Tulane University Biosafety Plan
**Purpose /Scope**

The following is the Tulane University Biosafety Plan addressing laboratory practices and procedures intended to reduce the risks associated with potential biological safety hazards encountered by the faculty, staff, and students working in laboratories where infectious agents, toxins, or recombinant DNA studies are performed. This plan is designed to provide guidance for personnel working in such laboratory facilities at Tulane University and is intended to supplement additional SOPs adopted for these studies as reviewed by the IBC and/or IACUC. In accordance with University practices and federal regulatory authorities such as the CDC, USDA, and NIH this plan will be reviewed annually and revised as needed. Furthermore the Biosafety Plan will be tested annually by drills, exercises, or incidents and will be modified as necessary to improve the effectiveness of the plan. A record of the result and any corrective action of the drills, exercises, or incidents will be maintained on file in the Office of Biosafety. The Office of Biosafety will annually inspect and certify to the current BMBL standard, all BSL-3, ABSL-2, and ABSL-3 laboratories under the authority of Tulane University. In addition, the Office of Biosafety will upon request inspect and certify BSL-2 laboratories to the current BMBL standard. The principal investigator to which any laboratory is assigned will be responsible for assuring that the Tulane University Biosafety plan is enforced and that all IBC protocols and IACUC protocols along with their associated SOPs are current for institutional approval and are strictly applied to all applicable work conducted under their supervision and authority.

**Definitions:**

**BSL-2** - Biosafety Level 2. Work must be conducted in accordance with the facility safeguards, standard microbiological practices, special practices, and safety equipment described in “Biosafety in Microbiological and Biomedical Laboratories”. BSL-2 is suitable for work involving agents that pose moderate risk to personnel and environment. Laboratory personnel have specific training in handling pathogenic agents and are supervised by scientist competent in handling infectious agents and associated procedures. All procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

**BSL-3** – Biosafety level 3. Work must be conducted in accordance with the facility safeguards, standard microbiological practices, special practices, and safety equipment described in “Biosafety in Microbiological and Biomedical Laboratories”. Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents that may cause serious or potentially lethal disease as a result of exposure by inhalation. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by scientists experienced in working with these agents. All procedures involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment. The laboratory has special engineering and design features such as access zones, sealed penetrations, and directional airflow.

**ABSL-2** – Animal Biosafety level 2. Conforms to requirements of the Animal Welfare Act 1966 and follows recommendations outlined in the “Guide for the Care and Use of Laboratory Animals” published by the National Research Council in 1996. ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel.
and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

**ABSL3** - Animal Biosafety Level 3. Conforms to requirements of the Animal Welfare Act 1966 and follows recommendations outlined in the “Guide for the Care and Use of Laboratory Animals” published by the National Research Council in 1996. ABSL-3 involves practices suitable for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission, and agents causing serious or potentially lethal disease.

**ACL-2** Arthropod Containment Level 2 Practices for working with exotic and indigenous arthropods infected with BSL-2 agents associated with animal and/or human disease, or that are suspected of being infected with such agents. Uninfected genetically modified arthropod vectors also fall under this level provided the modification has no, or only negative effects on viability, survivorship, host range, or vector capacity.

**Recombinant DNA Guidelines (NIH)** - The purpose of the NIH Guidelines is to specify practices for constructing and handling: (i) recombinant deoxyribonucleic acid (DNA) molecules, and (ii) organisms and viruses containing recombinant DNA molecules.

**Select Agents (SA)** - Biological agents that may be used for the purposes related to bioterrorism and which are thus regulated by the Select Agent Program (42 CFR Part 72).
Biosafety and recombinant DNA technology

Recombinant DNA technology involves combining genetic material from different sources thereby creating genetically modified organisms that may have never existed in nature before. Experiments involving the construction or use of recombinant DNA should be conducted after performing a biosafety risk assessment. The pathogenic properties and any potential hazards associated with such organisms may be novel and not well-characterized. The properties of the donor organism, the nature of the DNA sequences that will be transferred, the properties of the recipient organism, and the properties of the environment should be evaluated. These factors should help determine the biosafety level that is required for the safe handling of the resulting genetically modified organism, and identify the biological and physical containment systems that should be used. In response to the previously described considerations the NIH has developed a guideline for research involving recombinant DNA molecules. All work with recombinant DNA molecules at Tulane University is required to be registered with the IBC and, depending upon the research proposed, will require institutional and/or governmental review and approval, as set forth in the current revision of the “NIH Guidelines for Research with Recombinant DNA Molecules.”

1. Biosafety considerations for biological expression systems

Biological expression systems consist of vectors and host cells. A number of criteria must be satisfied to make them effective and safe to use.

A. Biosafety considerations for expression vectors

Higher biosafety levels may be required when:

1. The expression of DNA sequences derived from pathogenic organisms may increase the virulence of the transformed organism.
2. Inserted DNA sequences are not well characterized, e.g. during preparation of genomic DNA libraries from pathogenic microorganisms
3. Gene products have potential pharmacological activity

B. Viral vectors for gene transfer

Viral vectors, e.g. adenovirus vectors and lentivirus vectors, are used for the transfer of genes to other cells. Such vectors generally lack certain virus replication genes and are propagated in cell lines that complement the defect. Stocks of such vectors may be contaminated with replication-competent viruses, generated by rare spontaneous recombination events in the propagating cell lines, or may derive from insufficient purification. These vectors should be handled at the same biosafety level as the parent virus from which they are derived.

C. Transgenic and “knock-out” animals

Animals carrying foreign genetic material (transgenic animals) should be handled in containment levels appropriate to the characteristics of the products of the foreign genes. Animals with targeted deletions of specific genes (“knock-out” animals) do not generally present particular biological hazards. Examples of transgenic animals include animals expressing receptors for viruses normally unable to infect that species. If such animals
escaped from the laboratory and transmitted the transgene to the wild animal population, an animal reservoir for that particular virus could theoretically be generated.

D. Transgenic plants
Transgenic plants expressing genes that confer tolerance to herbicides or resistance to insects are currently a matter of considerable controversy in many parts of the world. Transgenic plants expressing genes of animal or human origin are used to develop medicinal and nutritional products. A risk assessment should determine the appropriate biosafety level for the production of these plants.

2. Risk assessments for genetically modified organisms
Risk assessments for work with artificially transformed organisms should consider the characteristics of donor and recipient/host organisms. Examples of characteristics for consideration include the following.

A. Hazards arising directly from the inserted gene (donor organism)
Assessment is necessary in situations where the product of the inserted gene has known biologically or pharmacologically active properties that may give rise to harm, for example:

1. Toxins
2. Cytokines
3. Hormones
4. Gene expression regulators
5. Virulence factors or enhancers
6. Oncogenic gene sequences
7. Antibiotic resistance
8. Allergens.

The consideration of such cases should include an estimation of the level of expression required to achieve biological or pharmacological activity.

B. Hazards associated with the recipient/host

1. Susceptibility of the host
2. Pathogenicity of the host strain, including virulence, infectivity and toxin production
3. Modification of the host range
4. Recipient immune status
5. Consequences of exposure.

C. Hazards arising from the alteration of existing pathogenic traits
Many modifications do not involve genes whose products are inherently harmful, but adverse effects may arise as the result of alteration of existing non-pathogenic or pathogenic traits. Modification of normal genes may alter pathogenicity. In an attempt to identify these potential hazards, the following points may be considered (the list is not exhaustive).

1. Is there an increase in infectivity or pathogenicity?
2. Could any disabling mutation within the recipient be overcome as a result of
the insertion of the foreign gene?
3. Does the foreign gene encode a pathogenicity determinant from another
organism?
4. If the foreign DNA does include a pathogenicity determinant, is it foreseeable
that this gene could contribute to the pathogenicity of the recombinant organism?
5. Is treatment available?
6. Will the susceptibility of the recombinant organism to antibiotics or other
forms of therapy be affected as a consequence of the genetic modification?
7. Is eradication of the artificially transformed organism achievable?

