BIOSAFETY MANUAL

Institutional Biosafety Committees

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Version 1.1

In Case of Emergency Call 999

NAME	WORK TELEPHONE	HOME TELEPHONE	CELL TELEPHONE
(Name) Principal Investigator	+603 9101 8880 Ext:		
(Name) Lab Manager	+603 9101 8880 Ext:		
(Name) Lab Tech	+603 9101 8880 Ext:		
UCSI University Student Affair & Alumni Division Office	+603 9101 8880 Ext: 3451 / 3162 / 5052	Nil	Nil
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Emergency Telephone Numbers	8 am - 5 pm	After 5 pm, Weekends
National Poison Centre	+604 657 0099	+6012 430 9499
Guard House (North Wing)	+603 9101 8880 (Ext: 5401)	
Guard House (South Wing)	+603 9101 8880 (Ext: 3189)	
Ambulance (HUKM)	+603 9145 5588	
Police/Rescue	+603 2031 9999 / +603 2266 3333	
Fire Department	+603 9132 9490 / +603 9132 9491	

Annual Review/Revision Status

Date	Revision #	Comments	Signature

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1. HOW TO USE THIS BIOSAFETY MANUAL

- All information in this manual applies to work with material and recombinant DNA (rDNA) molecules that may pose a moderate hazard to personnel and the environment. All work will be in compliance with the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition and NIH Guidelines for Research Involving Recombinant DNA molecules

- All information in this manual is understood to be specific to the laboratory named below. Additional specific information can be found in the appendices.

- *ESSENTIAL INFORMATION TO THE MANUAL:*

  1. **Section 13** - Risk Assessment and Material Safety Data Sheet (MSDS) for BSL-1 & 2 agents. Similar agents may be grouped into one Risk Assessment as long as it is clear what information is for each specific agent.

  2. **Section 14** - Documents for all BSL- 1 & 2 agent-specific hazards/precautions requiring strict compliance by laboratory personnel which are not already covered in the manual or your risk assessments (if there is any).

  3. **Section 15 -** Additional information for higher risk procedures that will be performed (If there is any).

     - Include a chart with a list of all rooms where work will be performed or material stored and the procedures that will take place in each room.
     - List all types of sharps that will be used, the procedure for each and describe the specific precautions in place to minimize sharps-associated risk.

  4. **Section 16** - Lab-specific BSL-1 & 2 procedures used in the laboratory and any general building, department or area SOPs that are common for all labs and are NOT otherwise addressed in this manual.

  5. **Section 17** - ”Agent and Materials Summary” IBC document, a current and accurate lab sketch and other information necessary for the safe operation of the lab.

2. LABORATORY SUMMARY

This laboratory-specific Manual is intended to:

1. Supply biosafety information, guidelines, policies, procedures and practices for using potentially infectious material and/or recombinant DNA (rDNA) molecules in the laboratory of:

   enter PI name, Building, Room #s**.**

2. Document procedures that are intended to minimize or eliminate the exposure of research personnel and other persons and the environment to potentially hazardous material

3. Provide a complete list of all BSL-2 agents/materials/samples currently used in this laboratory.

   Enter list of agents/materials/samples here:
   1.
   2.
   3.
   etc.

   NOTE: Specific information for these agents will be included in Section 13 of this manual by providing a risk assessment, or MSDS sheet if available, for each. The following website provides a source of MSDS information for many BSL-2 agents: http://www.phac-aspc.gc.ca./msds-ftss/.

4. Provide a complete list of all personnel who work directly with or in close proximity to BSL-2 agents / materials / samples and /or rDNA molecules in this laboratory.

   Enter list of personnel here:
   1.
   2.
   3.
   etc.

   Add names of personnel below as needed:
   (Fill out training sheet for each.)

   _____ _____
   _____ _____
   _____ _____
   _____ _____

5. IBC approval must be obtained before BSL-2 work begins.

Enter IBC approval number here:

3. EMERGENCY CONTACT REQUIREMENTS

- The access doors to all areas where potentially infectious material is used or stored will be posted with current emergency contact information and biohazard information specific to the BSL-2 agents used therein.

- Posted information include:

    a. BSL safety level, biohazardous agents(agent list will be posted on inside of lab door), & specific precautions (in brief) associated with those agents

    b. Principal Investigator, Lab Manager, other responsible personnel information

    c. Telephone numbers for all responsible personnel

- Emergency contact sheets will be kept current.

4. SAFETY EQUIPMENT AND PRACTICES

4.1 PERSONAL PROTECTIVE EQUIPMENT (PPE)

MINIMUM REQUIREMENTS:

- While working with BSL-1&2 agent/material/samples and/or rDNA molecules in this laboratory, personnel will wear:

    1. Closed-toe shoes
    2. Street clothing to fully cover the legs
    3. Disposable or cloth laboratory coat
    4. Disposable gloves
    5. Eye and face protection (goggles, mask, face shield or other splatter guard) for any anticipated splashes or sprays of infectious or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.

PPE WILL BE LOCATED IN THESE AREAS: Donning and doffing area

PPE RESTRICTIONS:

- **PPE worn while working with BSL-1&2 agents/samples and/or rDNA molecules in the laboratory or work area will not be worn *outside of the laboratory or work area (This include administration area in the laboratory) unless specific procedures are approved by the Biosafety Office.***

PPE DISPOSAL, DECONTAMINATION AND CLEANING

- **Gloves:**
  - Gloves will be changed when contaminated, integrity has been compromised, or when otherwise necessary.
  - Disposable gloves will not be washed or reused.
  - Gloves will be disposed of in Biohazard waste containers, NOT household trash.
  - Personnel will remove gloves and wash their hands after working with hazardous materials and before leaving the laboratory.
- **Lab Coats:**
  - Disposable lab coats may be reused for a period of 3 months if not contaminated during work processes and integrity is maintained. If they are contaminated they must be placed in a Biohazard waste container located within the laboratory work area.
  - Cloth lab coats contaminated from work with BSL-2 materials and/or rDNA molecules will be autoclaved or otherwise decontaminated before they are sent to the laundry.
  - Lab personnel will not take lab coats home to launder them.
- **All PPE will be removed in a manner that minimizes transfer of BSL-2 materials and/or rDNA molecules outside of the BSL-2 work area. These practices include:**
  - Donning and doffing of clean PPE shall be made at the designated area.
  - Doffing of contaminated PPE such as gloves and mask must be made at the nearest Biohazard waste container located within the laboratory work area.
  - Please refer to the illustration in Appendix # for a proper donning and doffing of PPE.

4.2. EYEWASH STATION

- Located by sink on Bench #. Eyewash will be flushed for a minimum of 5 minutes weekly.
- A log sheet is kept up to date by the laboratory technologist in charge.
- In case of exposure, proceed to nearest eyewash station. Hold eyelids open with thumb and forefinger and rinse for at least 15 minutes. Wash from the outside edges towards the inside to prevent washing chemicals back into the eye.
- Rinse should be aimed at the inner corner of the eye (near the nose) not directly at the eyeball. “Roll” eyes around and up and down to ensure full rinsing.
- Contact lenses (if worn) should be removed as soon as possible. Have another member of the lab call for emergency response immediately. The area around the eye wash station must remain clear at all times.

4.3. SAFETY SHOWER

Located in Room #s, near enter text. Please refer to the laboratory sketch map posted on the information board next to the main door for exact location.

4.4 FIRE EXTINGUISHERS AND FIRE ALARMS

- Extinguishers - Please refer to signage posted on top of each extinguisher for the exact location.
- Fire alarms are located near each exiting doors of the laboratory.
- Extinguishers which are located in laboratories *are checked monthly for proper charge by lab personnel*.
- Those located in hallways are checked monthly by the Logistic staff.

