

UNIVERSITY OF ROCHESTER
ENVIRONMENTAL HEALTH & SAFETY

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| Policy No.: BS020 | Approved by: UR IBC |
| Title: Biosafety Level Requirements for BSL1, ABSL1, BSL2, ABSL2, BSL2+ or ABSL2+ | Date: December 30, 2020 |
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| Prepared by: Sonia Rosenberger | |

I. PURPOSE

Biosafety Levels (BSL) are a set of work practices, equipment and facility design appropriate for working with infectious agents, and are assigned by the Institutional Biosafety Committee based on agent risk (1 being the least hazardous, 4 being the most hazardous; UR does not perform BSL4 work).

This procedure establishes the biosafety requirements for laboratories working at:

- Biosafety Level 1 (BSL1) and Animal Biosafety Level 1 (ABSL1)
 - Biosafety Level 2 (BSL2) and Animal Biosafety Level 2 (ABSL2)
 - Biosafety Level 2+ (BSL2+) and Animal Biosafety Level 2+ (ABSL2+)
- Animals means small animals (including invertebrates) and nonhuman primates.
- This procedure does not include large animals (e.g. livestock) covered by the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (Appendix Q) or those regulated by the United States Department of Agriculture (USDA) for agricultural pathogens.

II. PERSONNEL AFFECTED

University of Rochester employees, students, and visitors working in laboratories

III. DEFINITIONS

Biosafety Level means a set of work practices, safety equipment and facility design appropriate for working with an infectious agent. Levels range from 1 to 4 with 1 being the lowest hazard and 4 being the highest hazard. For University research, the Institutional Biosafety Committee assigns project-specific Biosafety Levels.

Biosafety Level 1 (BSL1) is assigned to work involving well-characterized agents 1) not known to consistently cause disease in immunocompetent adult humans, and 2) present minimal potential hazard to laboratory personnel and the environment. Examples: Adeno-associated virus (and vectors, if delivering non-toxic or non-oncogenic inserts), plasmid DNA administered in vivo, nonpathogenic *E. coli* strains (e.g. K-12 derived strains like DH5alpha, and some non-K-12 derived *E. coli* strains like BL21).

Biosafety Level 2 (BSL2) is assigned to work involving human pathogens that pose moderate hazards to personnel and the environment. This does include agents that can cause fatal disease, but many times, vaccines or treatments are available.

Biosafety Level 2 + (BSL2+): Biosafety Level 2 plus additional precautions required by the IBC

CDC: Centers for Disease Control and Prevention

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CDC/NIH BMBL: Biosafety in Microbiological and Biomedical Laboratories (publication)

LAB/L form: IBC form that provides the IBC and Environmental Health & Safety (EH&S) lab-level information about where biological agents and materials are used and stored, engineering controls (aerosol containment equipment), and work practice controls (disinfection practices, medical surveillance/occupational medicine).

NIH OSP: The National Institutes of Health Office of Science Policy administers the NIH Guidelines.

NIH Guidelines: NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

OSHA: Occupational Safety and Health Administration

UCAR: University Committee on Animal Resources

UCAR Protocol Review for Hazardous Substances – Biohazards, ‘NIH Guidelines’: A letter written by the Biosafety Officer outlining Institutional Biosafety Committee (IBC) requirements for research personnel and animal care staff when working with animals and biohazards requiring BSL2 or above and/or biologicals covered by the NIH Guidelines.

UR IBC: University of Rochester Institutional Biosafety Committee, composed of faculty, staff, and community members; required by the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

IV. RESPONSIBILITIES

The University of Rochester Institutional Biosafety Committee (IBC) determines Biosafety Levels for research covered by the NIH Guidelines, and any research for which CDC recommends BSL2 or higher (including materials covered by OSHA’s Bloodborne Pathogens standard).

The Principal Investigator or the laboratory supervisor ensures the applicable BSL requirements are implemented and followed.

Environmental Health and Safety (EH&S) uses these requirements as a basis for auditing laboratories for compliance.

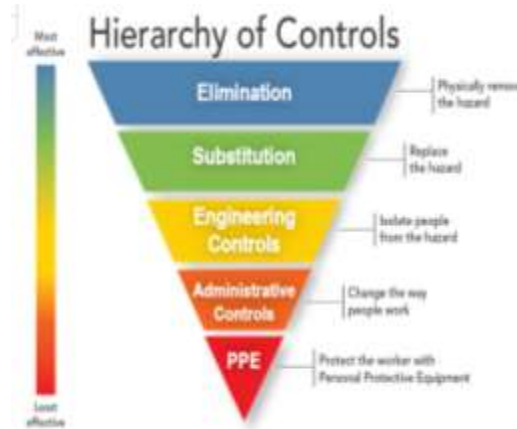
V. PROCEDURES

The following requirements are from CDC/NIH’s Biosafety in Microbiological and Biomedical Laboratories, 6th edition (2020), the NIH Guidelines, OSHA’s Bloodborne Pathogens standard and from additional Institutional Biosafety Committee deliberations.