3. Further considerations
The use of whole animals or plants in experiments involving the use of recombinant DNA
molecules requires careful consideration and investigators must comply with all Tulane
University policies as set forth by the IBC, as well as the NIH Guidelines concerning research
involving recombinant DNA molecules. Risk assessment is a dynamic process that takes into
account new developments and the progress of science, as well as observations made in the
course of a study. The performance of appropriate risk assessments will assure that the benefits
of recombinant DNA technology will be weighed against the potential risks associated with the
research prior to performing the research.
Prescribed Laboratory Practices by Biosafety Level.

BSL-2 Practices

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

1. laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures;
2. access to the laboratory is restricted when work is being conducted; and
3. 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:

**A. 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 are not 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:
a. 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.

b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware 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:
   a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
   b. 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 for all RG2 and RG3 agents.

10. An effective integrated pest management program is required.

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 childbearing age are 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. For all non-select agent registered laboratories the PI is responsible for maintaining all training records for the previous three years.
B. 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. Where appropriate serum samples should be stored.

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 the facility.

7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
   a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
   b. 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 must be conducted within a BSC or other physical containment devices.

C. 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:
   a. 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.
b. 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. Laboratory clothing should 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 shall be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
   a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
   b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
   c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

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

D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors should be self-closing and have locks in accordance with the security 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.
   a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
   b. 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 allowed.

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 must be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may also be required for aspiration of contaminated liquids.

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).
BSL-3 Practices

Biosafety Level 3

Biosafety Level 3 is applicable to research where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure. All laboratory personnel will receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures. All procedures involving the manipulation of infectious materials within a Tulane University BSL-3 facility will be conducted within BSCs or other physical containment devices by trained personnel wearing appropriate personal protective equipment. All BSL-3 facilities at Tulane University will be inspected and re-certified by the Office of Biosafety annually.

The following standard and special safety practices, equipment, and facility requirements apply to BSL-3 laboratories at TNPRC designated for Select Agent work:

A. Standard Microbiological Laboratory Practices

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

2. 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.

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

4. Puncture wounds cause most serious biological accidents. Examples of sharps used in a lab or chemical area are: hypodermic needles, glass pasteur pipettes, razor blades, capillary tubes, scapels, broken glass, suture needles. The best way to avoid sharps injury is to avoid using sharps. Substitute plastic when possible. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps.

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

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

7. All cultures, stocks, and other potentially infectious materials will be decontaminated by autoclaving before disposal.

8. All laboratory wastes will be double bagged labeled, sprayed with bleach, autoclaved prior to disposal.

9. A sign incorporating the universal biohazard symbol will be posted at the entrance to the laboratory.
when infectious agents are present. Posted information must include the infectious agents present the supervisor’s name (or other responsible personnel), telephone number, and PPE s required for entering and exiting the laboratory.

10. An effective integrated pest management program is maintained by Tulane University Facilities Services.

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. The PI is responsible for maintaining training records for all personnel working under their direction in any BSL-3 laboratory for the previous three years. In addition, training records for all personnel with access to a BSL-3 facilities at Tulane University will be maintained by the Office of Biosafety. The Office of Biosafety will train all personnel with access to BSL-3 laboratories in the basic principles and practices of biosafety and biosecurity. In addition, the Office of Biosafety in cooperation with the PI will assure that all personnel receive agent specific training for all agents in use in the BSL-3 facility to which the individual has been granted access.

B. Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and wear the proper PPE.

2. Laboratory personnel are provided medical surveillance and are required to comply with the Occupational Health requirements for employees at TNPRC including regular TB skin testing and serum banking.

3. A laboratory-specific biosafety manual has been prepared and adopted as policy. A copy of the laboratory manual will be maintained in the laboratory and accessible for use as reference.

4. The laboratory supervisor is responsible to ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with Select Agents or RG-3 agents in the BSL-3.

5. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within the facility. All material containing viable select agents will remain within the control of the PI or other authorized individuals until decontaminated or returned to secure storage.

6. Laboratory equipment should be decontaminated after use, as well as, after spills, splashes, or other
potential contamination.
  a. Spills involving infectious materials must be contained, decontaminated, and cleaned up
     according to Spill Clean up procedures described in the laboratory safety manual and
     displayed in the laboratory.
  b. Equipment will be decontaminated before repair, maintenance, or removal from the
     laboratory.

7. 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 will be provided and appropriate records maintained.

8. Animals not associated with the work being performed are not be permitted in the laboratory.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. All procedures involving the manipulation of infectious materials must be conducted within a BSC
   (preferably Class II or Class III), or other physical containment devices.

2. Personal protective equipment required for work in any Tulane University BSL-3 designated
   laboratory will determined and required based upon a risk assessment of the agents in use and the
   specific requirements of the facility. A list of required PPE will be posted on the door and available
   for donning before entry into these laboratory spaces. All personnel will be trained in the proper use
   and the required PPE before allowing access to the laboratory. Change outer pair of gloves when
   contaminated, integrity has been compromised, or when otherwise necessary. Dispose of used
   gloves with other biohazardous waste.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors are self closing and have electronic locks and card access control in accordance
   with the Select Agent requirements. The laboratory is separated from areas that are open to
   unrestricted traffic flow within the building. Access to the laboratory is restricted to entry by a
   series of two self-closing doors including a clothing change room (anteroom) located in the
   passageway between the two self-closing doors.

3. The laboratory contains a hands-free sink for hand washing and a emergency eye wash station. An
   additional hand wash sink is located in the hallway near the exit door to the ante room.

4. The laboratory is designed so that it can be easily cleaned and decontaminated. Carpets and rugs
   are not permitted. Seams, floors, walls, and ceiling surfaces are sealed. Spaces around doors and
   ventilation openings are capable of being sealed to facilitate space decontamination.
   a. Floors are slip resistant, impervious to liquids, and resistant to chemicals and incorporate
      an integral cove base.
   b. Walls are finished with a sealed smooth finish that can be easily cleaned and
decontaminated.
c. Ceilings are constructed, sealed, and finished in the same general manner as walls.

5. Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment of the biological agents in use.

6. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.
   a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
   b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

7. BSCs do not interfere with proper operations and are located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

8. All Vacuum lines must be protected with HEPA filters. Filters must be replaced as needed. Liquid disinfectant traps may also be employed as required.

9. An eyewash station must be readily available in the laboratory as well as in the hall way connecting the animal holding rooms.

10. The BSL-3 laboratories have ducted air supply systems that provide sustained directional airflow drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas. The air supply control system provides that under conditions of exhaust fan failure the supply system shuts down to prevent the reversal of air flow within the facility.
   a. A digital readout monitoring device is located at the point of entry/egress to the BSL-3 laboratory or suite. Audible alarms will sound to notify personnel of air flow disruption.
   b. The laboratory exhaust air does not re-circulate to any other area of the building.
   c. The laboratory building exhaust air is HEPA filtered and the HEPA filters are tested and certified annually.

11. HEPA filtered exhaust air from Class II BSCs. All class II BSCs in Tulane University BSL-3 laboratories re-circulate HEPA exhaust air into the laboratory environment. These BSC must be tested and certified annually and operated according to manufacturer’s recommendations. In addition, two Class III BSC are located within the aerobiology suites at the TNPRC BSL-3 facilities. The Class III BSCs are HEPA filtered supply and double HEPA filtered exhaust ducted to provide maximum containment for the aerosolization studies involving RG-3 agents and biological toxins.

12. All laboratory wastes will be autoclaved before removal from the BSL-3 facilities at Tulane University.

13. Any large pieces of equipment will be autoclaved or decontaminated before removal from the
laboratory. If the equipment can not be autoclaved the Office of Biosafety should be contacted to arrange decontamination of the equipment.

