**This is a template and must be tailored to the individual’s specific lab. Please review the manual and make changes accordingly. For questions, contact the University’s Biosafety Office at hoffman55@marshall.edu.**

Marshall University

BSL-2 BIOSAFETY MANUAL



Dr. \_\_\_’s Lab

\_\_\_ Building

Rooms \_\_\_

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# I. INTRODUCTION AND DESCRIPTION OF RESEARCH

Insert brief description of research.

**Biological agents/organisms used:**

(include bacterial, viral, fungal, recombinant, human/non-human primate unfixed tissues or cells)

*List organisms/biohazards here*

**Risk Assessment:**

Please provide a brief protocol-specific risk assessment. Include consideration of parent and recombinant agent pathogenicity, virulence, infectious dose, route of transmission, host range, and stability, as well as the likelihood of exposure and consequences of exposure. How will identified risks be controlled (e.g. PPE, work practices, etc.)? Note that “replication incompetent” does not mean “non-infectious.”

All research involving recombinant DNA (rDNA), synthetic nucleic acids (sDNA), infectious agents, human cell lines, select agents or other biological toxins must be reviewed by the Institutional Biosafety Committee (IBC) regardless of funding source according to the *NIH Guidelines for Research Involving Recombinant DNA Molecules*. Since the university receives funding from NIH, all research at UM must comply with the *NIH Guidelines*.

Applicants must complete Sections I-VII as directed. If you have questions, please contact Vincent Sollars, IBC Chair, at 304-696-7357 or sollars@marshall.edu. Consult the NIH Guidelines for information on rDNA regulations. Approved applications will be valid for a period of three years from the time of approval.

This plan was designed as a supplement to the University Biosafety Manual to meet the requirements of *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed.

# II. TRAINING

All researchers, staff, and students of submitting a proposal to the Biosafety Committee are required to complete an educational course. The educational course utilized by Marshall University is the Collaborative Institutional Training Initiative (CITI). The steps for completing the educational requirements can also be found on the ORI educational website at <http://www.marshall.edu/ori/human-subject-research/education/>. All personnel who work in the laboratory must receive adequate instruction from their supervisor prior to beginning work. Some training is required annually. Each lab will require different training. For biosafety, the IBC and EHS recommends: (tailor to your own specific lab needs; some general policies are below)

* General Lab Safety Training provided by Marshall within the last year.
* All lab personnel working in BSL 2 labs or with nonexempt rDNA have taken Biosafety/Bloodborne Pathogens training provided by Marshall University within the last year.
* The IBC requires all MU investigators with pending or active rDNA-infectious agent applications to complete the CITI Initial Biosafety Training course. All members of an applicant’s lab (including postdocs, students and staff) who are listed on the rDNA-infectious agent application must also complete Initial Biosafety Training. The applicant’s certification is valid for three years. CITI will provide reminders as the applicant’s certification end date approaches.
* If an applicant checks yes to Question 14 (Does your project involve use of rDNA or synthetic nucleic acids as defined by the NIH Guidelines?) in Section II of the application, the applicant and members of the applicant’s lab must also complete the NIH Recombinant DNA Guidelines course in CITI
* If an applicant checks yes to question 41 (Does this project involve human sera or tissue?) of the application or question 46 (Does this project involve human cell lines), the applicant and members of the applicant’s lab must also complete the OSHA Bloodborne Pathogens course. This does not apply to cell lines that have been verified to be free of any human pathogens.
* If a faculty member doesn’t have a pending or active rDNA application, the IBC recommends that the faculty member and members of the applicant’s lab complete the CITI Initial Biosafety Training course and if appropriate the NIH Recombinant DNA Guidelines and the OSHA Bloodborne Pathogens courses.
* The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, potential hazards present in the laboratory, and exposure evaluation procedures. **This training must be documented,** including general site-specific training and biological site-specific training.

Some work may require an occupational health plan, including annual physicals, pulmonary function test and fit test for use of a respirator, vaccinations, serum testing, and/or other elements of a medical plan.

The PI is required to inform laboratorians and animal handlers of any risks to immunocompromised individuals and should encourage those individuals to consult with their health care provider about these risk

**III. LABORATORY SIGNAGE**

To assist Emergency Responders, all rooms and laboratories at Marshall University that house biohazard materials must be labeled with a Biohazard sign. A [Biohazard Sign Template](https://view.officeapps.live.com/op/view.aspx?src=https%3A%2F%2Fwww.marshall.edu%2Fsafety%2Ffiles%2F2013%2F03%2FBiohazard-Sign-Template.doc&wdOrigin=BROWSELINK) is available in Word and instructions are provided.

