

P3 FACILITY RULES AND GUIDELINES

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1. SCOPE

The present Biosafety Level 3 (BSL3 or P3) Manual describes the rules and guidelines for the use of the BioSafety Level 3 facility of the Instituto de Medicina Molecular (IMM), Lisboa, Portugal. The BSL3 laboratories are a potentially hazardous work area due to pathogen species research projects proposed to take place therein. All rules, definitions and procedures in this manual follow CDC and/or WHO guidelines. All laboratory procedures will follow the guidelines of BSL3 for this facility and BSL3 practices.

Because of the biohazards associated with research on BSL3 pathogen species, all such operations will be confined to the designated research area. Adherence to proper standard operating procedures protects everyone from exposure to infectious agents in the BSL3 laboratory. Working with BSL3 pathogens requires diligence from the laboratory worker to maintain safe laboratory conditions. This includes extensive knowledge of both the pathogen and the procedures, proper training and certification, and rigorous adherence to safety practices. Failure to meet any of these expectations will result in removal of BSL3 access privileges. The user is responsible for being well versed in the experimental design and execution of each protocol to be done in the BSL3. Also, all procedures must be approved by the Scientific Committee and the Facility Supervisor prior to being performed in the BSL3 facility.

Training of users will take place prior to any work in the BSL3. Each user is required to take BSL3-specific training. An unsafe worker jeopardizes the health and safety of all workers in the BSL3. Any reports of unsafe working conditions will be critically evaluated and appropriate measures will be taken, including retraining/certification, reduced privileges, or banning from the facility.

This document describes all standard operating procedures associated with the Research Facility.

Remember that safety is a shared responsibility. Attitude and work practices are critical for one's own health and safety, and for the welfare of others and the environment.

2. ACRONYMS

BSL3 – Biosafety Level 3

FS – Facility Supervisor

SAS – Ventilated compartment with interlocked doors, that serves as passage between two areas of different containment level.

PPE – Personal Protective Equipment

CDC – Center for Disease Control, USA

WHO – World Health Organization

SOP - Standard Operating Procedures

TB - Tuberculosis

BSC - BioSafety Cabinet

3. DESCRIPTION

I. INTRODUCTORY REMARKS

I.1. ORGANISATION AND CONTACT NUMBERS

I.1.1. Facility Supervisor

Miguel Prudêncio - Unidade de Malária, Ext 47341 (mprudencio@fm.ul.pt)

The Facility Supervisor has the responsibility, authority, and support from the Scientific Committee for establishing and maintaining policies and procedures, training personnel and maintaining the facility and equipment. The BSL3 Facility Supervisor will be responsible for managing the BSL3 Facility's operation, for supervising the technical conditions of the Facility and for issuing authorizations to use the BSL3 Facility.

I.1.2. Scientific Committee

Dolores Bonaparte, Ext. 47244 (doloresbf@fm.ul.pt)

Joana Marques – Animal House Director, Ext 47406 (joanammarques@fm.ul.pt)

Mário Ramirez - Unidade de Microbiologia Molecular e Infecção, Ext. 47205 (ramirez@fm.ul.pt)

Pedro Simas - Unidade de Patogénese Viral, Ext. 47211 (psimas@fm.ul.pt)

Ana Espada Sousa - Unidade de Imunologia Clínica, Ext. 47362 (asousa@fm.ul.pt)

The Scientific Committee is, together with the Facility Supervisor, responsible for helping to define the rules in the PG-P3 Manual, future amendments, and new procedures. They also evaluate projects that apply for the use of the BSL3 laboratory, advise facility users on policies related to BSL-3 containment and monitor registration of research protocols. The Scientific Committee will also issue opinions and assist the Facility Supervisor in decision-making whenever requested by the latter.

I.1.3. Animal Biosafety Officer

Dolores Bonaparte, Ext. 47244 (doloresbf@fm.ul.pt)

The Biosafety Officer provides technical support and consultation on biological safety practices, laboratory facilities and engineering controls, and regulatory matters. In particular, the Biosafety Officer has responsibility for the issues relating to health and safety such as reviewing and monitoring the BSL3 Facility design and renovation projects, advising project managers on acceptance criteria and tests, initial and periodic assessment of critical facility and component performance, consultation and technical assistance in response to incidents and facility decontamination. In addition, the Biosafety Officer will be involved in the safety training and testing of candidate BSL3 Facility users.

I.1.4. Technical Supervisor

Inês Matos - Ext.47408 (imatos@fm.ul.pt)

The Technical Supervisor will ensure the day-to-day running and operation of the BSL3 facility. The Technical Supervisor will carry out periodic rounds of the BSL3 Facility (2-3 times daily), during which they will be responsible for replenishing stocks, disposing of waste containers, autoclaving material,

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checking installations and equipment, checking users' compliance with BSL3 facility rules, etc.. In addition, the Technical Supervisor will maintain the stocks of all disposable material provided by the BSL3 facility to the users and assist the Facility Supervisor and the Biosafety Officer in any matters related to improving and optimising work in the BSL3 Facility.

I.2. PRIVACY REMARKS

Access to the P3 facility requires users' biometric data (fingerprint).

The P3 Facility has a video surveillance system. Users must be aware that they are being monitored while inside.

I.3. RISK of INFECTION

Inside the BSL3 facility, there is a risk of infection by pathogens that are dangerous to humans (BSL3 agents, according to the CDC classification).

Infection may occur via the respiratory route, as well as by ingestion, skin/mucosal contact or accidental inoculation.

This risk is higher inside the procedure rooms, and higher still inside the Biosafety Cabinets (BSC).

II. GAINING ACCESS TO THE BSL3 FACILITY

II.1. GENERAL ACCESS RULES

- Access to the BSL-3 laboratories will be restricted to authorized and approved personnel. The Supervisor of the BSL-3 facility, has overall authority and responsibility for the laboratory.
- All individuals working in the laboratory must be fully trained and approved as capable of handling the pathogen(s) being studied. Individuals must be able to demonstrate proficiency in laboratory procedures prior to working with BSL3 agents. New personnel shall not perform any work in the BSL3 laboratory until they have completed training. Training shall include orientation and review of general safety practices, biosafety, BSL3 practices, emergency operations, waste management, medical surveillance, and site-specific SOP topics. In addition to the safety orientation, new personnel should familiarize themselves with the IMM BSL3 Manual.
- Individuals at an increased risk for acquiring infection must consult with the Facility Supervisor before working in the BSL3 laboratory area. Individuals at risk include pregnant women, individuals who are immunosuppressed or undergoing immunosuppressive therapy and individuals having recent surgical procedures or injuries in which the integrity of the skin has been compromised.
- Children under 18 years of age are not permitted in the laboratory area.
- Pregnant women are not allowed in the BSL3 Facility.
- Emergency crews and fire personnel may respond to emergencies in the area but may not enter unless the Technical or Facility Supervisor authorizes entry.

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II.2. RESEARCH PROJECTS

All research projects to be carried out at the BSL3 facility of the IMM require a written proposal to be submitted by the Principal Investigator responsible for the project (Form.P3F.001) and approved by the scientific committee. The proposal should describe all manipulations/operations involving BSL3 agents required by the project. No manipulations or operations involving BSL3 agents are allowed unless they are previously approved in the scope of a project proposal.

II.3. PRINCIPAL INVESTIGATORS

Principal investigators are responsible for the use of appropriate safety practices and procedures by their staff. It is the responsibility of each Principal Investigator to ensure that all individuals working under his or her direction are appropriately trained for work with pathogens.

II.4. USERS

Access to the BSL3 Facility requires previous training and authorization from the Facility Supervisor.

Research personnel must:

- Complete requirements for approval to work in the BSL-3 laboratory and ensure that all work is conducted in compliance with the BSL-3 Manual.
- Learn the operating procedures for the laboratory, the potential hazards of the infectious agents in use and emergency procedures.
- Assist in maintaining the BSL-3 laboratory in good working condition.
- Report to the Principal Investigator and BSL-3 Facility Director any medical restrictions, reportable illnesses, and any event that may cause a risk of exposure or result in the creation of a potential hazard.

In order to get permission to use the facilities, candidates need to:

1. Attend and complete the IMM BSL3 safety training module, or other training considered as equivalent by the Facility Supervisor
2. Be evaluated by a BSL3 user during a short trial period
3. Receive approval and biometric access to BSL3 facility

Following completion of the BSL3 safety training course, the user will be tested under mock BSL3 conditions and evaluated for knowledge of procedures and technique. A short evaluation period is also required before the user can work in the BSL3.

Adherence to safety practices is paramount to all BSL3 work. Researchers are not permitted to use the BSL3 facility unless accompanied by at least one other authorized user. Exemptions from this rule must be approved by the Facility Supervisor. At present, the only procedures that can be carried out by a single user are basic re-feeding and sample harvesting/extraction protocols in the non-aerosol

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room, in which case users must ensure that proper video monitoring of their activities is being carried out.

Aside from this exception, users wishing to enter the facility unaccompanied must notify the Facility Supervisor in advance and justify this request.

Access to the P3 facility may be canceled by the Facility Supervisor without prior warning in the event of non-compliance with the present rules and guidelines.

II.4.1. BSL3 safety training

The BSL3 safety training is required to work in the BSL3 facility. The applicant is expected to have thoroughly read this manual before training begins. Typically, two safety training courses will take place per year at the IMM.