14. Enhanced environmental and personal protection utilized by the TNPRC Select agent designated laboratory facility includes an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities, final HEPA filtration of the laboratory exhaust air, with appropriate decontamination procedures. The HEPA filter housings allow for leak testing of each filter and assembly. The filters and the housing are certified annually.

15. The BSL-3 facilities and all relevant equipment (BSCs, autoclaves, exhaust HEPAs, etc) must be re-certified and documented annually.
Animal Biosafety Level 2

Animal Biosafety Level 2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure. ABSL-2 requires that:

1. access to the animal facility is restricted;
2. personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of pathogenic agents;
3. personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures;
4. procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, should be conducted in BSCs or by use of other physical containment equipment.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Implementation of employee occupational health programs should be considered.

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

A. Standard Microbiological Practices

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergency situations. Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review. Prior to beginning a study animal protocols must be reviewed and approved by the IACUC. Studies involving the use of select agents or the introduction of recombinant DNA into an animal must also be reviewed by the Institutional Biosafety Committee prior to beginning work.

2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals. The safety manual must be available and accessible. Personnel are advised of potential hazards, and are required to read and follow instructions on practices and procedures. Consideration should be given to specific biohazards unique to the animal species and protocol in use.

3. Supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.

4. Appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered.
   a. Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.
b. Personal health status may impact an individual’s susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of child-bearing age should be provided 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.

c. Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.

5. A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/or animals are housed or are manipulated when infectious agents are present. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor’s name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room. Security-sensitive agent information and occupational health requirements should be posted in accordance with the institutional policy. Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.

6. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or are manipulated. All persons including facility personnel, service workers, and visitors are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.

7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing. Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials and when handling animals. Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated. Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Eye and face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.

8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use should only be done in designated areas and are not permitted in animal or procedure rooms.

9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.

10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.

11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items.
These include:

a. Needles and syringes or other sharp instruments are limited to use in the animal facility when there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.

b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.

c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware must not be handled directly; it should be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.

e. Equipment containing sharp edges and corners should be avoided.

12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.

13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/or animals are housed or are manipulated.

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

15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof containers for appropriate disposal in compliance with applicable institutional, local and state requirements. Decontaminate all potentially infectious materials before disposal using an effective method.

B. Special Practices

1. Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment, and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms. When appropriate, a baseline serum sample should be stored.

2. Procedures involving a high potential for generating aerosols should be conducted within a biosafety cabinet or other physical containment device. When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used. Consideration should be given to the use of restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications, etc).

3. Decontamination is recommended for all potentially infectious materials and animal waste before movement outside the areas where infectious materials and/or animals are housed or are manipulated by an appropriate method (e.g. autoclave, chemical disinfection, or other approved decontamination methods).
methods). This includes potentially infectious animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse. Consideration should be given to means for decontaminating routine husbandry equipment, sensitive electronic and medical equipment. Materials to be decontaminated outside of the immediate areas where infectious materials and/or animals are housed or are manipulated must be placed in a durable, leak proof, covered container and secured for transport. The outer surface of the container is disinfected prior to moving materials. The transport container must contain a universal biohazard label. Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements. Autoclaving of content prior to incineration is recommended.

4. Equipment, cages, and racks should be handled in manner that minimizes contamination of other areas. Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

5. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. Properly maintained BSCs, personal protective equipment (e.g., gloves, lab coats, face shields, respirators, etc.) and/or other physical containment devices or equipment, are used whenever conducting procedures with a potential for creating aerosols or splashes. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals. When indicated by risk assessment, animals are housed in primary biosafety containment equipment appropriate for the animal species, such as solid wall and bottom cages covered with filter bonnets for rodents, or larger cages placed in inward flow ventilated enclosures or other equivalent primary containment systems for larger animal cages.

2. A risk assessment should determine the appropriate type of personal protective equipment to be utilized. Scrub suits and uniforms are removed before leaving the animal facility. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home. Gowns, uniforms, laboratory coats and personal protective equipment are worn while in the areas where infectious materials and/or animals are housed or manipulated and removed prior to exiting. Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.

3. Eye and face protection (mask, goggles, face shield or other splatter guard) are used for anticipated splashes/ sprays from infectious or other hazardous materials and when the animal or 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 should also wear eye protection when entering areas with potentially high concentrations or airborne particulates. Persons having contact with the NHP should assess risk of mucous membrane...
exposure and wear appropriate protective equipment (e.g., masks, goggles, faceshields, etc.) as needed. Respiratory protection is worn based upon risk assessment.

4. Gloves are worn to protect hands from exposure to hazardous materials. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available. Gloves are changed when contaminated, integrity has been compromised, or when otherwise necessary. Gloves must not be worn outside the animal rooms. Gloves and personal protective equipment should be removed in a manner that prohibits transfer of infectious materials. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste. Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing must occur after the removal of gloves.

D. Laboratory Facilities (Secondary Barriers)

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking. Access to the animal facility is restricted. Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.

2. A hand washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility. If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink must also be available for hand washing at the exit from each segregated area. Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant. Penetrations in floors, walls and ceiling surfaces are sealed, to include openings around ducts, doors and door frames, to facilitate pest control and proper cleaning. Floors must be slip resistant, impervious to liquids, and resistant to chemicals.

4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning. Furniture should be minimized. Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.

5. External windows are not recommended; if present, windows should be sealed and must be resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.

6. Ventilation should be provided in accordance with the Guide for Care and Use of Laboratory Animals. The direction of airflow into the animal facility is inward; animal rooms should maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms.
Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas, to facilitate cleaning and minimize the accumulation of debris or fomites.

8. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.

9. Cages should be autoclaved or otherwise decontaminated prior to washing. Mechanical cage washer should have a final rinse temperature of at least 180°F. The cage wash area should be designed to accommodate the use of high pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures, during the cage/equipment cleaning process.

10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

11. If BSCs are present, they 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, heavily traveled laboratory areas, and other possible airflow disruptions. 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. Correct performance of the BSCs should be recertified at least once a year. All BSCs should be used according to manufacturer’s recommendation, to protect the worker and avoid creating a hazardous environment from volatile chemical and gases.

12. If vacuum service (i.e., central or local) is provided, each service connection should be fitted with liquid disinfectant traps and an in-line HEPA filter, placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement.

13. An autoclave should be considered in the animal facility to facilitate decontamination of infectious materials and waste.

14. Emergency eyewash and shower are readily available; location is determined by risk assessment.
Animal Biosafety Level 3 (ABSL-3)

Animal Biosafety Level 3 involves practices suitable for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission and agents causing serious or potentially lethal disease. ABSL-3 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-2.

ABSL-3 laboratory has special engineering and design features.
ABSL-3 requires that:
1. access to the animal facility is restricted;
2. personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of potentially lethal agents;
3. personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures;
4. procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, must be conducted in BSCs or by use of other physical containment equipment where possible.
5. Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment.
6. Employee occupational health programs must be implemented.

The following standard and special safety practices, safety equipment, and facility requirements apply to ABSL-3 laboratories at Tulane University.

A. Standard Microbiological Practices

1. The Office of Biosafety in cooperation with the vivarial staff will establish and enforce policies, procedures, and protocols for institutional policies and emergency situations. Worker safety and health concerns are addressed as part of the animal protocol and biosafety review. Prior to beginning a study in the ABSL-3 facilities at Tulane University animal protocols must also be reviewed and approved by the IACUC and the Institutional Biosafety Committee.

2. Safety practices and procedures specific to the animal facility are prepared or adopted in consultation with the vivarial staff and the Office of Biosafety. The SOPs are available and accessible for reference. Personnel are advised of potential and special hazards, and are required to read and follow instructions on practices and procedures. Consideration is given to specific biohazards unique to the animal species and protocol in use.

3. The Office of Biosafety and the vivarial staff will ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedure, potential
hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.