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Requirements have been reordered to follow OSHA's hierarchy of controls (picture reference NIOSH):



In cases where requirements are either superseded by other UR policies (e.g. security) or are met by using existing UR policies, training or signage (e.g. Emergency Management Plan, Emergency 13 flipchart, annual EH&S Laboratory Safety Training), the regulatory language has been condensed. For reference, unedited regulatory language is provided in the appendices.

Questions about these requirements should be forwarded to the Biosafety Officer.

A. Incident Reporting (all BSLs)

The Principal Investigator must immediately report to UR's IBC all significant problems with and violations of these requirements, and all significant research related accidents and illnesses (i.e. overt/obvious exposures, environmental release, animal release, etc.)

For exposures, personnel complete an Incident Form. For near misses, personnel complete a Near Miss Report. Both are available on the EH&S website.

- Spills and accidents which result in obvious exposures to organisms containing recombinant or synthetic nucleic acid molecules in BSL2 labs must be reported to NIH OSP on the same day they occur or as soon as possible thereafter. The Biosafety Officer notifies NIH OSP by email.

B. Occupational Medicine/Medical Surveillance

See the LAB form for agent-specific vaccination offers, medical surveillance and instructions for when to consult UHS (University Health Service).

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C. Biosafety Level 1 (BSL1)

1. Overview

Biosafety Level 1 (BSL1) is assigned to work involving well-characterized agents 1) not known to consistently cause disease in immunocompetent adult humans, and 2) present minimal potential hazard to laboratory personnel and the environment.

Examples: Adeno-associated virus (and vectors, if delivering non-toxic or non-oncogenic inserts), plasmid DNA administered in vivo, nonpathogenic *E. coli* strains (e.g. K-12 derived strains such as DH5alpha, and some non-K-12 derived *E. coli* strains such as BL21).

Please note that the IBC may classify a pathogen at BSL2 even if it appears to meet the criteria for BSL1.

Work is typically conducted on open bench tops using standard microbiological practices. Even at BSL1, lab personnel receive specific training on procedures and are supervised by a scientist with training in microbiology or a related science.

Laboratory facilities require door(s) for access control, a sink for handwashing, an eyewash, lab finishes that can be easily cleaned (including chairs), and windows that are fitted with screens if they can open to the outside. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.

Personal Protective Equipment (PPE): lab coat, eye protection, and gloves.

2. Engineering controls (containment equipment, facility design)

- See BSL1 Overview
- Mechanical pipettors
- Appropriately-colored waste containers (with lids, closed when no one working or not in active use) and puncture-resistant sharps containers. Before full, or for sharps containers when the fill line is reached, these containers are closed and placed in durable, leak-proof containers for treatment (i.e. red totes, for treatment by UR's onsite Medical Waste Treatment Facility (Rotoclave®). If applicable, alternatives to these waste practices are described in the LAB form.
- Recommended: durable leak-proof secondary containers for transporting materials through the facility with an absorbent sufficient to absorb all liquid placed between the inner and outer container. Label the outer container with the biohazard symbol, if applicable.

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3. Work practice controls (Standard Microbiological Practices)

Standard Microbiological Practices (all BSLs) are basic work practices to reduce the risk of sustaining an exposure (ingestion or across mucous membranes or broken skin) and the spread of microorganisms inside or outside the lab. In many cases, these overlap with ‘good laboratory practice’ for working with chemicals (see UR’s Chemical Hygiene Program).

- Limit/restrict lab access, post signs. Templates are on the IBC and EHS Laboratory Safety Unit websites.
 - For labs assigned BSL1 only because the lab is performing experiments covered by the NIH Guidelines (e.g. non-K12 *E. coli*, AAV vectors, ecotropic MMLV vectors), at its 2/3/2016 meeting, the IBC determined that these labs may use standard laboratory door signage required by EH&S. No other signs are required (e.g. the universal biohazard symbol).
- Receive lab-specific training: duties, protocols, preventing exposures/safety techniques, exposure evaluation, how to deal with emergencies (exposures, medical emergencies, facility malfunctions, etc.), precautionary medical practices (like vaccinations), and additional training when procedures or policies change.

Emergency 13 flip charts are available from EH&S, and are available on the EH&S website accompanying the Medical Center/SMH and River Campus Comprehensive Emergency Management Plans.