4.5 DISINFECTION AGENTS

- Sufficient quantities of disinfectant(s) of choice (example: bleach, ethanol, or other appropriate disinfectants) are kept in the laboratory for disinfection purposes.

- Working dilutions of each disinfectant, (example: 10% bleach, 70% ethanol) are kept in sufficient quantities in the lab for disinfection purposes.

- Bleach dilutions will be prepared fresh weekly, are inactivated by organic matter and are corrosive to metal surfaces and the skin.

- For general disinfection of surfaces/ objects use a 1:50 dilution (1 part undiluted bleach to 49 parts water). This provides 1000 ppm available chlorine.

- The minimum contact time or times (in minutes) which will be allowed for disinfectant (s) to be effective is as follows : enter contact time for each disinfectant used

- **Expiration dates of undiluted bleach and all other disinfectants will be always checked; up-to-date stocks will be maintained in the laboratory.**

4.6 CONDUCT OF LABORATORY PERSONNEL

To ensure everyone's safety, all users of the facilities in this laboratory must agree to maintain the space in a clean and orderly state. Each individual will thoroughly clean up after him/her self. (Refer to *Section 9 for Training Record* and *Laboratory Usage Agreement* in this document.)

4.7 ROLES AND RESPONSIBILITIES OF LABORATORY PERSONNEL

- The Principal Investigator (PI) bears ultimate responsibility for laboratory BSL-1&2 practices, supplies, equipment, safety and procedural training, and maintenance of documentation/records.

- Under the supervision of the PI, a Laboratory Manager/Supervisor may be responsible for daily oversight of lab BSL-1&2 practices, supplies, equipment, safety and procedural training, and maintenance of documentation/records.

- PIs and/or Lab Managers are responsible for training laboratory personnel in lab-specific procedures and maintaining documentation of training. (Refer to Section *9. Training Record,* in this document.)

- Laboratory personnel are responsible for:
    a. Participating in all required training
    b. Being familiar with the biosafety manual contents and its location in the work area
    c. Following all approved departmental and lab-specific procedures as well as safety guidelines
    d. Informing supervisors of deficiencies in facilities, equipment or procedures
    e. Reporting accidents to supervisors

- Specific cleaning practices will be determined by PI and Lab Manager, and will be followed by all lab personnel.

- General cleaning practices for which lab personnel are responsible will include, but are not limited to:
    a. Decontamination of work surfaces
    b. Decontamination and cleanup of spills
    c. Decontamination and/or disposal of contaminated PPE
    d. Decontamination of lab equipment scheduled for repair or surplus
    e. Appropriate containment, removal and decontamination of biohazard waste
    f. Appropriate containment of chemical waste; making arrangements for disposal with EHS

- Laboratory personnel are responsible for advising visitors, custodians, and service providers per section 4.9 and 4.10 on any safety awareness issues and procedures *prior to their entry into the laboratory*.

- Lab personnel are responsible for decontaminating refrigerated equipment before having interior surfaces exposed for defrosting. They will document those actions and attach it to the equipment while defrosting is in process.

- Lab personnel are responsible for decontaminating and cleaning equipment scheduled for service or surplus, and they will document those actions. The decontamination document will be taped onto the equipment upon completion of the tasks. For liability purposes, the decontamination documentation must be kept in laboratory records following service on equipment.

4.8 FOOD AND DRINK POLICY

Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption is **not permitted** in any laboratory area. Food will be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

4.9 VISITORS

- Visitor access will be at the discretion of the PI.
- Prior to entry into the laboratory, all visitors will be made aware of the hazards and emergency procedures, including but not limited to:
    a) Instructions on avoiding physical contact with all research equipment, material and working surfaces, unless invited and/or approved to do otherwise by authorized personnel who will provide appropriate supervision.

    b) Information on any health hazards specific to the work occurring in the lab, as well as specific safety practices for avoiding those hazards.

    c) Infrequent visitors (i.e., those who enter the area less than once/month) must be made aware of the above items upon each visit.

    d) Frequent visitors (i.e., those who enter the area at least once/ month) can be trained once and updated as needed if anything changes.

    e) If visitors' time spent in the lab will exceed a short stay, they will be:
        - Shown the emergency exit route from laboratory
        - Given a review of the Emergency Procedures list posted in the lab
        - Shown the locations and proper use of emergency eyewash & shower
        - Shown the locations of the nearest fire alarm & extinguisher
- Documentation will be kept for all visitors and the training that they receive to minimize any lab liability issues.

4.10 CUSTODIAL AND SERVICE PERSONNEL

- Presence of custodians in the laboratory should be kept to a minimum. This will necessitate a greater level of housekeeping, cleanliness and equipment maintenance on the part of laboratory personnel.
- Custodians are responsible for:
    - Emptying regular household trash
    - Regular mopping of floors when requested
- Laboratory personnel are responsible for advising custodians and service providers on any safety awareness issues and emergency procedures *prior to their entry into the laboratory* and providing them with any necessary PPE.

Documentation will be kept for all custodial and service personnel and the training that they receive to minimize any lab liability issues.

5. BSL-1&2 WORK AREAS

5.1 LOCATION

- Use of BSL-2 materials and/or rDNA molecules will be limited to Biotechnology Laboratory and Molecular Biotechnology Research Laboratory only

- The biosafety cabinet(s) is (are) identified as enter biosafety cabinet make (i.e., Envirco, etc.), located in Room #, will be used for handling BSL-2 materials and/or rDNA molecules.

5.2. SIGNAGE

- Appropriate "BSL-2—Biohazard" signs or labels will be posted in/on:
  - entrance doors to work areas
  - biosafety cabinets
  - equipment such as centrifuges, refrigerators, freezers, etc.
  - transport containers
  - secondary containers for biohazardous waste
  - any other equipment used to store or manipulate BSL-2 agents

5.3 RESTRICTED OR LIMITED ENTRY

- Access to BSL-1&2 work areas or laboratories will be determined by the PI and will be restricted or limited in the following ways: enter text

- The access doors to BSL-1&2 work areas will be closed when any work is being performed with BSL-2 materials.

- All access doors to the BSL-1&2 areas will be locked when no one is in the lab for an extended period of time.

- Appropriate biohazard signage will be posted on doors when BSL-2 work is in progress.

5.4 MIXED BIOSAFETY LEVEL ACTIVITIES

- When there is BSL-1 work occurring in a BSL-2 laboratory, the PI or lab manager will provide clear documentation and training on what procedures to follow to avoid confusion with multiple containment levels. To protect personnel safety and to contain biological agents it is recommended that clearly defined areas be set up within the lab for each biosafety level. All such procedures will be described in Section 15 of this manual.

5.5 STORAGE OF BSL-1&2 MATERIALS

- A current and accurate list of each biohazardous agent or toxin held in storage will be maintained in the biosafety manual.

- All frozen BSL-1&2 samples/materials/agents will be stored in proper storage container/s located in the respective freezer/s (-20°C or -80°C) in freezer area. Refer List of Suggested Storage Container for BSL-2 samples/materials/agents.

- All other BSL-1&2 samples and agents (liquid cultures in tubes, cultures on solid media, tissue samples in formalin, etc.) will be secured on/within proper sealed storage containers in assign freezers/chillers located in freezer area.

- Note: Any chillers/freezers used for storage of BSL- 2 material that is located in an area accessible to the public or any non-BSL-2 lab personnel must be locked.

