General SOP for ABSL-2 Procedures

LABORATORY HAZARDS

Organisms that fall into this category can cause disease in healthy adults or pose a significant risk to pregnant women and/or immunocompromised individuals. Extra precautions such as the use of a biosafety cabinet and respirator are strongly encouraged for all individuals who will be in proximity of aerosolized pathogens.

GENERAL PRECAUTIONS FOR BSL-2+ PATHOGENS:

- Lab coat and gloves required (goggles when splash hazards are present)
- BSC use is required. N95 respirators and goggles are strongly encouraged when there is a high likelihood of aerosols.
- High titers and large volumes require addition disposable lab coat, double gloves, use of a BSC and goggles.
- Decontamination via steam sterilization or chemical (bleach) means before incineration for all contaminated materials including animals and animal waste. (NOT FOR PRIONS)
- Proper biohazard stickers and waste containers must be used.
- Double containment must be used for transport of cultures or infected materials including animals.
- Adequate signage should be at the entrance of every laboratory or animal to communicate hazards to non-research personnel.

Specific hazards and precautions of commonly used agents:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Precaution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter Baumannii</td>
<td>Risk of exposure to broken skin and mucus membranes. <strong>Large volumes and high titers require an upgrade to BSL-3 containment.</strong></td>
</tr>
<tr>
<td>Adeno-Associated Virus</td>
<td>BSL-2/ABSL-2 containment for 48 hours before downgrading to BSL-1</td>
</tr>
<tr>
<td>Aspergillus Fumigatus</td>
<td>Immunocompromised individuals are strongly encouraged to use a BSC and/ or N95 respirator as well as to take additional precautions such as double gloves and disposable lab coats. <strong>Inhalation Risk</strong></td>
</tr>
<tr>
<td>Candida Albicans</td>
<td>Opportunistic infections in immunocompromised individuals</td>
</tr>
<tr>
<td>Escherichia Coli (pathogenic)</td>
<td>No special precautions</td>
</tr>
<tr>
<td>Fusobacterium Nucleatum</td>
<td>Accidental parenteral inoculation and direct contact of mucous membranes constitute the primary hazards</td>
</tr>
<tr>
<td>Helicobacter Pylori</td>
<td><em>H. pylori</em> is a Class I human carcinogen according to the World Health Organization accidental ingestion poses the most common hazard</td>
</tr>
<tr>
<td>HSV-1</td>
<td>Droplet and aerosol exposure risk</td>
</tr>
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<table>
<thead>
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<th>Pathogen</th>
<th>Precautions</th>
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<tbody>
<tr>
<td>Legionella Pneumophila</td>
<td>No special precautions</td>
</tr>
<tr>
<td>Lentivirus</td>
<td>BSL-2/ABSL-2 containment for 72 hours before downgrading to BSL-1</td>
</tr>
<tr>
<td>Listeria Monocytogenes</td>
<td>Opportunistic infections in immunocompromised individuals. Extremely dangerous to pregnant women. <strong>Immunocompromised individuals and pregnant women are strongly encouraged to use a BSC or N95 respirator as well as to take additional precautions such as double gloves and disposable lab coats.</strong></td>
</tr>
<tr>
<td>M. Bovis-BCG</td>
<td>No special precautions</td>
</tr>
<tr>
<td>Measles Virus</td>
<td>No special precautions for immunized individuals. Individuals who have not received the MMR vaccine are encouraged to do so or to use extra precautions against droplets and aerosols.</td>
</tr>
<tr>
<td>Prions</td>
<td>Have special hazards that require unique handling, PPE and decontamination procedures. <strong>Use Prion protocol.</strong></td>
</tr>
<tr>
<td>Pseudomonas Aeruginosa</td>
<td>Multiple routes of transmission. Droplet and aerosol risk.</td>
</tr>
<tr>
<td>Staphylococcus Aureus (including MRSA)</td>
<td>Opportunistic pathogen can be transmitted via ingestion, animals bite and contact with infected individuals and contaminated materials.</td>
</tr>
<tr>
<td>Toxoplasma Gondii</td>
<td>Transmitted mostly through ingestion though there is a slight inhalation risk. <strong>Immunocompromised individuals should follow BSL-2+ protocol. Pregnant women are strongly encouraged not to work with this organism. In the event of pregnant women or a high likelihood of aerosolization, follow BSL-2+ protocol and wear a N95 respirator.</strong></td>
</tr>
</tbody>
</table>

For detailed information regarding a specific pathogen please reference the Pathogen Safety Data Sheets and Risk Assessment provided by the Public Health Agency of Canada (http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php)

**DETAILED EXPOSURE CONTROLS / PERSONAL PROTECTION**

**RISK GROUP CLASSIFICATION:** Risk group 2 and 3 agents.

**CONTAINMENT REQUIREMENTS:** Containment Level 2 facilities, equipment, and operational practices for work involving materials, animals, or cultures.