Shape

Description automatically generated

Complete the biohazard agent(s) and contact information, then post sign on the outside of the laboratory door to make emergency responders aware of the hazards inside the room. Update as needed, when contact person or biohazard agent(s) change. Contact Marshall Environmental Health and Safey or the IBC, with questions.

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# IV. USE OF BIOLOGICAL SAFETY CABINET

The biosafety cabinet (BSC) is the primary means of protecting the researcher, the product, and the environment from biological hazards. All work with infectious agents should be manipulated in the BSC, especially those practices which could generate aerosols. Using the BSC properly includes the following:

* 1. Turn on cabinet fan 15 minutes before beginning work
  2. Disinfect the cabinet work surface with 70% ethanol or other disinfectant such as 10% bleach and wipe surfaces.
  3. Place supplies in the cabinet. Locate container inside the cabinet for disposal of pipettes orange bags need to be used for biohazards. (Movement of hands in and out of the cabinet to discard pipettes into a container located outside of the cabinet creates turbulence and disrupts the air barrier that maintains sterility inside the cabinet.)
  4. Work as far to the back (beyond the air split) of the BSC workspace as possible.
  5. Always use mechanical pipetting aids.
  6. Avoid using open flames inside BSCs. If a flame is necessary, use a burner with a pilot light and place it to the rear of the workspace. Flames disrupt the airflow and contribute to the heat load inside the BSC. Flames have burned holes through HEPA filters and have caused explosions in BSCs.
  7. Do not work in a BSC while a warning light or alarm is signaling.
  8. Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC may affect proper airflow and the level of protection provided.
  9. Keep the front and rear grilles clear.
  10. When work is completed, remove equipment and supplies from the cabinet. Wipe the work area with disinfectant and allow the cabinet to run for 15 minutes. For biological waste, it is recommended to treat with 10% bleach and follow with autoclaving when working with any BSL 2 pathogen.
  11. Some BSCs are equipped with ultraviolet (UV) lights. If one is used, the tube should be wiped with disinfectant every two weeks, while turned off, to remove dust. UV radiation should not take the place of disinfectant of the cabinet interior.
  12. The UV lamp should never be on while an operator is working in the cabinet.
  13. Minimize traffic around the biosafety cabinet and avoid drafts from doors and air conditioning.
  14. The BSC is certified annually by a contractor. If certificate is out of date please contact the biosafety officer.
  15. Vacuum lines are protected with liquid disinfectant trap, in line HEPA filters, and drying agent trap.
  16. All biological waste, including aspirators, is stored in a secondary container.
  17. Ensure disinfection after work is complete with an approved disinfectant

# V. USE AND DISPOSAL OF SHARPS

To prevent needle stick injuries:

* Avoid using needles whenever possible.
* Replace glass materials with plastic (such as Pasteur pipettes)
* Do not bend, break, or otherwise manipulate needles by hand.
* Do not recap needles by hand. Do not remove needles from syringes.
* Immediately after use, discard needle and syringe (whether contaminated or not) into puncture resistant sharps containers. RECAPPING OF NEEDLES IS PROHIBITED.
* Never discard sharps into regular trash.
* Never discard sharps into bags of biological waste.
* Use care and caution when cleaning up after procedures that require the use of syringes and needles.
* Do not overfill sharps containers. Close completely when 2/3-3/4 full.
* Take biohazard sharps to be autoclaved in BBSC 119
* Locate sharps containers in areas in which needles are commonly used. Make containers easily accessible.

In the event of a needle stick injury:

Wash the area thoroughly with soap and water. Notify supervisor immediately and will out a Workplace Injury/Illness Report Form [Microsoft Word - HR-SERV-FORM-31 (marshall.edu)](https://www.marshall.edu/safety/files/2013/04/HR-SERV-FORM-31.pdf).

# VI. SPILL RESPONSE PROCEDURES

Use the guidelines below for response to spills of biological materials outside of the biosafety cabinet.