5.6 HANDLING OF BSL-2 MATERIALS

We will open BSL-2 agent/material/sample containers inside a biological safety cabinet and perform subsequent procedures therein, whenever possible. Working within a biological safety cabinet minimizes the potential exposure to the user from the agent/material/sample.

Enter PI initials:_____

NOTE: All procedures performed with potentially infectious BSL-2 agents/materials/ samples and/or rDNA molecules outside of the biosafety cabinet are to be included in *Section 15: Procedures/Locations Summary.*

5.7 WORK SURFACE DECONTAMINATION

Work surfaces in biosafety cabinets and on bench tops will be decontaminated/disinfected with 10% of sodium hypochlorite or 70% ethanol spray immediately before and after work is complete.

5.8 EQUIPMENT

- All equipment must be located in a BSL-2 approved area and posted with appropriate biohazard signage.

- Equipment used with potentially infectious material will be decontaminated:

    1. Routinely (e.g., daily or weekly depending on frequency of use)
    2. After any spill or splash and
    3. Before any repair, maintenance or removal from the lab, contact Biosafety Office for approved decontamination methods

- In the event of equipment failure during use of a biosafety cabinet with BSL-2 material and/or rDNA molecules, the cabinet's fan, filters and airflow plenums may need to be decontaminated by formaldehyde gas procedures or another approved method. Contact the Biosafety Office for assistance.

- Equipment failure experienced with refrigerators, freezers, water baths, centrifuges, etc. which are used with BSL-2 materials also require decontamination and cleaning before being serviced and put back into use.

5.9 INCUBATION

- Liquid bacterial cultures which may or may not contain rDNA molecules will be incubated in appropriate Erlenmeyer/conical flask volume according to the working volume of the culture

with sterile gauze or other appropriate closures. Cultures will be incubated in the incubator room in the assign incubator.

- Tissue cultures which may or may not contain rDNA molecules will be incubated in appropriate cell culture flask volume according to the working volume of the culture with screw cap or other appropriate closures. Cultures will be incubated in Cell Tissue Culture Room in the assign incubator.

5.10 TRANSPORTATION OF BSL-2 MATERIALS

Moving BSL-2 Materials/Agents/Samples/Extracts and/or rDNA Molecules from One Work Area to Another

- BSL-2 materials and/or rDNA molecules to be transported outside of Molecular Biotechnology Research Laboratory (MBRL), but within the building, will be placed into a durable, leak-proof container that is closed and disinfected on the outside before removing from the room.

- Lab personnel will use sealed and leaked proof container for containment of BSL-2 material and/or rDNA molecules for transport.

- BSL-2 materials and/or rDNA molecules to be transported outside the building will be sealed in a leak-proof primary tube/flask or other container and disinfected on the outside before being placed into a durable, leak proof secondary transport container. A biohazard symbol will be placed on the primary container. The secondary container will be disinfected and secured for transport.

- For added safety, containers should be transported on a cart to further minimize spill hazards.

Moving BSL-2 rDNA Waste to Autoclave/Glassware Rooms

- Waste will be collected in orange autoclave bags displaying biohazard symbol.

- Waste will be securely closed (at the end of the work session or when bag is 2/3 full), sprayed thoroughly with 70% ethanol, and allowed to dry.

- Waste will be placed within a secondary container (Nalgene or stainless steel pan) which is dedicated for this function with biohazard label.

5.11 PEST MANAGEMENT

- Food and drink are not allowed in any BSL-1&2 area.

- Routine cleaning and mopping of floors will occur daily with used of mild soap and bleach as disinfectant as will visual inspection for vermin.

- If insect or rodent pests are found in a laboratory work area/storage area, they present a possible contamination risk and containment breech. The PI or Lab Manager should contact Group Logistics Management Office (GLMO) to arrange pest control/removal by appropriate service providers and document any service provided.

6. CHARACTERISTIC OF BSL-1&2 LABORATORY

STANDARD PRACTICES	✓
Restricted access; authorized entry only	
Doors to lab kept closed and labeled with Biohazard sign	
Decontaminate work surfaces by following specific protocol	
Remove waste frequently using leak proof secondary containers	
Decontaminate solid Biohazard waste by autoclaving	
Decontaminate liquid waste with bleach or by agent-specific disinfectant method	
Dispose of Sharps in hard-sided Biohazard containers only	
Minimize aerosols and splashes	
No direct handling of broken glass	
No mouth pipetting or label-licking	
No eating, drinking, etc. in laboratory or storing food/drink in laboratory	
Wash hands before exiting laboratory	
Wash hands after handling hazardous agents	
Wear clean or disposable coats over street clothes in work areas	
Do not wear lab coats outside of lab	
Do not wear open-toed shoes	
Personnel must know all lab-specific SOPs,	
Personnel are responsible for lab cleaning & waste removal	
Personnel must read & understand Biosafety Manual	
MSDS, risk assessments & safety manuals must be kept at known locations	
All labeling must be clear & complete	
SPECIAL PRACTICES	
Specified lab entry policies; limit entry into work area when it is in use	
Visitors—by permission only; PPE maybe required	
Service personnel—PPE required; enter only upon arrangement by PI	
Wearing of gloves is mandatory	
Eye protection worn (including contact lens wearers) in areas of likely splash/spray	
Biohazard warning signs must be on all pertinent equipment	
Animals and Plants not involved in work not permitted in laboratories	
Remove BSL-2 agents from lab in closed, secure transport containers	
Dispose of decontaminated solid waste as Regulated Medical Waste	
Limit use of hypodermic needles/syringes	
Vacuum lines have in-line filters & traps with disinfectant	
Equipment is decontaminated before removal from area	
Spills/exposures reported to PI & IBC immediately	
Medical surveillance provided as necessary	
Specific training is required in all risks & hazards	
All training is documented, & updated annually	
Biohazardous Agents Inventory and associate Risk Assessments are kept up-to-date	
Contact Biosafety Office for help with shipments of BSL-2 or genetically modified material	
CONTAINMENT EQUIPMENT	
BSC function certified annually	
LABORATORY FACILITIES	
Impervious bench tops	
Sturdy furniture	
Good illumination	
Safe, adequate storage areas	
Work areas able to be easily cleaned	
Hand-washing sink must be located in lab	
Autoclave available in building	

7. INCIDENT RESPONSE AND REPORTING

7.1 INCIDENT/ACCIDENT RESPONSE PROCEDURE

INCIDENT REPORTING *– copy this introductory section and place it in a prominent location at the beginning of your lab's Biosafety Manual. A copy might also be place near the lab's telephone.*

INCIDENT / ACCIDENT REPORTING

1. As soon as any initial response is complete and incident is stable, ***immediately notify*** the Lab Manager and/or Lab Technologist, the Animal Facility or greenhouse Person-In-Charge (if applicable), and the University Biosafety Officer (UBO).

2. The UBO will acknowledge receipt of notification via email (to document notification) to the reporting person and other appropriate personnel.

    a. **NOTE: If UBO does not acknowledge receipt of notification within two (2) hours, notify an Associate Biosafety Officer (ABO).**

    b. If email is not available, the UBO/ABO will acknowledge receipt via phone call to the reporting person and other appropriate personnel.

3. If this is a reportable incident, the UBO/ABO will immediately report to the National Biosafety Board (NBB) and/or Institute of Medical Research (IMR) via phone or email. Ministry of Health (MOH) and/or Ministry of Natural Resource and Environment (NRE) may also be notified.

4. Reporting person and Lab Manager / Lab Technologist / Animal Facility PIC must complete the Lab Incident Report and submit it to the UBO/ABO via email **immediately**.