4. Appropriate medical surveillance program is in place through the Occupational Health Nurse, as determined by risk assessment. The need for an animal allergy prevention program will be considered. The Office of Biosafety will ensure that the Occupational Health staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, and animal care and manipulations. Personal health status may impact an individual’s susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of child-bearing age will be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions are encouraged to self-identify to the TNPRC’s Occupational Health staff healthcare provider for appropriate counseling and guidance. Personnel using respirators will be enrolled in an appropriately constituted respiratory protection program.

5. A sign incorporating the universal biohazard symbol is posted at the entrance to areas where infectious materials and/or animals are housed or are manipulated. The sign includes the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor’s name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is required for any agent used in the animal room.

6. Access to the animal room is limited to the fewest number of individuals possible. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or are manipulated. All persons including facility personnel, service workers, and visitors are advised of the potential hazards (natural or research pathogens, allergens, etc) and are instructed on the appropriate safeguards.

7. Protective laboratory coats, gowns, or uniforms are required for entry into the facility and additional gowning may be required to enter any animal room as determined by the specific agent and species of animals involved. Double-glove practices are required at all times in the ABSL-3 facilities. Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated. Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated. This practice will not be followed where the animal holding rooms are primary containment such as where NHPs are housed in open cages. Eye and face and respiratory protection are also required in the TNPRC ABSL-3 facilities.

8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use should only be done in designated areas and are not permitted in animal or procedure rooms.

   a. All procedures are carefully performed to minimize the creation of aerosols or

9. splatters of infectious materials and waste.
10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.

11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are required by the TNPRC sharps policy. When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
   a. Needles and syringes or other sharp instruments are limited to use in the animal facility when there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
   b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.
   c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
   d. Broken glassware must not be handled directly; it should be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
   e. Equipment containing sharp edges and corners should be avoided.

12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.

13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/or animals are housed or are manipulated.

14. An effective integrated pest management program is maintained by Facilities Services.

15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof containers for appropriate disposal in compliance with applicable institutional, local and state requirements. Decontamination using an effective method is required of all potentially infectious materials before disposal.

**B. Special Practices**

1. Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment, and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms.

2. All procedures involving the manipulation of infectious materials, handling infected animals or the generations of aerosols must be conducted within BSCs or other physical containment devices when practical. When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used. Consideration should be given to the use of restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications, etc).
3. The risk of infectious aerosols from infected animals or their bedding also can be reduced if animals are housed in containment caging systems (such as solid wall and bottom cages covered with filter bonnets, open cages placed in inward flow ventilated enclosures, HEPA-filter isolators and caging systems, or other equivalent primary containment systems).

4. Actively ventilated caging systems must be designed to prevent the escape of microorganisms from the cage. Exhaust plenums for these systems should be sealed to prevent escape of microorganisms if the ventilation system becomes static, and the exhaust must be HEPA filtered. Safety mechanisms should be in place that prevent the cages and exhaust plenums from becoming positive to the surrounding area should the exhaust fan fail. The system should also be alarmed to indicate when operational malfunctions occur. Furthermore, the system is tested and certified annually.

5. A method for decontaminating all infectious materials must be available within the facility, preferably within the areas where infectious materials and/or animals are housed or are manipulated (e.g. autoclave, chemical disinfection, or other approved decontamination methods). Consideration should be given to means for decontaminating routine husbandry equipment, sensitive electronic and medical equipment. Decontaminate all potential infectious materials (including animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse) before removal from the areas where infectious materials and/or animals are housed or are manipulated by an appropriate method. It is recommended that animal bedding and waste be decontaminated prior to manipulation and before removal from the areas where infectious materials and/or animals are housed or are manipulated, preferably within the caging system. Autoclaving of all waste from ABSL-3 is required before removal from the containment space.

6. Equipment, cages, and racks should be handled in manner that minimizes contamination of other areas. Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

7. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor and the Office of Biosafety. Medical evaluation, surveillance, and treatment will be provided as appropriate and records maintained.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. Properly maintained BSCs, and other physical containment devices or equipment, should be used for all manipulations for infectious materials and when possible, animals. These manipulations include necropsy, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals. The risk of infectious aerosols from infected animals or bedding can be reduced through the use of primary barrier systems. These systems may include solid wall and bottom cages covered with filter bonnets; ventilated cage rack systems; or for larger cages placed in inward flow ventilated enclosures or other equivalent systems or devices.
2. A risk assessment should determine the appropriate type of personal protective equipment to be utilized. Protective clothing such as scrub suits is worn by personnel within the animal facility. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing may not leave the ABSL-3 facility without decontamination. Disposable personal protective equipment such as non-woven olefin cover-all suits, wrap-around or solid-front gowns should be worn over this clothing, before entering the areas where infectious materials and/or animals are housed or manipulated. Front-button laboratory coats are unsuitable. Disposable personal protective equipment must be removed when leaving the areas where infectious materials and/or animals are housed or are manipulated. Scrub suits and uniforms are removed before leaving the animal facility. Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.

3. Appropriate eye, face and respiratory protection are worn by all personnel entering areas where infectious materials and/or animals are housed or are manipulated. To prevent cross contamination boots, shoe covers, or other protective footwear, are used where indicated. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.

4. Gloves are worn to protect hands from exposure to hazardous materials. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available. Procedures may require the use of wearing two pairs of gloves (double-glove). Gloves are changed when contaminated, integrity has been compromised, or when otherwise necessary. Outer pair of gloves must not be worn outside the animal rooms. Gloves and personal protective equipment should be removed in a manner that prohibits transfer of infectious materials. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste. Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

D. Laboratory Facilities (Secondary Barriers)

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking. Access to the animal facility is restricted. Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically. Entry into the containment area is via a double-door entry which constitutes an anteroom/airlock and a change room. Showers may be considered based on risk assessment. An additional double-door access anteroom or double-doored autoclave may be provided for movement of supplies and wastes into and out of the facility.

2. A hand washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility. The sink should be hands-free or automatically operated. If the animal facility has multiple segregated areas where infectious materials and/or animals are
housed or are manipulated, a sink must also be available for hand washing at the exit from each segregated area. Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning, decontamination and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant. Penetrations in floors, walls and ceiling surfaces are sealed, to include openings around ducts, doors and door frames, to facilitate pest control, proper cleaning and decontamination. Walls, floors and ceilings should form a sealed and sanitizable surface. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Flooring is seamless, sealed resilient or poured floors, with integral cove bases. Decontamination of an entire animal room should be considered when there has been gross contamination of the space, significant changes in usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the animal room must be based on the risk assessment.

4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning. Furniture should be minimized. Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.

5. External windows are not recommended; if present, all windows must be sealed and must be resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.

6. Ventilation to the facility should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*. The direction of airflow into the animal facility is inward; animal rooms must maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is HEPA filtered and discharged to the outside without being recirculated to other rooms. This system creates directional airflow which draws air into the animal room from "clean" areas and toward "contaminated" areas. Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process. Filtration and other treatments of the exhaust air may not be required, but should be considered based on site requirements, specific agent manipulations and use conditions. The exhaust must be dispersed away from occupied areas and air intakes, or the exhaust must be HEPA-filtered. Personnel must verify that the direction of the airflow (into the animal areas) is proper. It is recommended that a visual monitoring device that indicates directional inward airflow be provided at the animal room entry. The ABSL-3 animal facility shall be designed such that under failure conditions the airflow will not be reversed. Audible alarms should be considered to notify personnel of ventilation and HVAC system failure.

7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas, to facilitate cleaning and minimize the accumulation of debris or fomites.

8. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the
migration of vermin and gases.

9. Cages are washed in a mechanical cage washer. The mechanical cage washer has a final rinse temperature of at least 180°F. Cages should be autoclaved or otherwise decontaminated prior to removal from ABSL-3 space. The cage wash facility should be designed and constructed to accommodate high pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures, during the cage cleaning process.