BSL3 training will include a theoretical course during which all main aspects of working in a BSL3 facility will be presented and explained. Following this, an active BSL3 user (mentor) will be chosen by the Facility Supervisor to introduce the applicant to general BSL3 procedures and provide specific technical training based on the submitted protocols. All training must include identification and control of the hazards with which the person will be working. Furthermore, regardless of the techniques required for the proposed protocol, the training program must include performing mock procedures that cover the following topics:

- Correctly donning appropriate protective equipment.
- Adherence to general lab safety procedures, such as wearing double gloves and disinfecting gloved hands before removing them from the biosafety cabinet.
- Setting up, cleaning out, and properly using the biosafety cabinets.
- Bringing materials into and out of the cabinet.
- Culturing and manipulating airborne BSL3 pathogens safely, with emphasis on the importance of avoiding aerosol generation during all operations.
- Performing the essential procedures required of most protocols, such as centrifugation, plating, and incubating.
- Disposing of waste.
- Bringing materials out of the BSL3
- Following emergency procedures, especially what to do in the event of a spill. Particular emphasis must be placed on the reporting of spills and accidents, no matter how minor the incident may seem.

II.4.2. BSL3 safety testing

Following the training period, the applicant must demonstrate to the Facility Supervisor proper BSL3 safety practices. The test will require the applicant to demonstrate their ability to follow all the approved biosafety practices outlined in this manual. Typically, the applicant will have to complete a mock procedure that covers general aerosolisable pathogens culturing and manipulation. If the applicant fails to show competence in properly handling aerosolisable pathogens, more training will be

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required specifically focusing on problems that arise during testing and the applicant will have to be tested again by the Facility Supervisor.

II.4.3. Supervised trial period

Given the complexities and risks associated with BSL3 work, any new user must undergo a supervised trial period. For the first 2 sessions of work in the BSL3, an authorised BSL3 user (mentor) must be present to observe the user's technique and to help the new user if needed. The new user will be responsible for documenting the date, time and who supervised these initial sessions and for giving this information to the Facility Supervisor.

III. ENTERING and EXITING the BSL3 FACILITY

III.1. WORKING HOURS

Regular working hours are

- Monday to Wednesday - 8:00 to 17:00
- Thursday
 - Animal Room - 8:00 to 17:00
 - Aerosol Room - 8:00 to 17:00
 - Non-aerosol Room - 8:00 to 14:00 and 16:00 to 17:00
- Friday
 - Animal Room - 8:00 to 9:00 and 13:00 to 17:00
 - Aerosol Room - 8:00 to 14:00 and 16:00 to 17:00
 - Non-aerosol Room - 8:00 to 17:00

Bookings are made online using the IMM Booking System and require previous authorization from the Administrator.

Experiments that require the use of the BSL3 Facility outside regular working hours should be planned in advance and can only be carried out following authorization by the Technical or Facility Supervisor.

III.2. ENTERING THE BSL3 FACILITY

Entrance to the BSL3 facility requires authorization and granting of biometric access by the Facility Supervisor. Passage between two consecutive rooms is always done through an interlocked door system (SAS). This means that when entering or exiting the BSL3 facility, only one door of the air lock may be opened at a time.

III.2.1. Before Entering

Before opening the main door to the BSL3 facility, please:

- Check the pressures in the various compartments of the BSL3 Facility. These should be approximately +40 Pa in the first SAS, -40 Pa in the common corridor area and -60 Pa in the

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experimental handling rooms. If this is not the case, or if any of the alarms are signaling, DO NOT ENTER and contact the Technical Supervisor immediately.

- Ensure you have everything you need to take inside. Each entry is costly, time-consuming and constitutes a potential contamination hazard, so avoid unnecessary visits to the BSL3 facility.

III.2.2. Entering the Locker Room. Donning Personal Protection Equipment (PPE)

Enter the main door to the BSL3 facility, cross the first SAS and enter the appropriate vestibule. Leave your coat, heavy clothing, jewellery and other belongings, if any. PLEASE NOTE THAT THE USE OF MOBILE PHONES OR MP3 PLAYERS INSIDE THE FACILITY IS STRICTLY FORBIDDEN.

Designated personal protective equipment (PPE) must be worn by every person entering the BSL3. All PPE will be provided and will be stocked in the entry vestibule. Change into all PPE in the vestibule before entering the BSL3 facility. Required PPE includes:

- Face mask (FFP3)
- Gloves (nitrile and latex)
- Shoe covers
- Plastic ankle covers
- Autoclavable overalls
- Disposable apron
- Safety glasses

Correct donning of PPE is as follows:

- 1- Put on face mask
- 2- Put on one pair of shoe covers
- 3- Put on and zip up the overall
- 4- Put on plastic ankle covers and tape them to the overall
- 5- Put on second pair of shoe covers
- 6- Put on inner gloves (nitrile) and tape them over the cuffs of the overall
- 7- Put on the second pair of gloves (latex) over the first
- 8- Put on apron over the overall

Once you have double-checked that all PPE is correctly worn, pass the SAS into the BSL3 common corridor. MAKE SURE YOU'RE NOT CARRYING YOUR MOBILE PHONE OR MP3 PLAYER INSIDE.

Safety glasses are stored inside the experimental rooms. Wipe them with disinfectant BEFORE AND AFTER use.

III.2.3. Entering the Experimental Handling Rooms

There are three experimental handling rooms in the BSL3 facility: Non-aerosol room, Aerosol room and Animal room (see Appendix 1). Before entering the room where you will carry out your work, please

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- Check that the pressure inside the room is approximately -60 Pa. If this is not the case, or if any of the alarms are signaling, DO NOT ENTER and contact the Technical Supervisor immediately
- Check that there is no spillage or other warning signs on the door or window
- Check that the number of persons allowed in the room at one time (3 persons) will not be exceeded by you
- Collect and put on safety glasses or face shield

If you need to retrieve any samples from the -80 freezer, collect them before entering the room. A small trolley is available near the -80 freezer for your convenience. Samples must be carried from the -80 freezer to the room inside closed a secondary container (provided). Enter the room, verify that the door is closed behind you, and follow the room procedures. The containers can only be open inside the biosafety cabinet (BSC).

III.2.4. Moving from one Experimental Handling Room to Another

If you need to carry out work in more than one experimental handling room, make sure you observed all the room procedures regarding trash disposal (see relevant sections) before exiting the first room. If you are carrying samples, make sure you have observed the respective guidelines (see relevant sections).

- Before you exit the first experimental handling room, remove the apron and the outer pair of gloves and dispose of them in the appropriate bin
- Before exiting, put on a new pair of outer gloves
- Open the door and step into the common corridor
- Follow the procedure for “entering experimental handling rooms” (see previous section)
- Upon entering the second room, pick a clean apron and put it on

III.3. EXITING THE BSL3 FACILITY

Before exiting the experimental handling room, make sure you observed all the room procedures regarding trash disposal (section 3.IV.2.6). If you are carrying samples, make sure you have observed the respective guidelines (section 3.IV.2.2). Only then will you be ready to leave the BSL3 Facility.

- Wipe the outer pair of gloves with disinfectant after finishing the cleaning and waste disposal procedures
- Remove and wipe the safety glasses or the face shield with disinfectant and place them in the appropriate place
- Before you exit the experimental handling room, remove the apron and dispose of it in the appropriate bin
- Open the door and step into the common corridor
- Open the door to the Exit SAS. Remove outer pair of shoe covers, one foot at a time, as you enter the Exit SAS holding both shoe covers tightly in one hand
- Close the Exit SAS door behind you

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- Dispose of outer shoe covers in the appropriate bin
- Remove ankle tape and plastic protectors and hold them tightly in one hand
- Remove outer pair of gloves
 - using your free hand, remove the outer glove of the hand holding the plastic ankle covers in such a way that inside of the glove will be facing out and the plastic ankle covers will be enclosed in the glove.
 - move the bundle to your other hand and proceed as above to remove the second outer glove.
- Dispose of the bundle (two plastic ankle covers and two gloves) in the appropriate bin before proceeding to the next step
- Remove inner pair of shoe covers and cuff tapes
- Dispose of inner shoe covers and cuff tapes in the appropriate bin
- Remove your overall so that it comes off inside out and dispose it in the dedicated container
- Remove inner pair of gloves
- Dispose inner pair of gloves in the appropriate bin before proceeding to the next step
- Hold your breath, remove face mask and dispose of it in the appropriate bin
- Disinfect your hands with the solution provided using your elbow to dispense the solution
- Step into the shower stand
- Exit the SAS into the locker rooms
- Wash your hands, collect any personal belongings and leave the facility

IV. WORKING in the BSL3 FACILITY

IV.1. GENERAL RULES

The following rules apply to ANY work carried out in the BSL3 laboratory and must be adhered to at all times. Failure to do so may result in banning from use of the BSL3 facility.

IV.1.1. Restrictions

- Children under 18 years old and pregnant women are not allowed in the BSL3 facility.
- Use of chemical compounds and solvents must be specifically approved by the Facility Supervisor and the Scientific Committee.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption inside the BSL3 facilities is strictly forbidden.
- Mobile phones, electronic diaries, personal music players and other personal electronic devices, which will not remain in the BSL3 facility, are not allowed in the facility.
- Safety glasses (provided must be worn inside the BSL3 facility.
- Any skin cuts or abrasions on the hands, face, arms or other exposed areas of skin must be covered with a waterproof bandage or Band-Aid before entering the BSL3 facility.
- Only close-toe shoes can be work in the facility. No sandals or open-toe shoes are allowed.