5. UBO/ABO acknowledges receipt of report via email.

6. UBO/ABO completes appropriate state and/or federal reporting forms and submits them NBB and/or IMR.

7. **If an injury or exposure has occurred**, an Accident Report must be completed immediately by the supervisor. The hardcopy of the report is obtainable in the respective laboratory file for forms and reports.

    If the supervisor does not complete the report in a timely manner, injured/exposed individuals are encouraged to complete the Report themselves. Completed hardcopy must be submitted to the Lab Manager for filing.

Contact	Primary Method (cell phone)	Secondary Method (email)
UBO-Jason Lim	+6012 223 7197	limlh@ucsiuniversity.edu.my
ABO-		
ABO-		

7.5 SPILLS

CRITICAL THINGS TO KNOW

- **Major spill** --- a spill which, in your judgment, represents a significant health risk to people who may be exposed to a biohazardous agent as a result of the spill.

- Consider pathogenicity, concentration, volume, aerosol potential, etc. of the BSL-2 material when making this judgment. Consult the risk assessment for the BSL-2 agent(s) to find this information if necessary. Make this judgment as quickly as possible.

- Immediately notify everyone in the lab /area in the event of a major spill.

- Inform your PI or Lab Manager/Supervisor as soon as possible in the event of a major spill.

- In the event of a major spill, call 999 or 3189/5401 for campus security; ask that IBC be informed immediately.

IMPORTANT:

- **Chemical disinfectants require contact time with the spill to effectively decontaminate it. Be aware of the specific contact time of the disinfectant you use and allow that time to elapse before clean-up.**
- For metal surfaces, follow all bleach disinfectant treatments with a water rinse.
- In the event that a chemical disinfectant is not used (or cannot be used) with contaminated items, decontaminate by autoclaving or other method approved if items can withstand the process (example: contaminated lab coats).
- **For any spill involving broken glass**: DO NOT HANDLE BROKEN GLASS WITH YOUR HANDS. USE A DUSTPAN, FORCEPS OR OTHER DEVICE TO PLACE THE GLASS INTO AN APPROVED SHARPS CONTAINER TO BE AUTOCLAVED.

BIOHAZARD SPILL KITS

- BSL-2 work areas will have access to a Spill Kit which meets the critical needs of a Biohazard spill.

- Contents should be contained within a handled bucket and include a disposable lab coat or coveralls, disposable gloves, face shield/mask, protective footwear, spray disinfectant, clean-up supplies (forceps, dustpan, autoclave bags, spill pillows & socks) and a sign that reads" Biohazard Spill DO NOT ENTER".

- If respiratory hazard is indicated on Risk Assessment Forms for the BSL-2 agents in question, respiratory protection in the form of respirators or PAPR units should be provided separately by the laboratory; it will not be included in the Spill Kit.

- **LOCATION OF KIT:** Spill Kit will be located at MBRL to service all labs or work areas listed. Spill Kit directions must be displayed on the kit container and a wall sign for quickly locating the Kit must be in place.

- **TRAINING WITH KIT:** All personnel working with BSL-2 materials will receive training for Biohazard Spill Kit use.

SPILL OCCURING INSIDE THE BIOLOGICAL SAFETY CABINET:

1. Immediately notify everyone in the lab/area of the spill. Remove any contaminated PPE/clothing and place in Biohazard bag to be autoclaved.

2. Put on clean disposable Personal Protective Equipment prior to initiating clean up.

3. Continue operating blower to help control any aerosols.

4. Lesser spills, even the smallest amount, should be **immediately** treated with specified disinfectant. After sufficient contact time, wipe up with paper towels.

5. Surfaces treated with 10% bleach should be rinsed immediately with sterile water to avoid damage to the surface metal of the cabinet.

6. Spills of greater volume require more extensive decontamination of cabinet surfaces with greater volumes of specified disinfectant. Allow the appropriate contact time, and clean up with absorbent materials followed by a sterile water rinse. Use Spill Kit if necessary.

7. Inform all users of the biosafety cabinet, as well as the laboratory technician and/or Principal Investigator, about the spill and status of clean-up as soon as possible.

8. For a major spill of BSL-2 material within a cabinet, the cabinet's fan, filters and airflow plenums should be decontaminated by formaldehyde gas procedures. Contact UCSI University Biosafety Office to schedule this procedure.

9. *DISPOSAL OF ABSORBENT AND CLEANING MATERIALS SATURATED WITH DISINFECTANT:*
    a. Collect in a blue Chemical Waste bag; contact appropriate service provider for disposal.
    b. Bags should not be overfilled, and should be closed securely.
    c. Place bags in secondary, autoclavable containers (a Nalgene or stainless steel pan) until pickup.
    d. Spray bag surface liberally with 70% ethanol and allow to dry.

SPILL OCCURING INSIDE LAB AND OUTSIDE BIOLOGICAL SAFETY CABINET:

1. NOTIFICATION: Immediately notify everyone in the lab/area of the spill. Remove contaminated PPE/clothing and place in biohazard bag to be autoclaved.

2. *LESSER SPILLS:* Put on clean disposable Personal Protective Equipment prior to initiating clean up. Clean up immediately with paper towels soaked in disinfectant.

3. ***MAJOR SPILLS:***

    WARNING: If people in the area are at significant risk or hazard as a result of a major spill, call 999 or 3189/5401 for campus security; ask that IBC be informed immediately.

    a. Clear area of all personnel, apply absorbent material on spill if safe to do so, exit room, close door and mark it with NO ENTRY (sign included in Spill Kit).

b. Notify the principal investigator or lab manager of the spill.

c. Wait 30 minutes for aerosol to settle before entering spill area. Assemble clean up materials and personal protective equipment during this time, using Spill Kit if needed.

d. Don appropriate PPE

e. Initiate clean-up as soon as possible following the 30 minute wait by placing absorbent material on spill and flooding spill area with specified disinfectant. Work from the outside of the spill and finishing in the center.

f. Allow appropriate contact time (at least 20 minutes).

g. Pick up absorbent material and place it in a biohazard bag.

h. Repeat placing absorbent material and flooding with disinfectant until you are convinced the decontamination is complete (at least twice). Finish with a water rinse.

i. Place contaminated **reusable** items in biohazard bags, or lidded, heat-resistant pans/containers with lids before autoclaving. Place large equipment in separate bags and place bags on a lab cart for transport to autoclave. Initiate further clean-up, if needed, after autoclaving.

j. Expose non-autoclavable materials to disinfectant for 20 minutes.

Inform all lab personnel as well as the laboratory supervisor/principal investigator about the spill and status of clean-up as soon as possible.

4 *DISPOSAL OF ABSORBENT AND CLEANING MATERIALS SATURATED WITH DISINFECTANT:*

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c. Place bags in secondary, autoclavable containers (a Nalgene or stainless steel pan) until pickup.
d. Spray bag surface liberally with 70% ethanol and allow to dry.

5 ***AUTOCLAVING CONTAMINATED, HEAT-RESISTENT ITEMS NOT TREATED WITH DISINFECTANT:***

a. Collect in a Biohazard autoclave bag.
b. Bags should not be filled more than 2/3 full. When full, close the bag securely.
c. Place bags in secondary, autoclavable containers (a Nalgene or stainless steel pan).
d. Spray bag surface liberally with 70% ethanol and allow to dry.
e. Immediately transport to autoclave/glassware room and decontaminate by autoclaving as soon as possible.
f. Immediately prior to autoclaving, loosen bag closure to allow steam penetration within bag.
g. Dispose of all decontaminated waste in Regulated Medical Waste boxes.