10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

11. BSCs (Class II, Class III) must be installed so that fluctuations of the room air supply and exhaust do not interfere with its proper operations. Class II BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions. 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. BSCs should be certified at least annually to assure correct performance. Class III BSCs must supply air in such a manner that prevents positive pressurization of the cabinet or the laboratory room. All BSCs should be used according to manufacturers’ recommendations. When applicable, equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the animal facility. These HEPA filters should be tested and/or replaced at least annually.

12. An autoclave is available which is convenient to the animal rooms where the biohazard is contained. The autoclave is utilized to decontaminate infectious materials and waste before moving it to the other areas of the facility. If not convenient to areas where infectious materials and/or animals are housed or are manipulated, special practices should be developed for transport of infectious materials designated alternate location/s within the facility.

13. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.

14. Emergency eyewash and shower are readily available; location is determined by risk assessment.

15. The ABSL-3 facility design and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to use. Facilities should be re-verified at least annually against these procedures as modified by operational experience.

16. Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services, and the provision or effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment of the site conditions, or other applicable federal, state or local regulations.
ACL-2 Laboratories

Arthropod Containment Level 2 (ACL-2) must be practiced if working with exotic and indigenous arthropods infected with BSL-2 agents associated with animal and/or human disease, or that are suspected of being infected with such agents. *Uninfected genetically modified arthropod vectors also fall under this level provided the modification has no, or only negative effects on viability, survivorship, host range, or vector capacity* (see Risk Assessment). ACL-2 builds upon the practices, procedures, containment equipment, and facility requirements of ACL-1. It is more stringent in the physical containment, disposal, and facilities design. Moreover, access is more restricted than ACL-1. The decision to cultivate infected exotic arthropods under ACL-2 conditions in active transmission areas or in cases in which establishment is a possibility requires that measures that otherwise would only be recommended or preferred must be met.

A. Standard Practices

1. **Location of Arthropods.** Furniture and incubators containing arthropods are located in such a way that accidental contact and release by laboratorians, custodians, and service persons is unlikely. This may be achieved by locating arthropods in dedicated rooms, closets, incubators located out of the traffic flow or similar measures.

2. **Supply Storage.** The area is designed and maintained to enhance detection of escaped arthropods. Equipment and supplies not required for operation of the insectary should not be located in the insectary. All supplies for insect maintenance that must be kept within the insectary are located in a designated area and not on open shelves. It is recommended that a closed storage room, cabinets with tight-fitting doors or drawers be used. Doors and drawers are opened only for access. Insect diet should be kept in sealed containers.

3. **General Arthropod Elimination.** Accidental sources of arthropods from within the insectary are eliminated. Surfaces are cleaned after a spill of materials, including soil or water that might contain viable eggs. Pools of water are mopped up immediately.

4. **Primary Container Cleaning and Disinfestation.** In addition to cleaning cages and culture containers to prevent arthropod escape, containers are disinfected chemically and/or autoclaved if used for infected material. Autoclaving or incineration of primary containers is recommended for containers holding uninfected material.

5. **Primary Container Construction.** Cages used to hold arthropods are non-breakable and screened with mesh of a size to prevent escape. Containers are preferably autoclavable or disposable. Openings designed to prevent escape during removal and introduction of arthropods are recommended.

6. **Disposal of Arthropods.** All wastes from the insectary (including arthropod carcasses, and rearing medium) are transported from the insectary in leak-proof, sealed containers for appropriate disposal in compliance with applicable institutional requirements. All stages of arthropods are killed before disposal. Autoclaving or incineration of arthropod materials is recommended. Infected arthropods are autoclaved or incinerated.
7. Isolation of Uninfected Arthropods. Spread of agents to uninfected arthropods is prevented. Generally this is accomplished by isolating infected material in a separate room.

8. Primary Container Identification and labeling. Arthropods are identified adequately. Labels giving species, strain/origin, date of collection, responsible investigator, and so on are firmly attached to the container (and cover if removable). Vessels containing stages with limited mobility (e.g., eggs, pupae, hibernating adults) are securely stored.

9. Prevention of Accidental Dispersal on Persons or via Sewer. Before leaving the insectary and after handling cultures and infected arthropods, personnel wash their hands, taking care not to disperse viable life stages into the drainage system. No infected material is disposed of through the sewer. If uninfected materials are disposed of via the sewer, all material is destroyed by heat or freezing and preferably by autoclaving or incineration. Air curtains are recommended as appropriate.

10. Pest Exclusion Program. A program to prevent the entrance of wild arthropods (e.g., houseflies, cockroaches, spiders) and rodents effectively precludes predation, contamination, and possible inadvertent infection.

11. Escaped Arthropod Monitoring. Investigators assess whether escapes are occurring by instituting an effective arthropod trapping program to monitor the escape prevention program. Oviposition traps, ground-level flea traps, oil-filled channels surrounding tick colonies, light traps for mosquitoes and so on are recommended. Particularly in the case when exotic arthropods are used, exterior monitoring is recommended. Records of exterior captures are maintained.

12. Source and Harborage Reduction. Harborage and breeding areas are eliminated.

13. Furniture and racks are minimized and can be easily moved to permit cleaning and location of escaped arthropods. Equipment in which water is stored or might accumulate (e.g., humidifiers) is screened to prevent arthropod access, or contains chemicals to prevent arthropod survival.

14. Microbiological and Medical Sharps. In addition to minimizing arthropod sharps, these are restricted for use in the insectary if infected materials are used. Arthropod Sharps. In addition to minimizing arthropod sharps, these are restricted for use in the insectary if infected materials are used.

15. Routine Decontamination. Equipment and work surfaces in the insectary are routinely decontaminated with an effective chemical or by radiation (e.g., heat) after actual or potential contact with an infectious agent, and especially after overt spills and splashes of viable materials (including soil or water that might contain infectious agents or eggs).

16. Notification and Signage. Persons entering the area are aware of the presence of arthropod vectors. If infected material is present, a BSL-2 biohazard sign is posted on the entrance to the insectary listing all species handled within and is updated whenever new species are introduced or pathogenic infectious agents are present. The hazard warning sign identifies the arthropod species, agent(s) known or suspected to be present, lists the name and telephone number of the responsible person(s), and indicates any special requirements for entering the insectary (e.g., the need for immunizations or respirators).
17. **Procedure Design.** All procedures are carefully designed and performed to prevent arthropod escape.

18. **Safety Manual.** A safety manual is prepared, approved by the IBC, and adopted. The manual contains emergency procedures, standard operating procedures, waste disposal and other information necessary to inform personnel of the methods for safe maintenance and operation of the insectary.

19. **Training.** Laboratory personnel are advised of special hazards and are required to follow instructions on practices and procedures contained in the safety manual. Adherence to established safety procedures and policies is made a condition of employment and is part of the annual performance review of every employee. Personnel receive annual updates and additional training as necessary for procedural or policy changes. Records of all training are maintained.

20. **Medical Surveillance.** An appropriate medical surveillance program is in place. All personnel receive appropriate immunizations or tests for the agents handled or likely to be present. When appropriate, a serum surveillance system is implemented (see BMBL for guidance). Personnel are aware of the symptoms of infection and the procedure to follow in reporting these. In general, persons who may be at increased risk of acquiring infection, or for whom infection may be unusually hazardous (e.g., immunocompromised), are not allowed in the insectary unless special personal protection procedures are in place to eliminate extra risk.

21. **Access Restrictions.** Routine access is limited to trained persons and accompanied guests. Service persons are made aware of the hazards present and the consequences of arthropod release and contact with agents that may be present.

22. **Special Arthropod Handling Containers and Areas.** Infected arthropods are prevented from release into the laboratory area. This may be accomplished by secure glove boxes, biosafety cabinets, custom handling trays etc. These may vary from BSL recommendations insofar as necessary to safely contain both the arthropod and any agent. Such modifications should be made only in consultation with experts in handling the specific types of infected arthropods and biosafety experts. A dedicated area for handling infected material is recommended. This is preferably a separate cubicule, walkin incubator, or screen room.