**PROTECTIVE CLOTHING:** Lab coat. Gloves when direct skin contact with infected materials or animals is unavoidable. Eye protection must be used where there is a known or potential risk of exposure to splashes.

**OTHER PRECAUTIONS:** All activities with infectious material should be conducted in a biological safety cabinet (BSC) or other appropriate primary containment device in combination with personal protective equipment. Centrifugation of infected materials must be carried out in closed containers placed in sealed safety cups, or in rotors that are unloaded in a biological safety cabinet.

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The use of needles, syringes, and other sharp objects should be strictly limited. Open wounds, cuts, scratches, and grazes should be covered with waterproof dressings. The creation of aerosols should be avoided; if aerosols cannot be avoided, respiratory and eye protection should be considered. Additional precautions should be considered with work involving animals or large scale activities.

**HAZARD COMMUNICATION:** A sign incorporating safety information must be posted at the entrance to the areas where infectious materials and/or animals are housed or are manipulated. 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.

**SPILLS:** Allow aerosols to settle; wearing protective clothing, gently cover the spill with absorbent paper towel and apply suitable disinfectant, starting at the perimeter and working towards the centre; allow sufficient contact time before clean up.

**DISPOSAL:** Decontaminate all wastes before disposal; steam sterilization, chemical disinfection, incineration.

**STORAGE:** In sealed containers that are appropriately labeled. Long-term storage should have adequate security procedures in place.

**ANIMAL HUSBANDRY:** Animals inoculated with organism that fall under this protocol do not require any additional precautions beyond those of standard ABSL-2 procedures. Animals, bedding and cages should be treated as biohazardous and should be autoclaved before incineration.

**PRECAUTIONS FOR ARC STAFF:** Standard ABSL-2 protocols, including additional shoe covers, gloves and disposable lab coats should be donned before entry and should be removed upon exit of the ABSL-2 room. N95 respirators are strongly recommended for immunocompromised individuals and pregnant women.

**MEDICAL SURVEILLANCE:** Surveillance may be available for certain pathogens if desired.

A sign incorporating safety information must be posted at the entrance to the areas where infectious materials and/or animals are housed or are manipulated. 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.

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Practices outlined in the CDC’s *Biosafety in Microbiological and Biomedical Laboratories, 5th edition* should be followed. The relevant portion is excerpted below:

**Animal Biosafety Level 2**

Animal Biosafety Level 2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1. 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.

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; and 4) BSCs or other physical containment equipment is used when procedures involve the manipulation of infectious materials, or where aerosols or splashes may be created.

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 emergencies.
   - Each organization 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 also be reviewed and approved by the IACUC and the Institutional Biosafety Committee.

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. The 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. An appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered.
   - 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.
   - 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 childbearing 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.
   - 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 names of other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of all infectious agents is necessary 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 manipulated.

- All persons including facility personnel, service workers, and visitors are advised of the potential hazards (physical, naturally occurring, 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 prevents 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, 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 consumption must not be permitted in laboratory areas. Food must be stored outside of the laboratory in cabinets or refrigerators designated and used for this purpose.

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 must always be taken with sharp items. These include:

a. The use of needles and syringes or other sharp instruments in the animal facility is limited to situations where there is no alternative such 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. Plastic ware should be substituted for glassware whenever possible.

e. Use of equipment with 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 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 base line 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.
   - Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications) should be used whenever possible.

3. Decontamination by an appropriate method (e.g. autoclave, chemical disinfection, or other approved decontamination methods) is necessary for all potentially infectious materials and animal waste before movement outside the areas where infectious materials and/or animals are housed or are manipulated. This includes potentially infectious animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse.
   - A method for decontaminating routine husbandry equipment, sensitive electronic and medical equipment should be identified and implemented.
   - 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 have 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 a 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.

5. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

6. 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, splashes, or other potential exposures to hazardous materials. 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 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 manipulations or activities that may result in splashes or 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 NHPs should assess risk of mucous membrane exposure and wear protective equipment (e.g., masks, goggles, face shields) appropriate for the task to be performed. 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, glove integrity is 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 prevents 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.

- 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 disinfectant 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, including openings around ducts, doors and doorframes, 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 must be sealed and 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 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 directly to the outside through an independent, hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be recertified at least once a year to ensure correct performance.

• All BSCs should be used according to manufacturer’s specifications to protect the worker and avoid creating a hazardous environment from volatile chemicals 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 present 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.