SPILL OCCURING IN HALL OUTSIDE OF LABORATORY:

1. Warn personnel in the immediate area of the spill. Block off spill area as best you can.
2. See section "Spill Occurring Inside Lab and Outside Biological Safety Cabinet" for instructions.

SPILL OCCURING INSIDE A SHAKING INCUBATOR

IMPORTANT:

- **Proceed quickly! Immediately turn off power to unit and unplug power cord from wall socket**.
- **Immediately notify everyone in the lab/area of the spill.**
- **If spill volume is large (>2 L), then close lid of incubator and call PI, lab manager or UBO for assistance.**
- **If spill can be safely contained and removed by lab personnel, proceed as follows:**

1. Remove any clothing contaminated with spill and place in Biohazard bag to be autoclaved. If skin is contaminated, treat with non-bleach disinfectant and follow with a soap & water rinse.
2. Quickly place paper towels on spill inside incubator to absorb liquid before it leaks onto motorized parts below, then close lid.
3. Ask someone to contact the principal investigator or lab manager to advise them of the spill while you retrieve: a) the Biohazard Spill Kit from its storage location in the lab, and b) a sufficient quantity of specified disinfectant.
4. DO NOT LEAVE THE ROOM WITHOUT PUTTING A SIGN ON THE INCUBATOR THAT SAYS "HAZARDOUS SPILL INSIDE—DO NOT OPEN OR USE!"
5. DO NOT MIX DISINFECTANT TREATMENTS IN YOUR CLEAN-UP, ESPECIALLY BLEACH AND ETHANOL.
6. If you are wearing no Person Protective Equipment, put on the PPE in the Spill Kit.
7. Check to see if spill liquid is leaking out from the unit onto bench or floor. If so, apply disinfectant to the spill liquid as well as in a perimeter around the spill; wait for disinfectant to take effect, then clean up with paper towels or other absorbent material from Spill Kit. Discard soaked material into Biohazard waste.
8. Now direct your attention to the interior of the incubator once again. Spilled liquid cannot be absorbed all at once because of the way shaking incubators are constructed, therefore you must work from top to bottom. Spray disinfectant over the soaked paper towels you applied earlier, then position a Biohazard autoclave bag as close as possible for discarding the towels. Place wet towels in bag carefully, minimizing aerosols and drips however possible.
9. Immediately apply more absorbent material to the spill if needed. Use pads, socks or pillows from the Spill Kit according to the volume of the spill and the size of the area to cover.
10. Spray interior surface areas of unit with disinfectant, especially any broken vessels associated with the spill. Wait for disinfectant to be effective.

11. Remove the pieces of broken vessels from the incubator interior; use forceps to avoid skin injury. Place broken glass in Sharps container; decontaminate by autoclaving as soon as possible.

12. At this point you may need to remove the incubator's platform to get to lower regions for further spill cleanup. Removal is often accomplished by using a hexagonal T wrench on 4 platform screws; a Phillips screwdriver may be needed to move flask clamps if they are covering the platform's hex screws. These tools should be located on or near the shaking incubator.

13. Thoroughly spray platform with disinfectant before removal, and give disinfectant time to work.

14. Before taking platform out of incubator, spray paper towels with disinfectant and use them to cover an area on lab floor upon which to place the platform. Choose an area of the floor that is out of your way. Place removed platform onto paper towels and perform a more thorough clean up later. Spray tools with disinfectant.

15. Apply absorbent material to any spill liquid you see in lower regions of the incubator. TRY TO ABSORB AS MUCH OF THE SPILL FROM AS MANY SURFACES AS YOU CAN.

16. Flood the absorbent material in the incubator with enough liquid disinfectant to decontaminate, but not so much as will create a gross excess in the spill area. Wait for disinfectant to be effective.

17. Place absorbent material saturated with disinfectant into blue Chemical Waste bags and securely close the bags. Appropriate service provider must be contacted for disposal. Bags must be kept in secondary containers while awaiting pickup.

18. For materials that can be decontaminated by autoclaving (containing no alcohol or bleach), place directly into Biohazard autoclave bags and close bags securely. Place bags in secondary containers when transporting to autoclave room. Decontaminate by autoclaving.

19. After all spilled material has been removed, disinfect every surface of the incubator that is accessible and repeat if necessary. Use cotton-tipped swabs for hard-to-reach areas. Do not use bleach on metal parts. If decontamination of enameled surfaces is performed with a bleach solution, apply a water rinse.

20. Put cleaned, dried platform back into position.

21. Leave lid incubator open for additional drying out.

22. Mop lab floor with disinfecting agent.

23. Autoclave any contaminated PPE.

24. Have a technical service provider test the incubator for electrical safety and proper function before returning it to service.

SPILL OCCURING INSIDE CENTIFUGE:

1. Leave centrifuge closed for at least 30 minutes for aerosol to settle. During this time, get clean up supplies ready, including Spill Kit if needed.
2. Flood spill area with specified disinfectant. Allow contact time of 20 minutes. All exposed surfaces should be disinfected, including heads, cups, cushions, etc.
3. Absorb spill with paper towels.
4. Flood area again with specified disinfectant. Allow 20 minutes contact time, then repeat clean up and finish with a water rinse.
5. All disposable materials used in clean up must be collected in a blue Chemical Waste bag. Place bag in secondary container and contact the appropriate service provider for disposal.

SPILL OCCURRING IN WATER BATH OR SHAKER BATH

1. Turn power off.
2. Pour specified disinfectant directly into water bath in sufficient quantity to effect decontamination.
3. Replace cover and wait for 20 minutes.
4. Discard the water/disinfectant solution by pouring down sink drain, and flush sink drain with water.
5. Disinfect the surfaces of the water/shaker bath, and allow to dry before returning unit to regular use.

SPILL OCCURRING IN INCUBATORS OR REFRIGERATORS

1. Minor spills which have not generated significant aerosols may be cleaned up with a paper towel soaked in disinfectant.
2. In the event of a major spill, the door should be left shut for 30 minutes to allow any aerosol to settle.
3. Cleanup should be initiated with specified disinfectant. Allowing for a contact time of 20 minutes, all exposed surfaces should be disinfected, including equipment, racks, tubes, bottles, etc.
4. Absorb the spill with paper towels, and flood the area again with the disinfectant.
5. After another 20 minute contact time, absorb with paper towels and finish clean up with a water rinse.
6. All disposables used in the cleanup procedure should be collected in a blue Chemical Waste bag. Place bag in secondary container and contact the appropriate service provider for disposal.