23. **Safe Transport in the Laboratory.** All infectious and potentially infectious samples are collected, labeled, transported, and processed in a manner that contains and prevents transmission of the agent(s). Transfer of arthropods between manipulation and holding areas is in non-breakable secure containers.

**B. Special practices**

1. **IACUC and IBC Approval.** IBC approval is required for all ACL-2 studies. IACUC approval is required if vertebrates are used as hosts.

2. **Housing of Non-Arthropod Animals.** Other animals are not accessible to the arthropods. Animals used as hosts or blood sources generally are not housed with arthropods. If present, they are adequately protected from access by escaped arthropods, and protocols are approved by the IBC and
3. Containment During Blood-Feeding. Arthropods fed on host animals are prevented from accidental transfer to host cages. When handling/removing animals after exposure to arthropods, precautions must be taken to prevent arthropod escape through screens, covers, and by flying. Host animals are inspected closely (e.g., concealment in fur, ears, crevices), and the primary container is sufficiently robust to prevent escape during feeding.

4. Blood Source. The blood source is considered as a source of inadvertent arthropod infection and transmission. Only sterile blood or blood from sources known to be pathogen-free will be used.

5. Escaped Arthropod Handling. Loose arthropods must be killed and disposed of, or recaptured and returned to the container from which they escaped. Infected arthropods must not be killed with bare hands, and must be transferred using filtered mechanical or vacuum aspirators.

6. Accidental Release Reporting. A release procedure is developed and posted. This includes contacts and immediate mitigating actions. Accidents that result in release of infected arthropods from primary containment vessels, or that result in overt exposure to infectious material must be reported immediately to the insectary director who is responsible for ensuring that appropriate and documented action is taken to mitigate the release. Location, number, and type of material are prominently posted until the source is eliminated. Follow-up medical evaluation, surveillance, and treatment are provided as appropriate, and written records are maintained.

7. Movement of Equipment. All equipment must be appropriately decontaminated and disinfested before transfer between rooms within the insectary, and before removal from the insectary.

C. Safety Equipment (Primary Barriers)

1. Eye and Face Protection. Appropriate face/eye and respiratory protection are worn by all personnel entering the insectary.

2. Gloves. Gloves are worn when handling potentially infected arthropods, blood, and associated equipment and when contact with potentially infectious material is unavoidable.

3. Torso Apparel. White laboratory coats, gowns, and/or uniforms are worn at all times in the insectary when handling blood, vertebrate animals, and infected materials.

4. Personal Clothing. Clothing should minimize the area of exposed skin (e.g., skirts, shorts, open-toed shoes, sandals, tee shirts are inadvisable), since this can increase the risk of attracting and being bitten by a loose arthropod.

5. Arthropod-Specific Personal Protective Equipment. Personal protective equipment is worn as appropriate e.g., respirators for arthropod-associated allergies, particle masks, head covers. Agent specific personal protection equipment is used for all activities involving manipulations of infected or potentially infected arthropods.
D. Facilities (Secondary Barriers)

1. Location of Insectary. The insectary is separated from areas that are open to unrestricted personnel traffic within the building. It is recommended that this be accomplished by at least two self-closing doors that prevent passage of the arthropods. Increased levels of physical isolation are recommended, e.g., separate buildings, wings, suites.

2. Insectary Doors. Recommended entrance to the insectary is via a double-door vestibule that prevents flying and crawling arthropod escape. For example, the two contiguous doors must not be opened simultaneously. Internal doors may open outwards or be sliding, but are self-closing, and are kept closed when arthropods are present. Additional barriers (e.g., screened partitions, hanging curtains) are highly recommended. Insectary Windows. Windows are not recommended, but if present cannot be opened and are well sealed. Windows must be resistant to breakage (e.g., double paned or wire-reinforced).

3. Vacuum Systems. If a central vacuum system is installed, each service outlet is fitted with suitable barriers/filters to prevent arthropod escape. Filters are installed to permit decontamination and servicing. Other vacuum devices are appropriately filtered to prevent transfer and exhausting of arthropods.

4. Interior Surfaces. The insectary is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior walls are light-colored so that a loose arthropod can be easily located, recaptured, or killed. Gloss finishes, ideally resistant to chemical disinfectants and fumigants, are recommended. Floors are light colored, smooth and uncovered. Ceilings are as low as possible to simplify detection and capture of flying insects.

5. Floor Drains. Floor drains are modified to prevent accidental release of arthropods and agents. If present, traps must be filled with an appropriate chemical treatment to prevent survival of all arthropod stages (e.g., mosquito larvae).

6. Plumbing and Electrical Fixtures. Internal facility appurtenances (e.g., light fixtures, pipes, ducting) are minimal since these provide hiding places for loose arthropods.

7. Penetrations of walls, floors, and ceilings are minimal and sealed/caulked. Ideally, light fixtures are flush with the ceiling, sealed, and accessed from above.

8. HVAC. Ventilation is appropriate for arthropod maintenance, but does not compromise containment of the agent or arthropod. Examples include: exhaust air is discharged to the outside without being recirculated to other rooms; appropriate filter/barriers are installed to prevent escape of arthropods; the direction of airflow in the insectary is inward; a progressively negative pressure gradient is maintained as distance from the main entrance increases; fans located in the vestibule and internal corridor can be used to help prevent escape of flying arthropods; air curtains are located in vestibules and doorways.

9. Sterilization Equipment. An autoclave is available conveniently located to rooms containing
arthropods within the insectary building.

10. Sink and Shower. The facility has a hand-washing sink with hot water and with suitable plumbing to prevent arthropod escape.

11. Illumination. Illumination is appropriate for arthropod maintenance but does not compromise arthropod containment, impede vision, or adversely influence the safety of procedures within the insectary. Lighted (or dark) openings that attract escaped arthropods are avoided.

12. Facility Compliance Monitoring. The facility is evaluated annually for compliance to the ACL-2 level. The principle investigator or insectary director inspects the facility annually to ensure that alterations and maintenance have not compromised the containment characteristics. Adequacy of the practices and facility in view of changes in research protocols, agents, or arthropods are considered.
Operation of Laboratory Equipment

1. Operation of Biosafety Cabinets

Biological Safety Cabinets (BSCs) are the primary means of containment developed for working safely with infectious microorganisms. BSCs are designed to provide; **personnel protection** (protect laboratory worker from harmful agents inside the BSC), **environmental protection** (protect environment from contaminants contained in the BSC) and **product protection** (protect the work, product, or procedure performed in the BSC from contaminants that are outside the cabinet in the laboratory environment or from cross contamination inside the cabinet).

Most BSCs use high efficiency particulate air (HEPA) filters in the exhaust and supply systems. HEPA filters remove the most penetrating particle size (MPPS) of 0.3 µm with an efficiency of at least 99.97%.

A. Procedures- **BSC Certification Requirements**

1. It is required that BSCs are tested and certified on site:
   - At the time of installation
   - At least annually thereafter
   - At any time the BSC is moved

2. Contact the Office of Biosafety ((985) 871-6641 or (504) 988-0300) if the BSC is located at the TNPRC in Buildings 20 or the BSL-3/ABSL-3 facilities in Building 5. All BSCs that are located in other buildings are scheduled for certification through OEHS (504) 988-5486).

B. Procedures- **Preparing for Work in the BSC**

1. It is the responsibility of the principle investigator (PI) to provide training to laboratory workers on the proper techniques and practices of working in the BSC prior to the laboratory employee working in the BSC.

2. Prepare a written checklist of materials necessary before beginning work in cabinet; this will minimize the number and extent of air curtain disruptions which may compromise the air barrier of the cabinet.

C. Procedures- **Operating the BSC**

1. If the cabinet has been shut off, the blowers should be operating for 5-10 mins before beginning any work, this will remove any suspended particulates in the cabinet. Make sure the UV light is turned off and the fluorescent light is turned on before beginning. Note- BSCs in the BSL-3 suites should never be turned off.