A. Infectious medical waste spill containment and cleanup kits will be located in each satellite autoclave room (Rooms 238, 333, 432) and in the main autoclave facility on the first floor (Rooms 119 and 121 BBSC) to allow for rapid and efficient cleanup of spills

B. Immediately following a spill of infectious medical waste or its discovery, all personnel must leave the area until any aerosol settles or until the spill is cleaned up. Personnel must notify one of the following

* Julia Schreiber, Dept. of Biomedical Sciences, at 304-696-3714
* Austin Hoffman, in the MU Environmental Health and Safety office at 696-2563
* Vincent Sollars, IBC chair at 304-696-7357
* MUPD at 304-696-4357

Cleanup personnel shall implement the following proce­dures for cleaning up a spill:

(1) Secure the area from entry by unauthorized persons;

(2) Put on disposable cleanup lab coats appropriate for the level and nature of the spill, prepare disinfectant solution, and collect necessary equipment;

(3) Spray all broken containers and spills of infectious medical waste with disinfectant such as bleach and allow contact with the disinfectant for at least 15 minutes;

(4) Place broken containers and spillage in the packing bags in the kit;

(5) Remove liquids with absorbent material and wipe spill area dry;

(6) Disinfect area again and allow to air dry;

(7) Transport all waste to the autoclave room (Biotech Center Room 119A);

(8) Clean and disinfect non-disposable items and cloth­ing using the BBSC

Laundry Guidelines;

(9) Remove cleanup lab coats/outfits and place disposable items in a biohazard-labeled orange plastic bag; and

(10) Replenish the containment and cleanup kit.

(11) Complete a Spill Occurrence Report. Form is available on the Institutional Biosafety Committee web site: <http://musom.marshall.edu/biosafety/>

C. Small Spills. When a spill involves a single container of infectious medical waste with a weight of less than fifty (50) lbs., or a volume of spilled liquid of less than one (1) quart, the individ­ual responsi­ble for the cleanup should select protective equipment and proce­dures that are appropriate to the level and nature of the spill. Any proposed alternate proce­dures for small quantity spills should provide protection to the health of workers and the public equivalent to that provided by the procedures above. A Spill Occurrence Report must be submitted after cleanup is complete.

The BMBL recommends that chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant. If upholstered chairs cannot be replaced with non-porous ones, please address spills on these surfaces by developing a procedure for cleaning the chair cushion.

# VII. WASTE DISPOSAL PROCEDURES

All personnel are responsible for maintaining a clean work area. Only trained individuals should operate the autoclave.

1. Solid infectious materials

Solid infectious materials (used pipettes, flasks, Petri dishes, etc) must be disposed of in orange biohazard waste bags to be autoclaved. Waste should be double bagged and placed in one of the gray bins in 119. The autoclave should be run for one hour or sufficient time to fully decontaminate the waste. Contact Julia Schreiber for more information on Marshall’s autoclaves.

1. Liquid infectious waste

Liquid infectious wastes, such as spent media, is to be treated with 10% bleach for >30mins and then autoclaved before being disposed of in the sanitary sewer.

1. Uncontaminated waste

Uncontaminated non-sharp waste should be disposed of in the general lab waste stream.

Uncontaminated broken glass is disposed of in a sturdy cardboard box, lined with a plastic bag. When full, the box should be taped closed and disposed of in the dumpster. Housekeeping will not dispose of broken glass.

1. Sharps disposal

Sharps are items which pose a puncture or cutting hazard, such as glass, needles, pipette tips and razors. Sharps should be disposed of in approved autoclave-resistant puncture-proof containers. Please refer to section IV of this manual for more information.

1. Animal Carcasses

Place animal carcasses/tissues into a plastic bag. Animal carcasses/tissues that have been subject to biohazardous agents should be place in red biohazard bags. Non biohazardous animal carcasses/tissues may go out in the regular trash; however, disposal of biohazardous animal carcasses/tissues is coordinated through the Animal Resource Facility (ARF). Contact ARF for removal and disposal.

1. Disposal of waste into dumpsters

Waste bags should not be left sitting in the laboratory or autoclave room for more than a few hours. If the dumpster is full, trash bags may NOT be discarded outside the dumpster. Bags must be returned to the lab and disposed of when the dumpster has been emptied.

# VIII. EMERGENCY PROCEDURES

1. Fire evacuation procedures

During a fire emergency, lab staff should prioritize life safety. Cultures and animals may be put away if time allows; if not, walk to the nearest exit. Pull the fire alarm if necessary, and call 911 once outside the building.

1. Power outage

In the event of a power outage, put away cultures and animals. Remove PPE and exit the lab normally. Emergency lighting within the buildings should provide adequate visibility to exit the building. Notify the PI immediately.