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- No high-heel shoes are allowed in the facility, as they may pierce the safety shoes.
- Shorts and skirts cannot be worn in the facility.
- Long hair and/or bangles must be tied back.
- Once stationery (paper, pens, books, etc.), has entered the Facility, it must not be taken out. Minimize transit of written documents. Use the computers instead. Pens and work papers must not be used inside the Biosafety Cabinets.
- Jewelry cannot be worn inside the BSL3 facility. Rings must be removed as they may pierce the safety gloves.
- No more than three people are allowed inside any of the experimental handling rooms at any given time.
- Only one person may work in a cabinet at a time.
- Mouth pipetting is strictly prohibited; mechanical pipetting devices must be used.
- No glassware or sharps (including glass pipettes and needles) are allowed in the Facility (except for animal experimental handling room, see relevant section). Specific exemptions may be obtained from the safety committee for the use of glass chamber slides etc. where a suitable plastic alternative is not available.
- Styrofoam eskies must NOT be taken into the BSL3 Facility.

IV.1.2. Rules of Conduct

- All work in the facility must follow BSL3 procedures.
-
- Laboratory doors must be closed.
- All procedures involving the manipulation of infectious material are conducted within class II biosafety cabinets (BSC) or other physical containment devices.
- Appropriate personal protective equipment must be worn at all times. Two pairs of gloves must be worn at all times. Gloves must always be changed when contaminated and when integrity has been compromised.
- While working, do not touch your face, eyes, mouth or other exposed body parts. Never put any materials in your mouth. Once you are in the BSL3 Facility, the outer gloves should be considered to be "contaminated". If absolutely necessary, remove the outer glove first before touching mask, glasses, hair or any exposed skin and immediately put on a new outer glove.
- All aerosol-producing equipment, such as that used for vortexing, must be kept in the biosafety cabinet. All centrifugation must be performed with adapters designed for containment purposes. See centrifugation procedures. Aerosol resistant centrifuge canisters / buckets must only be opened in a biosafety cabinet (BSC).
- No work with open vessels containing BSL3 agents is allowed on the bench. When such a procedure cannot be performed within a BSC, please notify the Facility Supervisor in advance.

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- All BSCs must be maintained in a clean and orderly manner. Upon completion of a work session, the individual using the BSC is responsible for cleaning and decontaminating the inside surfaces.
- All benchtop and BSC surfaces must be decontaminated upon completion of each work session.
- Organise your work time to avoid rushing.
- Keep amount of work to be done realistic within the time frame. As a rule of thumb, you should double the amount of time you would normally require to carry out the same procedure(s) in BSL2 conditions.
- Negative pressure at -60 Pa could be unpleasant and some people may feel dizzy. Avoid long work sessions in the handling room. If you feel uncomfortable in the handling room please notify the Facility Supervisor or the Technical Supervisor immediately.

IV.2. TRANSFER OF MATERIALS

IV.2.1. Entry of Biological Materials

- Biological materials potentially containing BSL3 agents that must enter and be transported across the BSL3 Facility areas in a viable or intact state will be in sealed leakproof primary containers inside secondary, leakproof containers (double containment rule), through the Pass Cabinet.
- Packages containing viable agents may only be opened inside containment, and within the confines of a biological safety cabinet (BSC).

IV.2.2. Removal of Biological Materials

- Any biological material transported out of the BSL3 Facility must be inactivated and must be removed through the Pass Cabinet.
- Viable materials **MUST NOT** be removed from the BSL3 facility, except with specific permission of the scientific committee and the Facility Supervisor.
- Method of inactivation must be proved appropriate for agent and sample, requiring prior approval by the Facility Supervisor and the Scientific Committee.
- Any non-viable materials to be transported from the BSL3 facility require previous authorization from the Facility Supervisor and the Scientific Committee.
- Inactivated samples for lab processing must be bagged and double contained.
- Spray the primary container thoroughly with an appropriate disinfectant and place inside a sealable bag.
- Spray and disinfect the bag and place in leak-proof secondary container (brought by the user).
- Spray secondary container with an appropriate disinfectant and 70% ethanol and bring to the Pass Cabinet.

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- Open Pass Cabinet, place box inside, and spray the box and the Cabinet walls with disinfectant.
- Either arrange previously with the Technical Supervisor to have the material collected from the wash room side of the Pass Cabinet or collect it yourself after exiting the Facility following the appropriate exit procedures. In any case, allow at least 10 minutes before collecting the material.

IV.2.3. Carrying Ice Into the Facility

There are strict regulations concerning carrying ice into the facility. Please note them carefully.

- Ice must be contained inside sealed plastic bags transported into the facility inside appropriate containers (provided).
- Alternatively, freeze pads can be used. Please note that they must also be transported into the facility inside appropriate containers and that you will not be allowed to take them with you when you leave.
- You must bring the amount of ice you expect to need for your procedure already in (a) sealed plastic bag(s), and place it/them inside the appropriate container once you enter the locker room. The same rules apply for the freeze pads.
- Once you finish work, the ice bags or freeze pads must be treated and disposed of as solid waste (see relevant section below).
- In the event that you will be carrying out a long procedure during which you expect to need a significant amount of ice, and to avoid having to leave and re-enter the facility, you can bring the amount of bagged ice (or freeze pads) you expect to need and store it in the -20 freezer in the room. Alternatively, you can contact the technical supervisor in advance and arrange to have ice brought to you either during one of the technical supervision rounds or through the Pass Cabinet.

IV.2.4. Bringing Printed Protocols In / Taking Data Out

Transit of printed and stationery material in the Facility must be minimized and is tightly regulated. Once such material has been taken inside, it cannot be removed from the Facility either than through the autoclave. Likewise, no written notes or data produced inside can be removed from the Facility. The following rules must therefore be observed at all times:

Bringing Printed Protocols In

- Printed protocols must be taken inside enclosed in a plastic sheet protector, which must be SEALED with sello tape BEFORE entering the Facility.
- During work in the biosafety cabinet, protocols can be fixed with tape to the OUTSIDE of the cabinet. NEVER PLACE THEM INSIDE THE CABINET OR ON THE GRILL AT THE FRONT OF THE CABINET.

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- If you need to turn, change or otherwise touch the protocol during work **BE SURE TO DECONTAMINATE GLOVES FIRST.**
- Once work is completed, the protocol's plastic sheet protector must be surface decontaminated with disinfectant and the encased protocol stored in the appropriate folder in the room (provided). This should be done **BEFORE** exporting your data (see below).
- If you will be using (a) protocol(s) that is(are) already in the folder, **DO NOT** bring an additional one inside. Instead, collect the protocol(s) you need from the folder **BEFORE** you start working.
- If a protocol becomes obsolete and is to be replaced by an updated one, surface decontaminate the old plastic sheet protected protocol and discard as solid waste.

Taking Data Out

- As much as possible, avoid taking written notes during work.
- When absolutely necessary, use the pen and paper provided to take notes.
- **NEVER PLACE THE PEN OR PAPER INSIDE THE CABINET OR ON THE GRILL AT THE FRONT OF THE CABINET.**
- Once work is completed, enclose the written notes in a sealable bag and surface decontaminate it with disinfectant.
- Written data cannot be removed from the Facility. Use the computer to e-mail your data or save it on a network disk.
- Once you have finished using the computer to export your data, discard the encased notes as solid waste.
- Using the computer and discarding the notes are be the **LAST** things you should do before exiting the room following the exit procedures (see relevant section).

IV.2.5. Maintenance, Repair and Removal of Equipment

- Maintenance and repair work will be carried out inside or outside the BSL3 Facility depending on a size of the equipment, contamination on surface or internal components, ability to decontaminate (large) equipment, ability to remove (large) equipment and ability to bring tool and specialty equipment into the Facility.
- Equipment maintenance or removal must be supervised by the Facility Supervisor and/or the Technical Supervisor.
- All equipment must be decontaminated inside and out prior to removal from the BSL3 facility (including for repair or maintenance). Always contact the Facility or Technical Supervisor for these ends. Depending on the equipment, decontamination may be achieved by the use of an appropriate disinfectant. Biohazard cabinets and incubators must be fumigated before removal.
- All non-hazardous materials leaving the BSL3 facilities must be decontaminated.