2. The inside of the BSC is NOT sterile.

3. The germicidal UV-lamp is not a substitute for good cleaning practices.

4. The cabinet should be surface decontaminated before beginning any work with Vimoba® (or an approved disinfectant). All materials and containers placed in the cabinet must also be wiped down with 70% ethanol to reduce the introduction of contaminants to the cabinet environment.
5. The front grille must not be blocked with toweling, notes or discarded plastic wrappers, etc. Place equipment towards the rear of cabinet but still keeping it at a comfortable distance to work.

6. After placing equipment and materials needed for procedures make sure that the sash is returned to the correct height (usually 8”-10”) which should be illustrated on the front of the BSC.

7. When setting up necessary equipment and cultures in the BSC, it is recommended that potentially infectious organisms or cultures be the last items to be placed on a BSC work surface and the first items decontaminated and removed.

8. Place a receptacle in the BSC for disposing of biohazard waste, this container should be lined with a red biohazard bag and should contain approximately 200 ml of disinfectant. All contaminated materials (pipettes, tips, paper towels) used in the cabinet will be placed in this bin for subsequent autoclaving.

9. The work flow should be from ‘clean to dirty’ direction with the discard receptacle being on the end, and one should limit the movement of ‘dirty’ items over ‘clean’ items, this is to avoid passing dirty items over clean items. The biohazard discard receptacle should be located in the back of the cabinet to one side.

10. **Use proper aseptic technique.** For example, frequently disinfect gloves with disinfectant and immediately dispose of contaminated item/materials in biohazard waste container.

11. Contaminated gloves should be removed and disposed of in the discard receptacle located in the BSC prior to removing hands from the BSC. Also, in any situation where the outer gloves may have become contaminated within the BSC, the outer gloves should be removed, and new pair should be put on.

12. Avoid using techniques or procedures that disrupt the air flow pattern of the cabinet, i.e. crossing arms, fast sweeping motions, avoid moving arms in and out of the cabinet.

13. Open flames are **NOT PERMITTED** in BSC

D. **Procedure- Unloading Materials and Equipment**

1. ALL items and materials used within the BSC must be disinfected with appropriate disinfectant and wiped down before being removed from the cabinet.

2. Place all disposable items in the biohazard trash receptacle, located in the BSC and disinfect the outer surfaces of the biohazard bag, remove outer pair of gloves, place in the biohazard waste container, and don a fresh pair. Tape the bag and place in an autoclave bin.

3. All trash must be autoclaved before exiting the BSL-3 facility. It is the responsibility of the PI or laboratory tech for proper disposal of their own waste each and every time that work is completed in the cabinet.

4. Wipe down the entire (side wall, back walls, tray and sash) BSC with disinfectant for the final cleaning.

5. Turn off the fluorescent light, turn on the UV lights, and allow the blower to continue operating. Close the sash so that the opening is about 3-4 inches.
E. Procedure - Emergencies or Alarms
If at any time the BSC alarm sounds or there is problem with air flow, immediately close, secure and store all open containers with infectious agents, remove outer pair of gloves, and tightly close the sash. Contact the Office of Biosafety immediately at (985) 871-6641 or (504) 988-0300.

F. Procedure - Spills in the BSC
The inadvertent spillage requires proper containment and cleanup immediately. If a spill occurs while working in the BSC:
1. Stop work immediately.
2. Cap or close all hazardous materials you are working with.
3. If a spill has contaminated PPE, remove contaminated PPE. DO NOT remove any respiratory protection (N-95 or PAPR).
4. All contaminated clothing is placed in biohazard waste bin.
5. Notify others in the area of the spill. Notify the Principal Investigator or Laboratory Supervisor of the incident.
6. Cover spill with paper towels or other absorbent material and flood with a disinfectant (e.g. Vimoba).
7. Allow for at least 20 to 30 minutes contact time to ensure germicidal action of the disinfectant.
8. Remove broken glassware with forceps or broom and dustpan and dispose in the sharps container. Do not pick up a contaminated sharp object with your hands.
9. Pick up the paper towels and any other absorbent material and dispose in a biohazard bag for decontamination.
10. Wipe off any residual spilled material and reapply the disinfectant before final clean-up. Allow 20 to 30 minutes contact time.
11. Wipe equipment with an equipment-compatible disinfectant and allow 20 to 30 minutes contact time. Rinse with water where necessary.
12. Allow the fan to run during cleanup and for at least 15 minutes flowing cleanup.
13. Re-open the area to general use only after the spill clean-up and decontamination are completed.
14. Inform all personnel and laboratory supervisors about the spill and successful clean-up as soon as possible.
15. If a small spill occurred and the spill is contained to a small area, the spill may be cleaned up using an appropriate disinfectant.
16. For large spills or any spills involving a select agent or toxin contact TNPRC Biosafety Officer (985) 871-6641 or the Office of Biosafety immediately at (504) 988-0300 after hours (318 286-3732).
17. Whole room decontamination (if involving the room) or biological safety cabinet (BSC) decontamination (if contained in the BSC) may be necessary. An assessment of the affected area will be conducted by a Biosafety Officer.
G. Procedure- Incident Reporting

1. Individuals with known or potential exposure to a RG-3 agent or a select agent or toxin must report immediately to the occupational health nurse to file an “Incident Report”. A surveillance program and post-exposure follow up plan will be instituted.

2. All spills or release of RG-3 agents must be reported to the Office of Biosafety and an adverse incident report filed with the Biosafety Office.

2. Autoclave operation for decontamination of biohazardous waste

A. Background

An autoclave is a commonly used piece of equipment in biomedical laboratories. Autoclaves pose many hazards including physical (e.g. heat, steam and pressure) and biological hazards. Different autoclave models may have unique characteristics for loading, load sizes, and cycle types and settings. The types of materials you sterilize will determine the appropriate cycle use.

Autoclaves use pressurized steam to destroy microorganisms, and are the most dependable systems available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media and reagents.

Do not autoclave items containing corrosives (e.g. acids, bases, phenol) solvents or volatiles (e.g. ethanol, methanol, chloroform), radioactive materials or aerosol cans.

B. Procedure- Container Selection

Polypropylene is a plastic capable of withstanding autoclaving, but resistant to heat transfer.

1. Polypropylene bags (autoclavable biohazard bags) are tear resistant, but can be punctured or burst during the autoclaving process. Therefore, these bags must be placed in a solid (leak proof), rigid container during autoclaving.

2. Polypropylene containers and pans. Materials autoclaved in a polypropylene pan will take longer to autoclave than the same materials autoclaved in a stainless steel pan. To decrease the time required to sterilize material in polypropylene containers, remove the lid (if applicable), turn the container on its side when possible, and select the container with the lowest side height and widest diameter.

3. Stainless steel containers and pans. Stainless steel is a good heat conductor and is less likely to increase sterilizing time.

C. Procedure- Personnel Protective Equipment (PPE)

At a minimum the following PPE should be worn when operating an autoclave:

1. Always wear long-sleeved gloves designed for heat when using an autoclave.

2. Wear safety glasses or goggles.

3. When handling liquids, wear close toed shoes/rubber boots with a protective apron.
D. **Procedure - Preparation and Loading Materials**

1. Before use of the autoclave always clean the drain strainer before loading the autoclave.
2. Ensure that plastic materials are compatible with the autoclave.
3. If autoclaving liquids:
   a. To prevent from shattering during pressurization, the caps of containers with liquids must be loosened before loading.
   b. Add ¼ to ½ inch of water to the tray so the bottles will heat evenly.
4. Polypropylene bags are impermeable to steam, and therefore, must not be twisted or taped shut, but gathered loosely at the top and secured around the neck of the bag with a tie or autoclave tape. This will allow the steam to enter the bag through the opening.
5. Always put biohazard bags in a pan or container to catch spills and prevent the bags from sticking to the inside of the chamber.
6. Leave space between items to allow steam circulation.
7. Every load must be validated by either a biological or chemical method
   a. Place the chemical integrator strips or test pack in a place that will allow the user to easily read when unloading the autoclave. Note: this may be on the opposite side than the side that the materials were loaded.
   b. Reference TU SOP TU SOP 6.2.55 Biohazard Waste Disposal and Autoclave Validation in the BSL-3 Laboratory Suites, RBL and TU SOP 6.2.68 Biohazard Waste Disposal and Autoclave Validation in the ABSL-3 Suites, RBL.