8. ROUTINE PROCEDURES

8.1 BIOSAFETY CABINET WORK PRACTICES

- Cabinet blowers should be operated at least 5 minutes before beginning work to allow cabinet to “purge”. This purge will remove any particulates in the cabinet.
- The work surface, the interior walls (not including the supply filter diffuser), and the interior surface of the window should be wiped with a solution of 70% of ethanol.
- The surfaces of all materials and containers placed into the cabinet should be wiped with 70% ethanol to reduce the introduction of contaminants to the cabinet environment.
- Place all necessary materials in the biosafety cabinet before beginning work. This will serve to minimize disruptions across the fragile air barrier of the cabinet.
- Disruption of the air curtain occurs with rapid movement of a worker’s arms into and out of the cabinet, compromising containment provided by the BSC. Moving arms in and out slowly, perpendicular to the face opening of the cabinet, to reduce this risk.
- Other personnel activities in the room (e.g., rapid movement, opening/closing room doors, etc.) may also disrupt the cabinet air barrier. For this reason, access to the work area is restricted when work is in progress.
- Before beginning work, adjust stool height so that your face is above the front opening.
- Manipulation of materials should be delayed for approximately 1 minute after placing the hands/arms inside the cabinet. This allows the cabinet to stabilize and to “air sweep” the hands and arms to remove surface microbial contaminants.
- When the user’s arms rest flatly across the front grille, room air may flow directly into the work area, rather than being drawn through the front grille. Raising the arms slightly will alleviate this problem. The front grille must not be blocked with research notes, discarded plastic wrappers, pipetting devices, etc.
- All operations should be performed on the work surface at least 4 inches from the inside edge of the front grille.
- Equipment that causes turbulence (centrifuge, vortex, etc.) should be placed in the back 1/3 of the work surface. All other work in the cabinet should stop while the apparatus is running.
- **The use of open flames such as Bunsen burners inside the biological safety cabinet is not recommended** as the open flame creates turbulence that disrupts the laminar HEPA filtered airflow and could expose personnel to potentially infectious material. Use of a Bacti-Cinerator, glass bead sterilizer or disposable loops is recommended.
- Separate clean and contaminated items. Minimize movement of contaminated items over clean items (work from clean to dirty).
- Only the materials and equipment required for immediate work should be placed in the BSC. Do not use as a storage area.

- Prior to removal from the cabinet pipettes and other material will either be decontaminated or placed into a sealed container which is disinfected on the outside,
- All vacuum lines must have in-line filters and traps containing disinfectant; all vacuum filtering takes place in the biosafety cabinet. (See In-Line Filters SOP for details).

- At the end of the work session, all materials are surface decontaminated and removed from the cabinet. The work surface, the interior walls, and the interior surface of the window are again wiped with 70% ethanol. UV light may be turned ON for 30 minutes as an additional precaution.

8.2 POTENTIALLY INFECTIOUS WASTE

Contaminated items generated in a biosafety cabinet can be removed after:

- Being decontaminated with disinfectant (rinsed in bleach solution or sprayed with 70%ethanol).
- Placement in a small SHARPS container, disposable pipette box or small Biohazard autoclave bag located inside cabinet, which will be closed and wiped with disinfectant before removal

Liquid BSL-1/2 and/or rDNA Molecule Wastes:

- Liquid waste will be discarded into a container containing a sufficient quantity of 100 ml household bleach
- After an exposure time of 30 minutes in a biosafety cabinet the liquid waste + disinfectant may be disposed of down the lab sink drain, followed by a water flush.
- Alternatively, liquid waste may be poured into a well-labeled autoclavable container (containing no bleach), decontaminated by autoclaving on a liquid cycle, at 121 degrees C, with 15 minutes sterilize time and then flushed down the drain.
- Liquid decontamination method(s) will be verified and documented.
- Waste containers must be appropriately labeled as such, with biohazard signage.

Solid BSL-1/2 and/or rDNA Molecule Wastes:

- Are collected in an ORANGE autoclave bag with a Biohazard symbol.
- Autoclave bags for filling should be kept inside appropriately labeled biowaste container, equipped with a closeable lid.
- Container should remain closed except when in use.
- At end of session or when 2/3 full, the autoclave bag is securely closed, placed in a secondary container labeled for this purpose, and sprayed with 70% ethanol prior to transport on a cart to the autoclave room.

- Bags will be autoclaved on a pre-vacuum cycle at 121 degrees C with 15 minutes sterilize time

- Bags will be autoclaved daily when possible, or as soon as an autoclave is available. No full bags should be left in the laboratory or the autoclave/glassware room OVER THE WEEKEND awaiting decontamination.
- Bags should remain securely closed *until going into the autoclave, at which time their closures should be loosened to allow steam penetration.*
- All BSL-2 solid waste is to be decontaminated by autoclaving and disposed of as Regulated Medical Waste.

8.3 USE OF SHARPS

- Use of sharp objects such as Pasteur pipettes, syringe needles, razors, glass slides, etc. will be utilized only when an adequate, less hazardous substitute cannot be found. Plastic ware will be substituted for glassware whenever possible.
- **In Section 13, identify each sharp being used, the procedure involved and describe what precautions (e.g., PPE, equipment, technique/procedure) will be taken to minimize the risk(s) associated with the sharp.**
- Personnel will be trained for safe use and disposal of sharps in work areas.
- Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
- Contaminated sharps will be disposed of in designated hard-sided Sharps containers; containers will be securely closed before transport to autoclave room for decontamination. All sharps containers are to be autoclaved before being disposed of in Regulated Medical Waste.
- Broken glassware will not be handled directly. It must be removed using a brush and dustpan, tongs or forceps.

NOTE: In situations when collection of Sharps is intermittent or of smaller volume, avoid collecting them in large sharps containers. Use smaller Sharps containers whenever possible, as these will fill more quickly and thus can be decontaminated more expeditiously.

8.4 PIPETTES

- Disposable pipettes used in the biosafety cabinet will be discarded directly into lidded sharps containers, submerged in a horizontal container with appropriate disinfectant, or discarded into a disposable pipette collection box within the biosafety cabinet.
- It is important for disposable pipettes to be discarded into appropriate receptacles inside biosafety cabinets to avoid repeatedly breaking the air barrier of the cabinet by discarding in a receptacle outside the cabinet.
- Aspirating 5% bleach solution through a pipette before discarding is recommended.
- Pipette receptacles will be closed when full and/or at the end of a work session. Autoclave in a timely way to decontaminate, then discard in Regulated Medical Waste.

- In situations when Sharps use is intermittent or of lesser volume, they will be discarded in smaller Sharps containers. These will fill and be removed more quickly than a larger Sharps container which may harbor contaminated materials for weeks before being filled removed from the lab.

8.5 PIPETTING

- *Never mouth pipette*. Automatic pipettors are to be used for all material.
- Automatic pipetters utilized in the BSL-2 Biosafety Cabinet will be properly identified and reserved for BLS-2 work. They will be sprayed within the hood with 70% ethanol disinfectant before work begins, and after work is finished.
- Larger volume pipettes to be utilized with the pipette aid device are recommended to be the disposable plastic type, with cotton plugged ends; this prevents aspiration of fluid or aerosols into the pipetting device.
- Avoid the following practices when pipetting:
  - Do not mix biohazardous fluids by repeated suction and expulsion from pipettes, which generates aerosols.
  - Do not bubble air through biohazardous fluids, which also generates aerosols.
  - Do not forcibly expel liquids from pipettes. Discharge as close as possible to the fluid or down the side of the container.
  - Avoid accidentally dripping infectious liquids from pipettes.

8.6 CENTRIFUGATION

- Be completely familiar with your project's risk assessment and lab-specific SOPs, and follow their guidelines when undertaking a centrifugation step with BSL-2 materials and/or rDNA molecules. (Example: loading rotors with BSL-2 materials inside a biosafety cabinet when indicated.)
- Do not run BSL-2 materials in the centrifuge without taking appropriate safety precautions and using required PPE.
- Make sure a Biohazard Spill Kit is available when using the centrifuge.
- All centrifuges used with BSL-2 materials and/or rDNA molecules must be labeled with the Biohazard symbol.
- If sealed rotors or aerosol tight safety caps are not available, wait at least 5 minutes after the spin has stopped before opening the centrifuge lid.
- Upon opening the centrifuge for spinning BSL-2 materials and/or rDNA molecules, if it is discovered that there has been a release of liquid (outside of the sealed rotor bucket), close the centrifuge immediately and wait at least 15 minutes before initiating clean up to allow aerosols to settle. Then follow "**Spill Procedure Inside Centrifuge**," found in this manual.
- Some BSL-2 agents require that aerosol protection, such as respirators, be available in case of a spill when centrifuging. Consult with EHS to fulfill this safety requirement, if applicable.
- After using a centrifuge for a BSL-2 agent and/or rDNA molecules, it is recommended to always wipe down the instrument with disinfectant.