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- Equipment that is to be serviced in-house should be decontaminated with an appropriate disinfectant and service representatives apprised of any potential hazards that may exist.
- It is the responsibility of the Technical or Facility Supervisor to advise non-IMM personnel of the hazards and safety issues applicable to them while working in the facility.
- Maintenance/repair personnel will be specifically trained for work inside the Facility.
- Whenever possible, maintenance or repair work in the BSL3 Facility will be carried out first thing in the morning, before any users have used the facility on that day.
- No research work is allowed in the Facility while maintenance or repair is being carried out in any area of the Facility, unless specifically authorized by the Facility or Technical Supervisor.
- Personnel who will be working in the BSL3 laboratory area must be accompanied while working in the BSL-3 laboratory area and a 'decontaminated' area will be provided for their instruments.
- Members of the BSL3 Facility staff will always enter the facility AHEAD of any maintenance or repair workers, wearing similar PPE.
- When possible, a set of dedicated tools for maintenance/repair work will be kept inside the BSL3 Facility.
- When possible, tools shall be wiped with an appropriate disinfectant before removal from the area.
- Decontamination of highly technical or sensitive equipment or equipment with limited access to contaminated areas may not be possible. The equipment will be decontaminated to the degree possible.
- In particular instances, maintenance or repair tools or equipment will be fumigated prior to being returned to maintenance or repair staff.
- Affix a tag to the equipment indicating when the equipment was decontaminated, what disinfectant was used, and the name of the person who performed the decontamination. The label must indicate what portions of the equipment remain potentially contaminated and include the biohazard symbol as well as the term "biohazard".

IV.2.6. Periodic Cleaning/Maintenance, Waste Removal, and Autoclaving

No waste can leave the BSL3 facility without being autoclaved. Please follow the instructions on waste removal in the relevant sections. Please remember that:

- Users are responsible for strictly following rules about cleaning BSCs as well as all surfaces and equipment used in the experimental rooms.
- All waste must be double bagged, marked with autoclave tape, and placed in covered plastic bins for autoclaving.
- Each worker is responsible for correctly bagging their own waste.
- Autoclaving of waste and PPE will be carried out regularly by the Technical Supervisor. Unless otherwise instructed, users are not to use the autoclave.
- Equipment in the Facility will undergo periodic maintenance, as detailed in Form.P3F.002.

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- Regular working/maintenance procedures will be carried out by the Technical Supervisor with the periodicity detailed in Form.P3F.008.

IV.3. BIOSAFETY CABINET (BSC) PROTOCOL

All procedures involving the manipulation of infectious materials must be conducted within Biosafety Cabinets (BSCs). The following rules constitute General Procedures that apply to work carried out in the BSC in ANY of the experimental handling rooms. BSCs are to be left on all day and will be switched off by the Technical Supervisor at the end of the day.

IV.3.1. Preliminary checks

Before you start work, please

- Check that the alarm in the room is not signaling
- Check that there are enough autoclave boxes, autoclave bags and waste containers
- Check that fresh disinfectant solution is prepared
- Check that there is enough disinfectant solution inside spray bottles

IV.3.2. Setting-up the BSC

To set up the cabinet for work, please

- Adjust the height of your chair so that your armpits are approximately level with the bottom of the front sliding sash
- Disinfect cabinet surfaces
- Micropipettes and tube holders are already inside the BSC. Disinfect them before starting work.
- Place a fresh benchcoat sheet inside the cabinet (absorbent side up); make sure that the grid at the front is not covered.
- Spray all other the materials with disinfectant and 70% Ethanol before placing them inside the cabinet.
- Place a squirt bottle also containing fresh disinfectant (for small/surface decontamination) inside cabinet.
- Place pipette boat containing fresh disinfectant for tip and pipette disposal inside cabinet.
- Put a pot 1/3 full with disinfectant for disposal of liquid waste inside the cabinet.
- Put a plastic bag held in tripod inside the cabinet for the disposal of solid waste.
- Place materials and equipment that will be used including pipettors, pipettes, pipette tips, sterile culture flasks, centrifugation tubes, media, etc. inside the BSC.
- After cabinet set up, work is performed following protocol and guidelines previously approved by the Facility Supervisor and the Scientific Committee.

IV.3.3. Working in the BSC

During work, please:

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- Be organised, practical and work safely at all times.
- Never work with the UV lights on.
- Work centrally in the cabinet well back from the grill.
- No items should be resting on any part of the cabinet grill as this will affect air flow and therefore the functioning of the air curtain.
- Do not store excess materials in the hood as this will affect air flow.
- Avoid a cluttered work area in the cabinet- it will minimise accidents and contaminations.
- Always move your arms slowly and avoid swaying or sudden swift movements. Do not cross your arms while working.
- Always walk slowly in front of a BSC to avoid disturbing the airflow.
- Avoid creating aerosols.
- Always change or decontaminate gloves (spray them with disinfectant) when taking hands out the BSC.
- Never touch equipment with potentially contaminated gloves.
- Never reattach apron, scratch nose/eyes, get hair out of face with gloves on.
- Never overfill waste containers.
- TAKE PARTICULAR CARE when opening cryotubes. Preferably centrifuge cryotubes before opening to remove supernatant from around the lid. Always open cryotubes slowly. Be aware that supernatant may leak or spurt out when opened. If outer gloves become contaminated in this way, discard them into the bin within the hood and apply fresh outer gloves.

IV.3.4. Clearing the BSC after work

When work has been completed, disinfect the exterior surfaces of potentially contaminated materials and supplies with an appropriate disinfectant before removing them from the BSC. Objects are not to be removed from the BSC if there are open containers with pathogen. Objects to be removed will be wiped thoroughly with disinfectant and can be carried directly out, if gloves have been wiped with disinfectant.

- Allow the cabinet to run for 5 minutes with no activity
- Close or cover open containers before removing them from the cabinet
- Always decontaminate all surfaces and material coming out of the BSC
- All biological or non-biological materials that need to be incubated should be wiped down.
- Ensure that there is enough disinfectant to cover all the materials in the pipette boat. Then put lid on pipette boat, surface decontaminate it and leave it inside the BSC
- Discard liquids into pot with disinfectant (do not fill above 2/3 of capacity, if needed get another pot with disinfectant). Please beware that this procedure is prone to generating aerosols and spills. Exert extreme caution in discarding contaminated liquids
- Close the liquid waste pot with the lid, spray and wipe THOROUGHLY with disinfectant, remove it from cabinet and place it inside an autoclave box

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- Put all solid waste material inside solid waste bag
- Paper material, including benchcoat, pipette wrappers and paper towels must be sprayed with disinfectant and treated as all other solid waste
- Disinfect the interior BSC surfaces, including the inside of the view screen, with disinfectant followed by 70% alcohol. Discard waste papers into solid waste bag. NEVER PLACE YOUR HEAD INSIDE THE BSC
- Close solid waste bag with a rubber band, spray it with disinfectant and place inside a second autoclave bag
- Remove outer pair of gloves them in the second autoclave bag with the waste bag
- Don a new pair of gloves
- Close second autoclave bag with rubber band, spray it with disinfectant, remove it from cabinet and place it inside an autoclave box
- Decontaminate plastic protocol protector and return protocol to the folder
- Use the computer to export data
- Follow appropriate instructions for either moving to a different room in the Facility or for exiting the Facility

IV.4. PROCEDURES FOR CENTRIFUGATION

IV.4.1. General centrifugation procedures

The following procedures must be followed whenever working with a centrifuge in the BSL3 facility:

- In the BSL3 laboratory all centrifugation steps are performed with sealed rotors or buckets. The rotors and buckets are designed to contain any aerosols that may be generated during centrifugation. Buckets must only be opened in a biosafety cabinet. O-rings must be inspected regularly for integrity and coated with vacuum grease.
- Examine tubes and bottles for cracks or stress marks before using them. Cracked or damaged aerosol resistant canisters MUST NOT be used as they can fail during centrifugation.
- Fill and decant all centrifuge tubes and bottles inside the biological safety cabinet. Wipe outside of tubes with disinfectant before placing in aerosol containers or rotors.
- Do not over or underfill tubes or bottles and be aware of splash levels. Leakage may occur if tubes collapse due to underfilling or when overfilled. The maximum fill level for centrifuge bottles is 2/3 full unless otherwise specified by the manufacturer.
- Centrifuge canisters must be balanced. Use balance tubes as required. When placing tubes inside ENSURE that the caps are on firmly. Take buckets to the scales inside secondary containment.
- Use biosafety cabinets to load and unload centrifuge safety buckets. When using the microcentrifuge tubes must be placed on a tube holder inside the BSC and disinfected before being taken to the centrifuge. After centrifugation, tubes must be carried to the BSC in a tube holder and only opened inside the biosafety cabinet. The O-rings should regularly inspected and coated with vacuum grease.

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- When removing canisters from the cabinet, decontaminate by spraying with disinfectant and wiping dry before placing canisters in the centrifuge.
- The centrifuge must be monitored until it reaches the selected speed.
- Thoroughly inspect the buckets at the completion of work for any spills. If any liquid is present, decontaminate the buckets. Plastic canisters should be decontaminated in freshly prepared disinfectant solution. Metal centrifuge buckets and microcentrifuge rotors should be decontaminated and dried thoroughly.
- Centrifuge buckets should be regularly decontaminated. Buckets and inserts should be thoroughly rinsed and dried before reassembling. O-rings should be inspected for integrity and coated with vacuum grease.

IV.4.2. Ultracentrifugation procedures

In addition to the procedures above, please note the following rules for using the ultracentrifuge:

- Use of the ultracentrifuge requires specific training. Please contact Technical Supervisor in advance if you plan to use the ultracentrifuge.
- All use of the ultracentrifuge must be logged in the appropriate file ("logbook.pdf"), available on the desktop of the computer in the non-aerosol room.
- Should any spill occur inside the ultracentrifuge, the lid must be immediately closed and must be kept closed for at least 12 hours.
- A spill in the ultracentrifuge requires fumigation of the whole room (to be carried out by the Facility Staff).