D. **Procedure - Cycle and Time Selection**

1. Use liquid cycle (slow exhaust) when autoclaving liquids, to prevent contents from boiling over.
2. Select fast exhaust cycle for glassware.
3. Use fast exhaust and dry cycle for wrapped items.
4. All waste must be autoclaved for an exposure time of 60 mins at 121°C to assure decontamination.

E. **Procedure - Removing the Load**

1. Check that the chamber pressure is zero.
2. Check the autoclave cycle printout to ensure that the proper cycle was selected and the cycle was successful before opening the door.
3. Wear lab coat, eye protection, heat insulating gloves and closed-toe shoes when removing a load from the autoclave.
4. Open the door slowly, keeping head, face and hands away from the opening.
   a. Stand to the side (or behind door) when opening it.
   b. Wait at least 30 seconds after opening the door before reaching or looking into the autoclave.
5. Check the chemical integrator result before removing autoclaved items
   a. If the chemical integrator failed, do not remove items and repeat cycle.
   b. All validation results must be recorded in the Autoclave Validation logbook.
6. Wait 5 minutes for loads that contain dry goods, and 10 minutes for autoclaved liquid loads before removing them from the autoclave. Remove waste slowly and gently.
7. Clean up any spills immediately.
8. Report any malfunctions or accidents to facilities services and the Office of Biosafety immediately.

3. Special practices for BSL-3/ABSL-3 Pass-Through Autoclaves

A. Operation

1. The pass through autoclaves are used to sterilize biohazard waste from the BSL-3, and ABSL-3 facilities and to prepare sterilized glassware, plastics, media, and other materials for use in both the BSL-2 and BSL-3 laboratories.

2. Autoclave operation procedures and log sheet are posted next to the autoclave.

3. Monitoring of the operation of the autoclave will be performed by a designated employee and any operational problems will be reported promptly to Facility Services and a sign posted on the autoclave as to the problem.

B. Monitoring- performed by the laboratory personnel.

1. Each load- Place one temperature indicator strip in the center of one load on each day used. Save strips that don't meet temperature and place in log book. Notify the supervisor to evaluate the situation.

2. Weekly: Use temperature-indicator strips to monitor the temperature attained during dry-sterilization cycle. Place one strip in the bottom of each of 3 boxes located in the middle and the outside of a full load. Place strips in the log book.

3. Monthly: Use Bacillus spore test strips to test killing of spores by autoclave.

   a. For dry loads, place bacterial spore strips within several positions located in the middle and outside of a full load. Label with the date, location of the strip, and type of run (dry or liquid) and by sterile technique transfer to media containing culture tubes for viability testing. Place test tubes in 37 incubator and read results after 5 days of culture.

   b. For liquid loads, place bacterial spore strips in each of 2 water containers, one located at the middle and the other in the outside of a full load. Label with the location of the spore strip and and by sterile technique transfer to media containing culture tubes for viability testing. Place test tubes in 37 incubator and read results after 5 days of culture.
c. Any Positive Results will require a repeat of these procedures. If they are still positive, the autoclave will be taken off-line and will be evaluated.

C. Records

1. Monitoring and maintenance procedures are to be recorded in a log book and stored in the laboratory. Results of microbiological monitoring are maintained by the laboratory manager.

4. Pass Box Boxes in BSL-3 /ABSL-3 facilities

Pass boxes located in Buildings 20 and 5 allow sealed and decontaminated samples to be passed outside of the containment area for transport to other facilities on or off campus at the TNPRC. The proper operation of this pass box is necessary to maintain containment of the BSL-3 facility and prevent the inadvertent release of agents within the containment area.

Operation Procedures

1. In order to prevent the simultaneous opening of both doors of the pass box it is imperative that prior to opening the door to the pass box the individual visually confirm that the door on the opposite side of the pass box be closed.

2. All objects placed into the pass box must be within a sealed container and additionally placed within a secondary container or leak proof bag. The surface of each sealed container will be decontaminated before being place in the pass box.

3. Upon removing an object from the pass box the outer surface will be decontaminated with an appropriate decontamination solution.

5. Biohazard Spill Clean-Up

Background
Tulane University - operates Biosafety Level BSL-2, BSL-3, ABSL-2, ABSL-3, and ACL-2 laboratory and animal holding facilities. The inadvertent spillage or release of biologically hazardous material from the primary biocontainment area requires proper containment and cleanup.

A. Procedures – Spill(s) Inside the Biosafety Cabinet (BSC)

1. Stop work immediately.
2. If a spill has been contained entirely within the BSC then clean up the spill using paper towels or other appropriate absorbent material and the appropriate disinfectant within the BSC. After allowing the appropriate contact time for the disinfectant used place the absorbent material in a biohazard bag and add a small volume of disinfectant.
3. Remove your outer pair of gloves and place them in the biohazard bag, close the bag, and remove it from the BSC and place it in the biohazard bag outside of the BSC.
4. Replace your outer pair of gloves and continue with your work.
B. **Procedures – Spill(s) Outside of Primary Containment (i.e. BSC)**

1. Stop work immediately.
2. Notify others to leave the area, close the door, and post a warning sign on the entrance to the laboratory. No re-entry is to occur while cleanup is proceeding.
3. Notify the Principal Investigator or Laboratory Supervisor of the incident.
4. If a minor spill occurred and the spill is contained to a small area, the spill should be cleaned up by the personnel in the lab using absorbent materials in the lab and an appropriate disinfectant.
5. Place all contaminated material in a biohazard bag. Remove any contaminated PPE, other than your respirator protection (i.e. PAPR/N95), and place in a biohazard bag with the other contaminated materials.
6. Double bag all material and after completion clean up and decontamination of PAPR (if applicable), PPE, and biohazard bag containing the contaminated materials. Keep the bag(s) containing the contaminated material in the laboratory until such time as it may be transported to an autoclave for immediate sterilization.
7. Whole room decontamination (if involving the room) or biological safety cabinet (BSC) decontamination (if contained in the BSC) may be necessary. An assessment of the affected area will be conducted by the Office of Biosafety.
8. If a spill has contaminated PPE (or clothing under the PPE), it is imperative that it be removed prior to exiting contaminated area. Never remove respirator protection.
   a. If PAPR has been contaminated decontaminate the PAPR with Vimoba prior to exiting the contaminated area.
9. All contaminated clothing is bagged and is decontaminated by autoclaving (contaminated clothing is not to be removed from the containment facility).
10. Wash as much of the material off the skin with soap and water for a minimum of 15 minutes at the sink in the BSL-2 area before exiting and reporting to Occupational Health.
11. Inform all personnel and laboratory supervisors about the spill and successful clean-up as soon as possible.
12. All spills must be reported to the Biosafety Officer (318) 286-3732 or the Office of Biosafety immediately at (504) 988-0300.

C. **Procedure - Incident Reporting**

1. Individuals with known or potential exposure to any infectious agent or toxin must notify their supervisor immediately. Individuals with known or potential exposure to a RG-3 agent or a select agent or toxin must report immediately to the occupational health nurse to file an “Incident Report”. A surveillance program and post-exposure follow up plan appropriate to the agent will be instituted.
2. All spills or release of RG-3 agents must be reported to the Office of Biosafety and an adverse incident report filed with the Biosafety Office.
3. If the spill or release involves a select agent then immediate notification of RO and Biosafety Office is required. CDC or APHIS will be immediately informed and APHIS/CDC Form 3 must be filed with APHIS or CDC within 7 days.