9. PERSONNEL

9.1 TRAINING REQUIREMENTS

- All personnel working in a BSL-2 laboratory will receive general awareness training which relates to all infectious organisms/material and/or rDNA molecules being used within the lab whether they are working with the material or not.

- At a minimum the following training will be completed by each person directly performing work with the infectious material and/or rDNA molecules:

  - Biosafety and Biosecurity online training through the Collaborative Institutional Training Initiative (CITI)

  - Specific training on the NIH Guidelines for Research Involving Recombinant DNA molecules

  - Specific training on aseptic techniques used in the experiments

  - Specific BSL-2 training provided by the PI or Laboratory Manager/ Supervisor (see BSL-2 Training Record)

  - Introduction to Biological Safety Cabinets

  - Selected additional training provided by other relevant bodies/institute/government agencies of topics related to biosafety and biosecurity.

  - Individual BSL-2 training records (found in this manual) must be completed for each person working directly with BSL- 2 agents.

- All personnel will abide by the BSL-2 protocols learned in training.

- The PI or Laboratory Manager will ensure and document that laboratory personnel demonstrate proficiency in standard and special microbiological practices at BSL-2 before working with any potentially infectious material.

- Personnel will receive annual updates or additional training when procedural or policy changes occur.

- The BSL-2 Training Record is found on the next page. This table is the required document for use as an individual training record for every person working in a BSL-2 laboratory.

9.2 TRAINING RECORDS FOR LAB PERSONNEL

- Signed training records will be retained for the duration of each person's employment and for at least 3 years after.

- All BSL-2 training will be documented and kept in Biosafety Manual for the laboratory.

9.3 MEDICAL SURVEILLANCE

- All personnel working with BL2/BSL-2 material will complete a medical survey questionnaire and update it at least annually or whenever their exposure to hazardous material changes.

- Laboratory personnel will be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory as determined by the risk assessment.

- Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly, but not limited to, women of child-bearing age, immunocompromised individuals, persons suffering from chronic inflammatory conditions, cancer patients, organ transplant recipients, patients undergoing chemotherapy, radiotherapy, or immunosuppressive therapy will be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these or other medical conditions will be encouraged to self-identify to MOH or the institution's healthcare provider for appropriate confidential counseling and guidance.

- All personnel will monitor their daily health status and for signs and symptoms of disease consistent with the agents being manipulated or worked with in the lab. Self-reporting of symptoms to the PI and/or lab manager or MOH is encouraged.

- A medical evaluation will be provided for individuals following an exposure incident to blood, blood products or other potentially infectious material.

- Records of medical surveillance testing, including any testing deemed necessary, are maintained by MOH.

- Records of medical surveillance, if any, will be kept for duration of each person's employment and for at least 3 years after.

10. BSL-2 TRAINING RECORD FOR _____

P.I. LABORATORY _____

TRAINING TOPIC	LOCATION OF INFORMATION ON TOPIC:	REVIEWED ON: (DATE/TRAINEE INITIALS)	REVIEWED BY: (TRAINER INITIALS)
BSL-2 Agent or Agents Used in the Lab:			
1. Specific hazards of BSL-2 agents (e.g., modes of transmission, signs and symptoms of disease, etc.)	Risk Assessment/MSDS		
2. Methods of disinfection & decontamination for specific BSL-2 agents	Risk Assessment/MSDS		
3. Rationale for use of containment equipment with BSL-2 agents to minimize exposure risks.	Risk Assessment/BSL-2 Manual		
4. Ordering, shipping & receiving procedures for specific BSL-2 agents			
5. Handling and storage for specific BSL-2 agents			
6. NIH guidelines for Research involving Recombinant DNA molecules	NIH Guidelines for Research Involving Recombinant DNA molecules		
Personal Protective Equipment, Lab Equipment & Lab Safety:			
1. PPE needed; location where PPE supplied in lab; proper use of PPE	Biosafety Manual		
2. Operation procedures for biosafety cabinets	Biosafety Manual/SOP		
3. Use & maintenance of pipetting aids	Lab-specific SOP		
4. Safe use & maintenance of centrifuges, incubators, etc.	Lab-specific SOP		
Emergency Response Training			
1. Procedure for spills & leaks; use of spill kit	Biosafety Manual		
2. Decontamination techniques	Biosafety Manual		
3. Fire/disaster response	Biosafety Manual		
4. Power failure procedure	Biosafety Manual		
Waste Disposal Procedure			
1. Collection, storage & disposal procedures for BSL-2 waste	Biosafety Manual		
2. Segregation & destination of BSL-2 waste	Biosafety Manual		
3. Decontamination/disinfection methods & efficacy	Biosafety Manual		

This individual under my supervision has demonstrated proficiency in standard and special BSL-2 procedures used in this laboratory.

_____***(PI or Lab Manager signature)*** _____***Date***

SIGNATURE PAGE

This manual MUST be read before beginning work in this Laboratory.
Please acknowledge that you have read this manual by printing and signing your name below.

PRINT NAME	SIGN NAME	DATE

12. SOURCES

Material in this manual has been obtained from the following sources:

1. *BSL-2 Laboratory Operations Manual,* VA-MD Regional College of Veterinary Medicine, October 2006.

2. "Primary Containment for Biohazards," Office of Health and Safety Information System, CDC.

3. "Biosafety Cabinet Work Practices," University of Maryland Biosafety Office.

4. *Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition,* U.S. Department of Health and Human Services Public Health Services, Centers for Disease Control and Prevention and National Institutes of Health.

5. *Laboratory Biosafety Manual*, World Health Organization, 3rd edition, 2004.

6. *NIH Guidelines for Research Involving Recombinant DNA Molecules*

13. RISK ASSESSMENT

Scope / Area / Section : Research Molecular Lab

Note:
"**R**"= Routine (daily activity)
"**NR**" = Non-routine / Ad-hoc.
"**E**" = Emergency Situation

Overall Risk Ranking
= **Severity (S) X Likelihood (L)**

Risk	Description	Action
15 ~ 25	High	A HIGH risk requires immediate action to control the hazard as detailed in the hierarchy of control. Actions taken must be documented and include date for completion.
5-12	Medium	A MEDIUM risk requires a planned approach to controlling the hazard and applies temporary measure if required. Actions taken must be documented and include date for completion.
1-4	Low	A risk identified as LOW may be considered as acceptable and further reduction may not be necessary. However, if the risk can be resolved quickly and efficiently, control measures should be implemented and recorded.