IV.5. INCUBATORS, REFRIGERATORS AND FREEZERS (-20 AND -80)

- ANY material in incubators or stored in the refrigerators or freezers MUST be properly labeled. Proper labeling includes a description of the contents of the vial/flask/plate/etc., the name, extension and unit of the user, and the name of the pathogen (if any).
- Absent or incorrect labeling of ANY ITEM in the incubators, refrigerators or freezers MAY RESULT IN THE ITEM BEING DISCARDED WITHOUT PRIOR WARNING.
- All items must be surface decontaminated before being placed in incubators, refrigerators or freezers
- ALL ITEMS in incubators MUST be placed in a secondary container (box or tray), which must be surface decontaminated before being placed in the incubator.
- Materials are to be transferred between the incubator and the BSC on plastic trays.
- A small trolley is available near the -80 freezer for your convenience.
- Storage of samples in the -80 freezer must follow the rules above. In addition:
 - Samples can only be transported to and from the -80 freezer inside secondary containers with lids (provided).
 - Users must respect the space allocations to their respective units.

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- Vials containing concentrated stocks of pathogen MUST be stored inside a secondary container (for example, a Falcon tube).
- The contents of the -80 freezer must be recorded in the appropriate form (Form.P3F.009).

IV.6. THE AEROSOL EXPERIMENTATION HANDLING ROOM

Work with airborne organisms must be carried out in the aerosol experimentation handling room. *Mycobacterium tuberculosis* is an airborne pathogen. In a laboratory setting, mechanical aerosol generation can lead to the release of particles which can cause infection if inhaled. The following rules, **in addition to those defined in Sections 3.IV.1 to 3.IV.5 of this Manual**, apply to work carried out inside the aerosol experimental handling room. The most important aspects of working with equipment in the BSL3 are to maintain secondary containment of pathogens and avoid generating aerosols.

IV.6.1. General rules for working with airborne BSL3 pathogens in aerosol experimental handling room

- Direct manipulation of *M. tuberculosis* is only allowed inside a prepared Biosafety Cabinet (BSC).
- Once sealed containers of *M. tuberculosis* have been placed inside the BSC nothing is to be removed without being wiped with disinfectant.
- The outer layer of gloves should be discarded, and a new pair donned, whenever there is the possibility of contamination with *M. tuberculosis*.
- All disposable materials that come into contact with *M. tuberculosis* are decontaminated with disinfectant before being placed into the biohazard bag. Liquid waste is to be disinfected by addition of equal volumes of disinfectant in tightly sealed containers (leaving 1/3 of the pot empty) and placed into the biohazard bag after thorough surface wiping with disinfectant.
- Objects are not to be removed from the BSC if there are open containers of *M. tuberculosis*. Objects to be removed will be wiped thoroughly with disinfectant and can be carried directly out, if gloves have been wiped with disinfectant.

IV.6.2. Pipetting

Pipetting can generate aerosols. All pipetting must be done inside the BSC.

- Only pipettes and pipette tips with aerosol barriers are allowed in the BSL3.
- Aerosols are minimized by avoiding blowing out the contents of a pipette completely and by preventing the formation of bubbles.
- To avoid aerosol generation, whenever pipetting TB into a container, pipette onto the side wall of the container, and be careful not to eject any air after all of the liquid has been ejected. Similarly, avoid accidental intake of air when drawing liquid into a pipette or pipette tip.

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- After pipetting, rinse the inside and outside of the pipette by drawing disinfectant into the pipette and then allowing it to drain back into the pot of disinfectant. After the pipette has been rinsed, it can be disposed of in the pipette boat.
- For micropipette tips, following ejection of liquid, do not release the micropipette piston allowing air to be drawn into the pipette tip. Instead, holding the pipetman depressed, immerse the pipette tip in disinfectant and draw disinfectant into the tip. The tip can then be disposed of into the pipette boat.

IV.6.3. Vortexing

Vortexing generates aerosols. All vortexing must be done inside the BSC and should be avoided if possible.

- The Vortexer is kept in a sealable container. Do not open this container outside the BSC.
- Turn on the BSC electrical outlets (on the control panel on the front of the BSC) and bring Vortexer container into the BSC. Take Vortexer out of container and plug into BSC outlets
- Check Vortexer settings – vortex at the lowest setting possible.
- Only use Screw-cap tubes for vortexing. Wipe surface of sealed tube with disinfectant
- Vortex for the minimal amount of time possible.
- Centrifuge tube briefly after vortexing or mixing by inversion.
- When complete, wipe Vortexer with disinfectant and place Vortexer back into the container.
- Seal container and disinfect thoroughly before taking it out of the BSC.

IV.6.4. Centrifuging

- All centrifugation shall be done using aerosol-containing centrifuge adapter buckets or sealed centrifuge tubes in sealed rotors.
- Rotors must be loaded and unloaded inside the BSC.
- If a small centrifuge is used and aerosol-containing rotors are not available, centrifugation shall be performed in the biological safety cabinet.
- Wait at least 15 minutes before opening any tube following centrifugation of aerosolisable material.

IV.7. THE ANIMAL EXPERIMENTAL HANDLING ROOM (ABSL3)

Work with animal models must be carried out in the animal experimental handling room. The following rules, **in addition to those defined in Sections 3.IV.1 to 3.IV.5 of this Manual**, apply to work carried out inside the animal experimental handling room (ABSL3). Previous experience with animal work is required. Please note that infection may occur following contact with inoculates, microbial cultures and/or tissues, body fluids, or secretions from infected animals through the following routes:

- Inhalation of infected aerosols
- Bite and/or scratch wounds

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- Oral route
- Skin/mucosal contact
- Accidental inoculation

Please note: **do not prepare inoculates in this room! Inoculates must be prepared in the appropriate BSL3 cell rooms (Aerosol or Non-Aerosol).**

IV.7.1. Sharps handling

- Restrict the use of hypodermic needles and syringes to injection or aspiration of fluids from laboratory animals.
- Needles must NEVER BE RECAPPED.
- Needles must not be bent, sheared, broken, removed from disposable syringes, or otherwise manipulated by hand before disposal.
- Used disposable sharps must be carefully disposed in appropriated sharps containers.
- Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination.

IV.7.2. Rules for Working with Animals

A. General procedures

- Prepare everything that is needed to work with the animals before retrieving them.
- Make sure you have enough room inside the BSC to accommodate the cage(s) and the equipments, AND handle the animals safely. Do not overestimate the space inside. Remember that you must work well inside the chamber, and not at its edge!
- If you need an empty cage to transfer the animals, take one from the shelves. Always use complete cages – never just the bottoms!
- After having sprayed the benchcoat inside the BSC with disinfectant, retrieve the cage(s) containing your animals from the IVC rack and place it (them) inside.
- Spray the outside of the cage, open it, and securely place the lid in a such a way as to ensure that its inner surface does not touch anything.
- Perform the tasks you need, being extra-careful not to be bitten and/or scratched by the animals. For these tasks, please refer to procedures in sections **B** and **C**, below.
- When you are done working with the animals of the first cage, make sure they are all inside, dispose of your outer gloves into the BSC trash, put on a new pair of gloves, put the cage lid in place without touching its inside surfaces, close it tight, and spray the outside of this cage.
- **Important Note: Never close cages or take them out of the BSC using gloves that have touched the animals or potentially infected materials!**
- If you have more cages inside the BSC, move on to the next cage, carrying out the same procedure.

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- After closing and spraying the last cage, spray your gloves with disinfectant and retrieve the cages to the IVC racks.
- Return to the BSC and take care of whatever is left inside.
- Finish according to section **3.IV.3.4** (“*Clearing the BSC after work*”)

B. Substance administration and /or in vivo sample collection

Important Note: Be extra-careful when handling used sharps (needles, scalpel blades, pins, etc.) and never recap used syringes. See section 3.IV.6.1 for further details on handling of sharps.

- Follow the procedures described in SOP.RDF.007 – Rodent Procedures
- **Important Note: be extra-careful not to be bitten and/or scratched by the animals.**
- Treat all sharps as infected after using them on the animals.
- Do not use needles, blades and other perforating materials across cages.
- When inoculating potentially infected products, take all precautions to avoid/minimize aerosolization. Keep infected inoculates contained (e.g.vials closed) as much as possible.
- When collecting potentially infected samples, contain them properly.
- **Important Note: Samples processed in the Animal Room can only be transferred to the appropriate experimental handling room (aerosol or non-aerosol) in double containment (see below).**
- **Important Note: Remember that infected samples –even if contained- cannot leave the BSL3 Facility without being inactivated!**

C. Euthanasia

- Wear safety goggles.
- Before starting, place a zip-lock plastic bag inside the BSC, wide open in a way you are able to dispose the dead animals inside without touching the outer surface of the bag.
- Follow the procedures described in SOP.RDF.007 – Rodent procedures.
- Dispose of the dead animals inside the zip-lock bag.
- Change your outer gloves, close the zip-lock bag without touching its inside, spray it with disinfectant and set aside.
- Move on to section **D**, below (“*Necropsy/organ collection/ex-vivo sample collection*”) or finish according to section **3.IV.3.4** (“*Clearing the BSC after work*”)
- When clearing-off cabinet, put the zip-lock bag with the dead animals inside an autoclavable bag, close it with a rubber band, and spray with disinfectant and store inside the dedicated freezer drawer.