1. Medical emergency

In the event of a medical emergency in the lab, follow appropriate procedures depending on the hazards present. If the emergency involves a spill of hazardous agent onto the clothing or body, assist the victim to the shower or eyewash station. If the victim requires medical attention, call 911 or go to Cabell Huntington Hospital ER for assistance.

1. Accidental exposure or needlestick

For splashes to the eyes, rinse the eyes under the eyewash for 15 minutes. If there has been a needlestick, wash the affected area thoroughly with soap and water, then report to the Health and Safety Department. Refer to the Post Exposure Fact Sheet.

# IX. Post-Exposure Fact Sheet

**Post-Exposure Procedures (EXAMPLE):**

***Salmonella typhimiruim***

**Characteristics:** Family Enterobacteriaceae; gram negative rod; motile, aerobic and facultatively anaerobic; serological identification of somatic and flagellar antigens; over 2000 serotypes capable of causing disease.

**Incubation Period:** Six to 72 hours, usually about 12-36 hours

**Symptoms:** Salmonellosis is an acute gastroenteritis; acute infectious disease with sudden onset of abdominal pain, diarrhea, nausea and vomiting; may progress to more serious septicemia, includes focal infections, abscesses, endocarditis, pneumonia; may also cause typhoid like enteric fever; some cases develop reactive arthritis (Reiter's syndrome) which may become chronic

**Infectious Dose:** 100 - 1,000 organisms - ingestion; varies with multiple factors

**What is a potential exposure?** Ingestion, needlestick or cut with contaminated sharp object, splash to the eye, contact with broken skin.

**Post-Exposure Treatment:** Skin exposure / Percutaneous: Wash affected area and apply antiseptic (3% H2O2), report to the Health Center. Mucous membrane exposure (splash to eye): flush eyes for 15 minutes using eyewash, then report to Health Center. Ingestion: Report to the UM Health Center. Antibiotic therapy may be required. If the Health Center is closed, the *After Hours NurseLine* is available at **(301) 314-9386**.

**If symptoms appear with no known incidence of exposure:** Seek medical attention and inform the health care provider of the microorganisms used in the workplace.

**Prevention:** Biosafety level 2 practices, containment equipment, and facilities; wear lab coat and gloves; frequent handwashing is essential. Never eat in the lab. Caution should be used with sharps.

**Reporting:** Make note of the date and time of the incident and any relevant details. Inform principal investigator, fill out a [First Report of Injury form](http://www.des.umd.edu/risk_comm/wcomp/form/wcomp.pdf) and also report incident to EHS (x5-3960). If recombinant, the incident must be reported to the IBC chair Vincent Sollars at (304) 696-7357.

# X. WORKING WITH ANIMALS

Animals must be housed, handled, and used in accordance with the federal Animal Welfare Act (P.L. 89-544, *et seq*) and the NIH *Guide for the Care and Use of Laboratory Animals*. All research involving animals must be done under a protocol approved by the Marshall University IACUC. The Director of the Animal Resource Facility is responsible for assuring the safety and wellbeing of the research animals.

Safety procedures for working with animals at BSL2 containment

• Access to the animal resource facility laboratory is restricted to personnel who have been advised of the potential hazard & who have a need to enter the room for program or service purposes.

• Staff will be advised of increased risks for persons who are immunocompromised, pregnant, or for whom infection might be unusually hazardous

• Personnel must wash their hands after handling cultures &/or animals, and before leaving the animal facility.

• Eating, drinking, handling contact lenses & applying cosmetics are not permitted in the animal rooms.

1. • Storing of food for human use is not permitted in animal rooms.
2. • Doors to animal rooms within the buildings are kept closed when animals are present. The building access doors are kept locked.
3. • Work surfaces are decontaminated after use or a spill of a viable material.
4. • An insect & rodent control program is in effect.
5. • Bedding & waste materials from animal cages are removed in such a manner as to minimize the creation of aerosols & disposed of by incineration.
6. • Cages are washed & decontaminated after use.
7. • All biohazard waste & animal carcasses from the animal rooms are bagged in red biohazard bags before removal from the building for incineration.
8. • Sharps shall be handled properly according to the relevant section of this manual.
9. • Broken glassware is not to be handled by hand but should be removed by mechanical means such as a broom & dustpan, tongs or forceps.
10. • Spills, which result in exposure to infectious materials, should be reported to the immediate supervisor.
11. • All personnel entering animal rooms shall wear appropriate protective equipment.