Severity	Ranking	Definition	Likelihood	Ranking	Definition
Catastrophic	5	Numerous fatalities, irrecoverable productivity.	Most Likely	5	The most likely result of the hazard / event being realized
Fatal	4	Approximately one single fatality / if hazard is realized	Possible	4	A good chance of occurring and is not unusual
Serious	3	Non-fatal injury, permanent disability	Conceivable	3	Might occur at sometimes in future
Minor	2	Disabling but not permanent injury	Remote	2	Has not been known to occur after many years
Negligible	1	Minor abrasions, bruises, cuts, first aid type injury.	Inconceivable	1	Is practically impossible and has never occurred

Process / Activity / Area	R / NR	Hazard (Hazard characteristics)	Risk Assessment					Ref to Applicable legal requirement/further actions to be taken	PIC (Due date / status)
			Current Control Measure	Severity (S)	Likelihood (L)	Overall Risk (L / M / H)	Legal & oths req. (LE)		
Research molecular lab, signage	R	Physical, biological and Chemical: no signage for fire extinguisher and first aid kit	none	1	2	L	Yes	Looking for supplier for signage	GLMO
Research molecular lab, transporting chemical to and from store.	R	Ergonomic and chemical: incorrect position of carrying heavy chemical, and chemical might drop and create spillage	Using bottle carry aid and trolley	2	3	M	Yes	Training on ergonomic and chemical handling	Lab assistance and lab tech
Research molecular lab, transporting waste chemical to chemical waste storage area	R	Ergonomic, chemical and biological: incorrect position of carrying heavy loads, waste bottle have leak fumes and waste storage area is bad in shape with grass and uneven surface	Use PPE like glove, face mask and lab coat.	2	2	M	Yes	Seeking advice and solution from GLMO	Lab manager and GLMO team
Research molecular lab, Washing of glassware.	R	Physical: might get cut from glassware's crack	Wear glove and replace defective glassware	1	3	L	Yes	Caution while performing task	Lab Assistance and lab tech

Signature: Prepared by: Jason Lim Lai Huat Date:	Signature: Reviewed and Approved by: Date:

14. BSL-2 AGENT-SPECIFIC HAZARDS/PRECAUTIONS

INSTRUCTIONS:

Please use this form to describe agent-specific procedural precautions, additional information, special handlings, PPE, concerns, etc. which are NOT covered in this manual. Fill out a separate form for each BSL-2 agent to which this stipulation applies.

FORM COMPLETED BY: Click here to enter text.

DATE: Click here to enter text.

BSL-2 AGENT: Click here to enter text.

HAZARDS/PRECAUTIONS: Click here to enter text.

15. HIGHER RISK PROCEDURES/LOCATIONS SUMMARY

BSL-2 containment includes

1) **Work involving recombinant DNA (rDNA) molecules and/or work involving agents that pose moderate hazards to personnel and the environment.**

2) **All work will be in compliance with the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition and NIH Guidelines for Research Involving Recombinant DNA molecules.**

IMPORTANT

Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly, but not limited to, women of child-bearing age, immunocompromised individuals, persons suffering from chronic inflammatory conditions, cancer patients, organ transplant recipients, patients undergoing chemotherapy, radiotherapy, or immunosuppressive therapy will be provided with information regarding immune competence and conditions that may predispose them to infection.

15.1 Location and Procedure-OUTSIDE Biological Safety Cabinet

List and briefly describe location of how containment will be maintained for ALL BSL-2 procedures taking place OUTSIDE THE BIOLOGICAL SAFETY CABINET.

(X)	**BSL2 Procedure(s)** (taking place outside BSC)	**Containment:** Briefly describe how containment will be maintained (e.g., sealed rotors)	**Containment is FULLY described in SOP_____ (specify SOP title/section)**	**Room #**
	Pipetting			
	Pouring			
	Shaking			
	Vortexing			
	Mixing			
	Homogenizing			
	Sonicating			
	Lyophilizing			
	Manipulation of animals (specimen collection, necropsy, surgery)			
	Opening Vacuum vials			
	Other			

15.2 Animal Use

Will animals be used for any procedure within the BSL-2 lab space? If YES, complete this table. If NO, skip to the next table.

***Animal**	**Procedure(s):**	**Describe the specific precaution(s)** (e.g., PPE, equipment, technique/procedure, disposal) that will be used to minimize risk.	**Room #**

***** *ABSL Hazard Summary Sheet must be completed and submitted to the animal facility supervisor or manager.*

15.3 Sharps

List all types of sharps that will be used, the procedure for each and describe the specific precautions in place to minimize sharps-associated risk.

Sharp (e.g., needle, scalpel, glass slide, razor, Pasteur pipette**)**	**Procedure** (e.g., injection, microscopy, isolating gel band, etc.)	**Describe the specific precaution(s)** (e.g., PPE, equipment, technique/procedure, disposal) that will be used to minimize the risk associated with the sharp

15.4 Centrifugation

Document centrifugation contaminant processes below.

1) **Select the option that describes the centrifugation procedure (1, 2, or 3).**
2) **The PI must initial next to the selected option and follow the process instructions for Option 2.**

Option	Centrifugation Procedure(s):	Process	P.I. INITIALS
1	• We will use **sealed rotors or aerosol-tight caps on rotor buckets** when centrifuging BSL-2 agents.	• We will follow the mandatory practice of loading and sealing all BSL-2 materials in rotors/buckets inside the biosafety cabinet, and wiping them with disinfectant before placing into the centrifuge. • We will unload rotors and/or buckets in the biosafety cabinet after centrifuging	
2	When centrifuging BSL-2 agents, we will use **non-sealed rotors or buckets that do not have aerosol-tight caps,** which we acknowledge is a practice that is not recommended and that creates an increased risk of potential exposure to infectious material. We will wait at least 5 minutes after the spin has stopped before opening the centrifuge lid.	**Provide an SOP in Section 16 detailing the centrifugation process for reduction of aerosols.** **(SOP must include all containment steps taken throughout the procedure)**	
3	We will not be centrifuging BSL-2 material.	**N/A**	

16. STANDARD OPERATING PROCEDURES (S0P)

INSTRUCTIONS:

1. Add specific procedures relating to **containment or disposal** of potential infections materials that are **NOT otherwise addressed in this manual**.

2. Add any building, area or department operating procedures that you will be following that are **NOT otherwise addressed in this manual**. (e.g., biowaste decontamination and disposal, biological safety cabinet use, using the biohazard spill kit, etc.

17. APPENDICES

INSTRUCTIONS:

1. Complete the below ***"Agent and Materials Summary"*** IBC document.

2. Attach and maintain a current **lab sketch** of labs included in this manual**.**

3. Attach any other relevant lab specific information not already included in this manual.

AGENTS AND BIOLOGICAL MATERIALS FORM

Please identify all biological agents and materials used in your program or study. Try to provide as much categorical, descriptive and source information as you can. Some categories may not apply to your study. Add rows if necessary. Submit the completed form to IBC.

Principal Investigator: Type of application: ☐New ☐Amendment

Program or Study Title:

Material Type	Agent or Material Name and/or Description	Strain/Isolate/Generation	Origin or Recipient	Source and/or vendor	Risk Group [1]	Biosafety Level [2]	Pathogen [3]		
(E.g. Animal, Bacterium, Cell Line, Fungus, Parasite, Plant, Soil, Tissue, Toxin, Virus, etc.)	Include Genus and Species. If virus, indicate if wild type or an engineered viral vector.	(E.g. Human adenovirus 4 *RI-67* or Acute T Cell Leukemia Jurkat, *Clone E6-1*)	Indicate whether agent or material is the origin or recipient of DNA, RNA, or rDNA pathogen.	(E.g. ATCC, NDRI, volunteers, Jax Mice, etc.)	1,2,3	1,2,3	Please check all that apply		
							Human	Animal	Plant
							☐	☐	☐
							☐	☐	☐
							☐	☐	☐
							☐	☐	☐
							☐	☐	☐
							☐	☐	☐

[1,2] **RG & BSL:** Identify the NIH Risk Group (RG) & Biosafety Level (BSL). In most cases the RG determines the BL (e.g., RG2 materials are usually handled at BL2).

[3] **BBP:** Blood-borne pathogen (BBP) materials are BBP agents & human or other materials covered by the OSHA BBP Standard (e.g., HIV, Hepatitis B, blood, human cells and cell lines, or unfixed tissues other than skin) BBP materials are categorized as RG2 & typically require BL2.