D. Necropsy/organ collection/ex-vivo sample collection

Important Note: Be extra-careful when handling used sharps (needles, scalpel blades, pins, etc.). See section 3. IV.6.1 for further details on handling of sharps.

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- Wear safety goggles.
- Do not perform necropsy or organ collection while cages or no longer required equipment are still inside the BSC.
- Avoid using boards and pins as much as possible. If you must use them, dispose of them as infected materials.
- Take all precautions to avoid/minimize blood spillage, fluid splashes, and aerosolization.
- Avoid working directly with your hands: use the appropriate tools instead, dipping them frequently in disinfectant.
- When collecting potentially infected organs/samples, contain them properly.
- Organ homogenizations must be carried out inside the BSC, using the available Ultra-Turrax equipment. When homogenizing organs in the Ultra-Turrax, the provided protection case MUST placed above the equipment.
- If you are processing more than one animal, prepare a zip-lock bag to dispose the carcasses, according to section **C**, above ("*Euthanasia*").
- Open the zip-lock bag containing the dead animal(s).
- Position and secure the animals in a way that is convenient.
- Perform the necropsy and/or collect the organs according the respective procedures.
- If collecting organs or samples, place them inside the appropriate containers, always avoiding touching the outer surfaces.
- When finished, place unwanted organs, tissues and carcasses inside the zip-lock plastic bag without touching the outer surface.
- Change your outer gloves and close the sample containers and the zip-lock bag without touching the inside. Spray gloves, zip-lock bag, and sample containers with disinfectant and set aside.
- Finish according to section **3.IV.3.4** ("*Clearing the BSC after work*")
- When clearing-off cabinet, put the zip-lock bag with the dead animals inside an autoclavable bag, close it with a rubber band, spray with disinfectant, and store inside the dedicated freezer drawer.
- Animal carcasses will be autoclaved prior to disposal and will not be kept for longer than 48 hours in the -20 freezer.
- **Important Note: Samples processed in the Animal Room can only be transferred to the appropriate experimental handling room (aerosol or non-aerosol) in double containment (see below).**
- **Important Note: Remember that infected samples –even if contained- cannot leave the BSL3 Facility without being inactivated!**

IV.8. TRANSPORTING MATERIAL BETWEEN ABSL3 AND BSL3 ROOMS

(Please see section "Moving from one Experimental Handling Room to Another" for details on procedures for discarding and donning PPE when using multiple rooms in the Facility).

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Samples from the ABSL3 room (liquid, homogenates or solid) must be further processed in the aerosol or non-aerosol BSL3 room, as appropriate. Inoculates must be prepared in the aerosol or non-aerosol BSL3 room, as appropriate, before being taken to the ABSL3 room. Transport of samples between rooms in the Facility must adhere to the following rules:

- Liquids and homogenates must be contained in leak/spill proof primary containers. Solids must be contained in closed primary containers.
- Primary containers must be surface decontaminated by wiping with disinfectant.
- Primary containers must be placed inside a zip-lock bag, which must be surface decontaminated by wiping or spraying with disinfectant.
- Zip-lock bags must be carried inside a spill proof container with lid (provided).
- If samples need to be refrigerated, ice in zip-lock bags or freeze pads (see section “**Carrying Ice Into the Facility**”) must be surface decontaminated and placed inside the spill proof container with lid.
- Containers should never be left unattended.
- Please observe the relevant rules for discarding and donning PPE when moving from one room to the next.
- Once in the destination room, prepare BSC for work (see “**Preliminary checks**” and “**Setting-up the BSC**” sections inside the “**Biosafety Cabinet (BSC) Protocol**” section) before opening the spill proof container with lid.
- Remove bagged samples and ice bags/freeze pads from spill proof container with lid and place them immediately in the BSC to continue work.

V. SPILLS, INCIDENTS AND ACCIDENTS; EMERGENCY PROCEDURES

Microbiological incidents include spills and leaks of infectious material and accidents with infectious agents, whether or not personal injury or contamination has occurred. ASSUME THAT ALL SPILLS ARE BIOHAZARDOUS.

In considering the response to **any** incident:

- **The FIRST PRIORITY** is the safety of the people involved. Your immediate task is to ensure that appropriate action is taken to attend to any serious injuries or life-threatening situations, and to ensure that yourself and others are not exposed to liquid or aerosols from the leaked material.
- **The SECOND PRIORITY** is to take whatever immediate action is required to limit the spread of infectious material where this can be done without increasing the danger to you or others. Action will depend on the nature of the substance (e.g. liquid or aerosol) and the type of incident.
- **As the THIRD PRIORITY**, the spilled or leaked material is to be cleaned up or disinfected, and the area or equipment made safe again. Whether the staff involved in the incident do this

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themselves, or whether the incident is immediately referred to the Technical Supervisor for action, will again depend upon what the material is and where the incident occurred.

- **Finally**, microbiological incidents must be reported to the PI of the project, the Technical Supervisor and the Facility Supervisor. The reporting procedure should **not** be seen as leading to punitive action; most incidents occur as a result of an accident, application of inappropriate procedure or equipment failure and rarely as a result of disregard for accepted practices and procedures. An incident report **must** be completed (For.P3F.004)

A hands-free telephone is available in all experimental rooms in the Facility. Each phone has preset numbers that will automatically dial and connect you to the Technical Supervisor or IMM Security, as appropriate.

V.1. THE EMERGENCY MASK

An emergency PAPR (Powered Air Purifying Respirator) is available and should be worn during cleaning of spills of aerosolisable material outside the BSC or whenever deemed necessary by the Facility Supervisor. These procedures will be carried out by the Facility Staff unless otherwise directed by the Technical or Facility Supervisor.

V.2. SPILLS INVOLVING NON-AEROSOLISABLE PATHOGENS

V.2.1. Spills Inside the Biosafety Cabinet.

In the event of a spill inside the BSC, always leave the BSC ON.

A. Small Spills

- Small spills (of less than 1ml) will probably be contained by the benchcoat on the workspace. Immediately add disinfectant, allow it to act for 20 minutes, and discard the benchcoat into the primary solid waste bag. Close the bag with a rubber band, spray it and place in the autoclave bag for disposal. Continue work, if necessary (using a fresh benchcoat sheet) after wiping the area clean with 70% ethanol.
- If the spill is not on the benchcoat, cover with paper towels and soak in fresh disinfectant, leaving the cabinet running. Use enough material to absorb the spill entirely, and allow the disinfectant to act for 20 minutes before thoroughly wiping the area clean with 70% ethanol and discarding waste into the primary solid waste bag. Close the bag with a rubber band, spray it and place in the autoclave bag for disposal. Continue work, if necessary.
- A small spill of a non-aerosolisable agent inside the BSC must be cleaned up immediately, but does not require any further notification.

B. Large Spills

- If your overall is contaminated, **immediately evacuate the laboratory**. Remove any contaminated clothing / PPE and discard in the work module as you leave. Then leave the BSL3 Facility:

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- Go into the shower SAS, remove PPE carefully, and place it in an autoclave bag with the inner gloves.
- Check personal clothing visually for contamination. If present or suspect, remove clothes, placing them in the autoclave bag. Seal bag. Take a shower, using the antiseptic wash gel.
- Put on emergency clothing provided.

- If your overall is not contaminated but your gloves and/or apron are, remove them and discard for autoclaving before proceeding.
- Large spills (e.g. broken culture flasks) of non-aerosilisable material are treated similarly to small spills, but require you to stop your work and clean all contaminated surfaces of the cabinet.
- All surfaces and items shall be surface decontaminated before being removed from the BSC. Discard any item that may have become contaminated and cannot be autoclaved.
- Use enough material to absorb the spill entirely, and allow the disinfectant to act for 30 minutes before thoroughly wiping the area clean with 70% ethanol and discarding waste into the primary solid waste bag. Close the bag with a rubber band, spray it and place in the autoclave bag for disposal.
- Large spills inside Class II Biosafety cabinets will run into the sump of the cabinet and must not be allowed to dry out. With the cabinet running, lift the work floor and clean its undersurface as above. Rinse with 70% ethanol and dry with towelling before re-assembly. Dispose of waste appropriately.
- Decontaminate inside of cabinet with disinfectant. DO NOT put your head inside the BSC.
- Report the spill directly to the PI of the project and the Technical Supervisor, and fill out an incident report.

V.2.2. Spills Outside the Biosafety Cabinet

- If your overall is contaminated, **proceed as above**.

- If your overall is not contaminated but your gloves and/or apron are, remove them and discard for autoclaving before proceeding.
- Prevent others from entering the laboratory involved, by placing the “STOP – Do not enter” sign on the door. In the case of a spill in the BSL3 corridor or anteroom, this will involve the entire facility.
- Cover spill with absorbent towels, working from the outside towards the inside of the spill.
- Spray the absorbent material thoroughly with disinfectant, until completely flooded.
- Allow at least 15 minutes contact time.
- Following the disinfection procedure, dispose of waste materials into a waste bag, close and spray the bag, and place it inside a second autoclave bag.

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- Wash affected surface with disinfectant followed by 70% Ethanol.
- Report the spill directly to the PI of the project and the Technical Supervisor, and fill out an incident report.

