with the contact time of 20 minutes and the surface will be thoroughly disinfected with Lizol solution. Fumigation of workspace determined based on the risk assessment of spillage occurred on floor.

5. The Users are not recommended to work alone, and maximum two person recommended to work on SARS CoV-2 in BSL-3 laboratory. Additionally, after office hour working is also not recommended.

6. The existence of SARS CoV-2 stock inventory is essential for facility; users, advised to update the facility about the stocks availability of SARS CoV-2 with them for their experiments.

7. Without approval from regulatory bodies of institute for culturing of SARS CoV-2 to any individual user/PI, the facility does not recommend for culturing of virus for any kind of assays. Further, the source of SARS CoV-2 is essential for facility for documents.

8. All the essential equipment used for SARS CoV-2 experiments should be thoroughly decontaminated with 2% Lizol followed with 70% ethanol. Micropipettes decontaminated with 70% Ethanol followed by 30 minutes of UV sterilization.

9. All PPE's used for SARS CoV-2 research should be discarded in Yellow biohazard bags, whereas the rest other consumables should be discarded in RED biohazard bags. All Biohazard bags should be Autoclaved thoroughly with the sterilization indicating tape stating COVID-19 laboratory waste with Date of autoclaving.

2.9. TRANSPORTING SAMPLES FROM THE BSL3

BSL3 facility approved methods of inactivation of virulent pathogens:

- Heating at 80ºC for 2 hours and other suitable methods.
- 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/pathogens:

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.

2.10. 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 by 2% Lizol for 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.

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 70% alcohol. 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 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.

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 2% 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 pathogens spilled. Depending on the characteristics of the spill (volume, number of pathogens, 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.

3. After a spill:
Medical surveillance may be required for potentially affected workers.

2.11: AUTOCLAVING THE INFECTIOUS SAMPLE

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 autoclave room, 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.

- 2% Lizol will be added to infectious material before disposal in autoclave bags. Pipette/tips will be rinsed with 2% Lizol (see SOP 8) once (in screw cap liquid waste container) and then placed in an autoclave bag.
- The discard bag must be inspected for leakage, wiped with Lizol and placed inside a second bag (in plastic barrel) as above.
- All waste is put into an autoclave bag. The bag is closed with a autoclave indicative tape 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 are not overfilled, as the sterilization will not be complete.

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°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.

2.12. BIOMEDICAL WASTE DISPOSAL OF COVID -19 RELATED WASTE

1. Solid waste (items contaminated with blood, serum, urine, stool, viruses) likes gloves, tissue papers, tube and others)

a. Disinfection with chemical treatment and double discard bags packing inside the biosafety cabinets

b. Autoclaving with autoclave tape indicator and labelled as covid-19 waste.

c. Incineration of autoclaved waste

d. incineration ash disposal in municipal land fill

(**Note- Waste disposal to be done by MEDICARE environmental management Pvt, Ltd. Bangalore)**

2. liquid waste: (like virus culture, blood, serum, urine, stool) - disinfection by chemical treatment in autoclavable bottle inside the biosafety cabinets, packing the bottle with discard bag, labelling, autoclaving and discharge into drains.

4. Animal Waste: double discard bag packing, autoclaving and incineration.

5. waste sharp: packing in puncture resistant container inside the biosafety cabinets, (use of needle cutter and electrical needle blunter) disinfection with chemical treatment, autoclaving and deep burial

2.13. RISK MANAGEMENT SOP-

Bio risk (Spill of infectious materials outside a BSC)

- Every person should immediately vacate the affected laboratory area.
- The laboratory manager 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 (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 (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 centrifuging, broken tubes must be discarded in puncture-resistant container and disposed immediately.
- Decontaminate centrifuge buckets by soaking them in a suitable disinfectant/ buckets may be decontaminated by autoclaving.

Bio risk (safe handling of sharps)

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

Bio risk (others 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

2.14. MEDICAL EMERGENCY PROCEDURES

1. 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:

- Allow wound to bleed, leave BSL3 and wash hand in washroom.
- Wash affected area with soap and water.
- Wash affected area with Betadine.
- Immediately or as soon as possible, contact the IISc health centre.
- *Symptoms associated with COVID-19 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 to be submitted.

Note: Keeping calm is extremely important when treating someone during an emergency.

2. 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

3. In case of complete incapacitation of BSL3 worker, a co-worker in BSL3/ BSL3 monitor should immediately phone the PI's Lab and report the incident.
4. If incident happens over the weekend, call BSL3 Supervisor and IISc Health centre/Ambulance at 2227/2234.
5. The BSL3 supervisor will contact CMO, IISc Health Centre for appropriate emergency response.
6. If injury permits, remove gloves, shoe covers, sleeves of injured person and move to "grey" area just outside BSL3 lab.
7. If injury does not permit removal of injured from the BSL3, stay with the

person.

2.15. FIRE EMERGENCY PROCEDURES

General:

- The fire alarm and extinguisher are 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 Firefighting 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.

2.16.BSL-3 INCIDENT REPORT

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

2.17. 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 video camera.

2.18. ANIMAL EXPERIMENTS

- For animal experiments, investigators have to submit a detailed work plan with time points and copy of IAEC / IBSC approval certificate to the virus BSL-3 in-charge, after confirming the availability of space in the BSL-3. After confirmation by virus BSL-3 in charge/virus 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 IAEC guidelines.

Review of SOPs by Research Personnel

By signing this document in the space below, I attest that I have received training on the Standard Operating Procedures for conducting research with SARS-CoV-2 within the Conventional Biocontainment Facility. I understand the entry and exit procedures, and rules governing my access to the Conventional Biocontainment Facility as described in the Biosafety/Biocontainment Plan. I acknowledge that my failure to abide by all rules and instructions may result in my removal and prohibition from future access to the VIRAL BSL3 Facility.

Review		
Review Date	Print Name	Signature