# XI. SAFE HANDLING OF CRYOGENIC LIQUIDS

**Danger!  Vials immersed in liquid nitrogen may explode violently when removed!**  Wear face and eye protection!   Plastic vials (even Nunc vials with silicon O-rings) used for storing cells in liquid nitrogen are designed to be used in the liquid nitrogen vapor phase.  When immersed in the liquid phase, the liquid nitrogen frequently enters vials around the cold O-ring.  When vials are removed to room temperature, the liquid nitrogen in the vial immediately begins to boil.  Usually it escapes harmlessly past the seal.  Occasionally (about 1 out of 1000 vials), the seal is too tight, and the pressure causes a violent rupturing of the vial, sending shards of sharp plastic rocketing in unpredictable directions with sufficient energy to lacerate the face and cause severe eye injury.  When removing vials from liquid nitrogen, it is mandatory that you wear full face shields, pulled in to touch your chin so that shards can't fly under the shield.  If they fit, wear goggles underneath the face shield.

# APPENDIX 1: EMERGENCY CONTACTS

**EMERGENCIES 911 (From campus phone)**

|  |  |  |
| --- | --- | --- |
| **Name** | **Office Phone #** | **Home/Cell Phone #** |
| Principal Investigator, |  |  |
| Backup Contact, |  |  |
| Facilities Management Work Control Center |  |  |
| Department of Public Safety | 304.696.4357 |  |
| Non emergency |  |  |
| EHS |  |  |
| Biosafety Officer (Austin Hoffman) | 304.696.2563 | 304.412.5788 |
| Radiation Safety Officer |  |  |
| Occupational Health Clinic |  |  |
| Urgent Care Clinic |  |  |

# APPENDIX 2: REFERENCES

*University Biosafety Manual:*

<https://jcesom.marshall.edu/research/institutional-biosafety-committee/>

*Biosafety in Microbiological and Biomedical Laboratories*, 5th edition

<http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>

*Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*

<http://oba.od.nih.gov/rdna/nih_guidelines_oba.html>

# APPENDIX 3: AGENT SUMMARY STATEMENT (OPTIONAL)

The BMBL contains summary statements for many bacteria, viruses, and zoonotics. If your agent is listed in the *BMBL*‘s Agent Summary Statements section, you may find it useful to paste it into the biosafety manual.

<http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>

# Appendix 4. BMBL BSL-2 Laboratory Criteria

***Biosafety in Microbiological and Biomedical Laboratories (BMBL)***

**5th Edition, February 2007**

**Centers for Disease Control and Prevention and National Institutes of Health**

**Biosafety Level 2** builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that:

* laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures;
* access to the laboratory is restricted when work is being conducted; and
* all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

The following standard and special practices, safety equipment, and facility requirements apply to BSL-2:

**Standard Microbiological Practices**

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.

2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

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

4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

1. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
2. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
3. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
4. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:

1. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
2. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory’s biosafety level, the supervisor’s name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.

10. An effective integrated pest management program is required. See Appendix G.

11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual’s susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution’s healthcare provider for appropriate counseling and guidance.

**Special Practices**

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.

3. Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.

4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.

5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.

6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.

7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.

1. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
2. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.

8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.

9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.

10. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

**Safety Equipment (Primary Barriers and Personal Protective Equipment)**

1. Properly maintained BSCs (preferably Class II), other appropriate personal protective equipment, or other physical containment devices must be used whenever:

1. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
2. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.

2. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.

3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.

4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:

1. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
2. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
3. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
4. Do not use gloves on surfaces others may touch such as keyboard, door handles, etc.
5. Do not wear gloves outside the laboratory unless absolutely necessary and ensure one hand method is used (one hand ungloved that touches things such as doors, elevator buttons, etc. and the other hand gloved to hold specimens)

5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

**Laboratory Facilities (Secondary Barriers)**

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.

2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.

3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.

4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

1. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
2. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

5. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.

6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.

7. Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.

8. An eyewash station must be readily available.

9. There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer’s recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

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# Appendix 5. LAB SAFETY AGREEMENT

Text, letter

Description automatically generated

# Appendix 6. Principal Investigator’s Registered Research

(Attach PDF copies of research forms submitted to IBC and approved. All work with infectious agents, r/s NAs, human or non-human primate unfixed tissues, cells or blood, or select agents or toxins must be registered with the IBC before the work begins.)