V.3. SPILLS INVOLVING AEROSOLISABLE PATHOGENS

In the event of ANY spill involving airborne pathogens STOP WORK IMMEDIATELY and ALERT OTHERS IN THE ROOM. Then act according to the instructions in the following sections.

V.3.1. Spills Inside the Biosafety Cabinet

In the event of a spill inside the BSC, always leave the BSC ON.

A. Small Spills

- Cover spill with tissue and flood with disinfectant.
- Place “Biohazard: Spillage - Do not use” sign on cabinet.
- Discard waste materials into the primary solid waste bag in waste container inside cabinet. Seal the bag, spray it and place in the autoclave bag for disposal.
- Wash contaminated work surface with disinfectant followed by 70% Ethanol, and continue work, if necessary (using a fresh benchcoat sheet).
- A small spill of an aerosolisable agent inside the BSC must be reported directly to the Technical Supervisor and the PI of the project, but does not require filling out an incident report.

B. Large Spills

- If your overall is contaminated, hold your breath to avoid breathing in any aerosols and **immediately evacuate the laboratory**. Inform all others in the area that an aerosol may have been generated. All persons shall evacuate the room immediately. Remove any contaminated clothing / PPE and discard in the work module as you leave. Then leave the BSL3 Facility:
- Go into the shower SAS, remove PPE carefully, and place it in an autoclave bag with the inner gloves.
- Check personal clothing visually for contamination. If present or suspect, remove clothes, placing them in the autoclave bag. Seal bag. Take a shower, using the antiseptic wash gel.
- Put on emergency clothing provided

- If your overall is not contaminated but your gloves and/or apron are, remove them and discard for autoclaving before proceeding.
- Do not attempt to clean the spill for at least 30 minutes (to allow aerosols to settle).
- Large spills inside Class II Biosafety cabinets may run into the sump of the cabinet. If that happens, DO NOT continue cleaning/decontamination procedure. Leave the Facility, and alert the Technical Supervisor. If not, proceed as follows.
- Place “Biohazard: Spillage - Do not use” sign on cabinet.

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- All surfaces and items shall be surface decontaminated before being removed from the BSC. Discard any item that may have become contaminated and cannot be autoclaved.
- Cover spill with tissue and flood with disinfectant. Use enough material to absorb the spill entirely.
- Allow the disinfectant to act for at least 30 minutes
- Following the disinfection procedure, dispose of waste materials into a waste bag, close and spray the bag, and place it inside a second autoclave bag.
- Wash affected surface thoroughly with disinfectant, again.
- Thoroughly wipe the area clean with 70% ethanol and discard waste into the primary solid waste bag. Seal the bag, spray it and place in the autoclave bag for disposal.
- Decontaminate inside of cabinet with disinfectant. **DO NOT** put your head inside the BSC.
- Report the spill directly to the Technical Supervisor and the Facility Supervisor, and fill out an incident report.

V.3.2. Spills Outside the Biosafety Cabinet

- **DO NOT ATTEMPT TO CLEAN THE SPILL. Presume a contaminated aerosol has been generated. The incident should be treated as a potential exposure.**
- Hold your breath to avoid breathing in any aerosols and **immediately evacuate the laboratory**. Inform all others in the area that an aerosol may have been generated. All persons shall evacuate the room immediately. Spills in individual laboratories require evacuation of the laboratory involved; spills in the BSL3 corridor or anteroom, require evacuation of the entire BSL3 Facility.
- Prevent others from entering the laboratory involved, by placing the “STOP –Do not enter” sign, on the door. A sign should also be placed on the exterior door of the BSL3 Facility, specifying the location of the spill, and which areas may not be entered. In the case of a spill in the BSL3 corridor or anteroom, this will involve the entire facility.
- Quickly remove any contaminated clothing / PPE and discard in the work module as you leave. Then leave the BSL3 Facility.
- Go into the shower SAS, remove PPE carefully, and place it in an autoclave bag with the inner gloves.
- Check personal clothing visually for contamination. If present or suspect, remove clothes, placing them in the autoclave bag. Seal bag. Take a shower, using the antiseptic wash gel.
- Put on emergency clothing provided
- Immediately notify the Technical and the Facility Supervisor, the PI of the project, and all BSL3 staff.
- Only after 30-60 minutes (to allow aerosols to settle/dissipate by the room ventilation changes) can the decontamination procedure be carried out. The Technical or Facility Supervisor will evaluate the situation and decide who will carry out with the decontamination procedure. Do

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not re-enter the Facility unless you have been instructed to do so by the Technical or Facility Supervisor.

- The Facility Supervisor will evaluate the situation and decide whether fumigation of the room is necessary.

V.4. SPILLS IN INCUBATORS

(Spills in incubators must be immediately reported to the Technical Supervisor or the Facility Supervisor, who will determine whether the user or the Facility Staff will carry out the decontamination procedures).

Spills in incubators should follow the procedures outlined above for major spills involving non-aerosolisable or aerosolisable pathogens, as appropriate. **In addition:**

- Stop work immediately. Notify Technical Supervisor.
- If spill is of aerosolisable material evacuate room for at least 30 minutes and label the area off-limits.
- All materials are to be removed from the incubator and decontaminated. All items shall be surface decontaminated with an appropriate disinfectant before being removed from the contaminated incubator and placed in another incubator.
- Decontaminate the water in the floor of the incubator with disinfectant in the incubator pan. Remove this water using the aid of a vacuum collection flask located within a BSC.
- Place absorbent material soaked with disinfectant on each shelf and let sit for at least 30 minutes.
- Wipe incubator surfaces with disinfectant, followed by 70% ethanol.
- The incubator will undergo a sterilization cycle in accordance with the manufacturer's instructions (to be carried out by the Technical Supervisor)

V.5. SPILLS IN CENTRIFUGES

(Spills in centrifuges must be immediately reported to the Technical Supervisor or the Facility Supervisor, who will determine whether the user or the Facility Staff will carry out the decontamination procedures).

- Stop work immediately. Notify Technical Supervisor.
- TURN THE CENTRIFUGE OFF. Spills in centrifuges should follow the procedures outlined above for major spills involving non-aerosolisable or aerosolisable pathogens, as appropriate. **In addition:**
- If unusual sounds from a centrifuge with aerosolisable material suggest that breakage and a spill has occurred, or, if breakage and a spill is discovered after the machine has stopped, CLOSE LID, evacuate room for at least 30 minutes, and label the area off-limits. This will allow hazardous aerosols to settle in the centrifuge.

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- Pour disinfectant onto and around the spill absorbent matting and let sit for at least 30 minutes.
- WITHOUT OPENING THE ROTOR decontaminate all exposed centrifuge (interior centrifuge sides and rotor surfaces) and environmental surfaces by wiping with an appropriate disinfectant.
- Remove rotor and place in a BSC. Spray and wipe rotor surfaces again with disinfectant.
- Open rotor inside BSC. Disinfect by submersion in disinfectant allowing at least 30 minutes contact time, followed by 70% ethanol, then again with disinfectant followed by 70% ethanol.
- After a spill is decontaminated, the area shall be thoroughly cleaned with 70% ethanol and dried, and decontaminated again with an appropriate disinfectant, followed by 70% ethanol.

V.6. SPILLS IN REFRIGERATORS/FREEZERS

(Spills in refrigerators/freezers must be immediately reported to the Technical Supervisor or the Facility Supervisor, who will determine whether the user or the Facility Staff will carry out the decontamination procedures).

Spills in refrigerators/freezers should follow the procedures outlined above for major spills involving non-aerosolisable or aerosolisable pathogens, as appropriate. **In addition:**

- Stop work immediately. Notify Technical Supervisor.
- If spill is of aerosolisable material evacuate room for at least 30 minutes and label the area off-limits.
- Pour disinfectant onto and around the spill absorbent matting and let sit for at least 30 minutes.
- Remove and disinfect each item separately. Transfer to a new box.
- Check all tubes, plates, etc. for cracks and other damage.
- Wipe surfaces with disinfectant, followed by 70% ethanol. Repeat.

V.7. GENERAL FACILITY DECONTAMINATION

Decontamination Procedure

(To be performed only by the Facility Staff, unless otherwise directed by the Technical Supervisor or the Facility Supervisor)

- Dress in protective clothing- rubber gloves, boots, disposable jumpsuit, head covering.
- Wear an Emergency Mask (see section 3.V.1)
- Cover spill with tissue and flood with disinfectant. Use enough material to absorb the spill entirely.
- Allow the disinfectant to act for at least 30 minutes
- Following the disinfection procedure, dispose of waste materials into a waste bag, close and spray the bag, and place it inside a second autoclave bag.
- Wash affected surface thoroughly with disinfectant, again.

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- Thoroughly wipe the area clean with 70% ethanol and discard waste into the primary solid waste bag. Seal the bag, spray it and place in the autoclave bag for disposal.
- Broken glass and sharp items are to be picked up with forceps.
- Swab area around spill, including floor, walls, counters, using paper towels soaked in disinfectant.
- During the clean-up process, all contaminated clothing, footwear and contaminated material used in cleanup must be double-bagged in clear autoclave bags and autoclaved at the soonest opportunity.

After a spill

- The Facility Supervisor will determine if gaseous decontamination is necessary.
- Medical surveillance may be required for potentially affected workers.
- No-one is to re-enter the Facility until authorization is granted by the Facility Supervisor.

V.8. VENTILATION CONTROL OR ELECTRICAL POWER FAILURES

Continuous maintenance of room-to-room differential pressures and appropriate directional bulk airflow through the BSL3 suite is essential to safe operations. If two or more biosafety cabinets go into an alarm condition for more than two minutes, or if the alarms activate, everyone in the BSL3 suite must:

- Stop all work and cover or secure exposed materials
- If working with animals, ensure that all live animals are inside the cages and that all cages are locked and on the ventirack or inside BSC.
- Exit the suite following normal exit procedures
- Place “do not enter” sign on door.
- Contact the Technical or Facility Supervisor.
- Not re-enter the suite until advised by the Technical or Facility Supervisor that it is safe to do so.

V.9. FLOODS OR LEAKS FROM CEILING

The ceiling of the BSL3 Facility is also the floor of the technical area above the facility. Any leaks from the ceiling must be treated as potentially contaminated.

- Immediately notify Technical Supervisor
- Place absorbent material soaked in disinfectant on any pools of liquid that are forming
- If leak is small and in an area that does not contain material or equipment finish work and then leave the facility following the normal exit procedures.
- If leak is substantial or above material or equipments, stop work immediately and leave the facility following the normal exit procedures.

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V.10. FIRE OR EMERGENCY EVACUATION PROCEDURES

- Stop all work and cover or secure exposed materials.
- If working with animals, ensure that all live animals are inside the cages and that all cages are locked and on the ventirack or inside BSC.
- Exit the suite following normal exit procedures.
- Do not re-enter the suite until advised by the Facility or Technical Supervisor that it is safe to do so.
- Fire responders must wear respiratory protection when accessing the area.

V.11. PERSONAL INJURY

- Needle sticks or cuts involving potential pathogen exposure:
 - Allow wound to bleed – express the wound
 - Wash area with disinfectant and 70% ethanol
 - Leave the Facility following exit procedures
 - If bleeding has not stopped, cover the wound with 70% ethanol soaked tissue until you reach the exit SAS
 - In the exit SAS, discard tissue in PPE container, keeping it inside removed gloves
 - Disinfect the wound again with disinfectant in SAS before removing and after your mask and exiting
 - Wash affected area with germicidal soap and water for at least 15 minutes
 - Report to Facility staff and PI
 - Seek medical attention
- Splashes to nose and eyes
 - Splashes to the nose and mouth require flushing with water or saline for at least 5 minutes
 - Splashes to eyes: remove contact lenses immediately (if worn). Rinse eyes and inner surfaces of eyelids continuously for 15 minutes
 - Report to Facility staff and PI
 - Seek medical attention
- Occupational injury such as exposure to hazardous chemicals
 - Report immediately to the Facility Supervisor
 - Report to Facility staff and PI
 - Seek medical attention

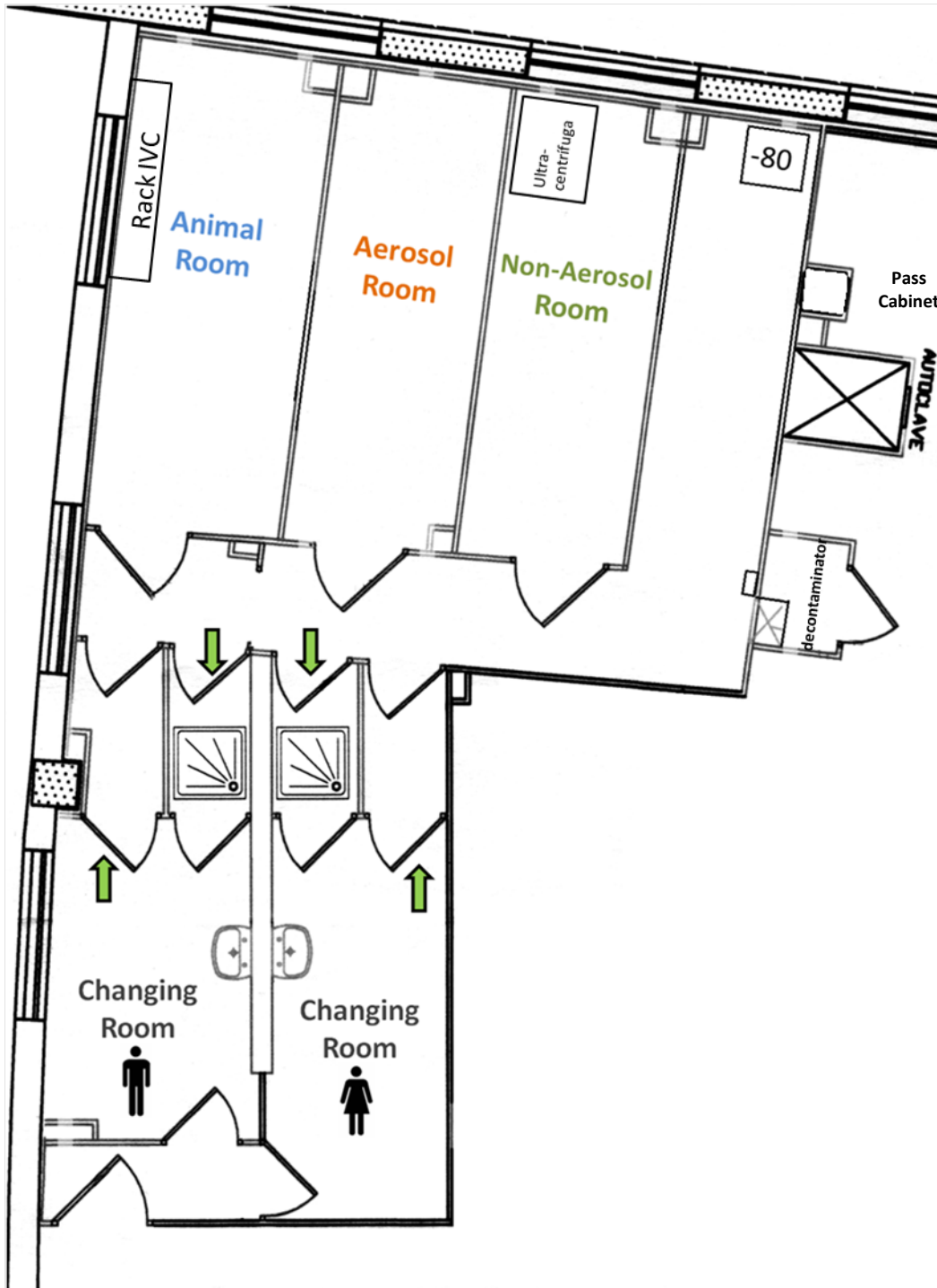
4. RECORDS

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Records' Identification	Indexation	Archive Responsible
FORM.P3F.001 – P3 Access	SI / SP	P3F
FORM.P3F.002 – Manutenção de equipamentos	SI / SP	P3F
FORM.P3F.004 – Incident Report	SI / SP	P3F
FORM.P3F.008 – Registo de Limpeza	SI / SP	P3F
FORM.P3F.009 - -80 Record	SI / SP	P3F
FORM.P3F.010 – Training Record	SI / SP	P3F

5. APPENDICES

I. APPENDIX 1 – BSL3 FACILITY BLUEPRINTS



II. APPENDIX 2 - HUMAN IMMUNODEFICIENCY VIRUS

HIV is spread by sexual contact with an infected person, by sharing needles and/or syringes (primarily for drug injection) with someone who is infected, or, less commonly (and now very rarely in countries where blood is screened for HIV antibodies), through transfusions of infected blood or blood clotting factors. Babies born to HIV-infected women may become infected before or during birth or through breast-feeding after birth.

In the health care setting, workers have been infected with HIV after being stuck with needles containing HIV-infected blood or, less frequently, after infected blood gets into a worker's open cut or a mucous membrane (for example, the eyes or inside of the nose).

HIV does not survive well in the environment, making the possibility of environmental transmission remote. HIV is found in varying concentrations or amounts in blood, semen, vaginal fluid, breast milk, saliva, and tears. To obtain data on the survival of HIV, laboratory studies have required the use of artificially high concentrations of laboratory-grown virus. Although these unnatural concentrations of HIV can be kept alive for days or even weeks under precisely controlled and limited laboratory conditions, CDC studies have shown that drying of even these high concentrations of HIV reduces the amount of infectious virus by 90 to 99 percent within several hours. HIV is unable to reproduce outside its living host, except under laboratory conditions.

III. APPENDIX 3 - MYCOBACTERIUM TUBERCULOSIS

Tuberculosis has existed for centuries, and used to be called "consumption". It is a serious disease that usually attacks the lungs, but can also affect other parts of the body, including the brain, the lymph nodes, and bones. Although most TB infections are curable with antibiotics, it continues to be a major health problem and kills as many as three million people worldwide every year. However, multiple-drug resistant TB is increasing world-wide, complicating treatment.

Mycobacterium tuberculosis is an airborne pathogen. It is usually spread through the air from person to person, when someone with infectious TB disease coughs or sneezes. In a laboratory setting, mechanical aerosol generation can also lead to the release of particles which can cause infection if inhaled. Likewise, direct inoculation via injection into the bloodstream can also cause infection.

IV. APPENDIX 4 – BSL3 FACILITY FORMS