The EH&S policy/procedure BS017 – ‘Emergency Procedures for Biological Spills in BSL1, BSL2 and BSL2+ Labs’ is online at www.safety.rochester.edu/labbiosafe/emergencyprocedures-biohazards.html

- Be aware that immune suppression or conditions that may predispose a person to increased risk of infection (e.g. pre-existing disease, medication, compromised immunity, organ transplant, pregnancy or breastfeeding) can make a person much more susceptible to pathogens that are 1) commensal (i.e. can live in your body usually without causing disease) or 2) worked with at BSL1.
- No eating, drinking, smoking, handling contact lenses, applying cosmetics, or storing food for human consumption. No mouth pipetting.
- Don’t transfer contamination to cell phones, personal possessions, your hair, or lab surfaces that then touch your lab coat/clothing. Some agents can survive days and months on fabric and plastics. If you have long hair, restrain it.
- Wash hands: after working with organisms, after removing gloves, and before leaving the lab.

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- Perform procedures to minimize splashes and/or aerosols
 - Develop and implement Sharps Safety Plan (see Lab-Specific Biosafety Manual and/or LAB form).
 - If you see insects or pests, call UR's Pest Control Program in EH&S at 275-3241.
 - Dispose of solid and liquid wastes per your Lab-Specific Biosafety Manual and/or LAB form.
 - Decontaminate work surfaces, lab equipment, and waste containers using disinfectant listed in your LAB form
 - 1) After procedures are done
 - 2) After any spill or splash of potentially infectious material
 - 3) Before equipment is disposed of, relocated, or worked on by an outside contractor (after all materials are removed from the unit). Attach "Equipment Hazard Tag/Decontamination Form". Exception: Biological safety cabinets are decontaminated by an outside contractor (formaldehyde gas or vaporized hydrogen peroxide), unless waived by EH&S.
 - 4) Red toters or other waste collection containers outside the lab, if those surfaces may be contaminated with infectious materials, so that contamination is not spread to the public or those with impaired immune systems.
4. Personal Protective Equipment (PPE)

The Principal Investigator (PI) determines the appropriate PPE to be worn for the particular activity, per the Job Hazard assessment.

- Lab coats:
 - CDC (for non-recombinant organisms) recommends lab coats.
 - The NIH Guidelines state that lab coats shall be provided "appropriate for the risk of exposure to viable organisms".
 - Lab coats and gowns may be worn throughout a period of work (unless visibly contaminated) but are removed before leaving the lab area.
 - Lab coats and gowns, which are used as personal protective equipment, are NOT taken home for laundering.
 - Gowns supplied by the Vivarium must be returned to the Vivarium for laundering.

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○ Eye protection:

- Engineering controls (best) or administrative controls (next best) are used to control aerosols, splashes or sprays. When neither are used, PPE is the next control measure available.
- If impact protection is required (e.g. protection from potential projectiles), either safety glasses or face shield used must be ANSI Z87.1 rated.
- Per CDC (for non-recombinant organisms), eye protection is worn when “conducting procedures that have the potential to create splashes or sprays of microorganisms or other hazardous materials”.
 - Eye protection would not be required if splashes and sprays are contained using engineering controls, e.g. biological safety cabinet.
 - Eye protection may be required if administrative controls are used to control splashes and sprays.
 - e.g. performing benchtop work close enough to the face with a volume of material that could result in a splash or spray to the eyes if an open container was dislodged or dropped.
- The NIH Guidelines do not address eye protection at BSL1.
- Dispose of eye and face protection (surgical mask, chin-length face shield, other splatter guard) with other contaminated lab wastes or decontaminate before reuse.

○ Gloves:

- CDC (for non-recombinant organisms) states that gloves “are worn to protect hands from exposure to hazardous materials”, and changed when contaminated or glove integrity is compromised.
- The NIH Guidelines (for recombinant or synthetic nucleic acid molecules) say that “gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable.”
- Select appropriate glove material, surface texture, thickness and cuff length based on the task and agent.
- Alternatives to latex are available.
- Remove gloves when contaminated, if integrity is compromised, when work is completed and before leaving the lab. Use a method that prevents contamination of hands or other parts of your person.
- Do not wash or re-use disposable gloves. Dispose of used gloves with other contaminated lab waste.

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- Wash hands: after working with organisms, after removing gloves, and before leaving the lab.

D. Animal Biosafety Level 1 (ABSL1)

1. Overview

Animal Biosafety Levels (ABSLs) include animal-specific hazards:

- Aerosols are generated during animal activities and husbandry,
- Biologicals and/or biohazards may be shed in excretions,
- Infectious diseases may be transmitted in bites/scratches, etc.

For materials that require IBC approval, the Biosafety Officer writes a “IBC Approval/UCAR Biohazards, NIH Guidelines” letter.

2. Engineering controls (containment equipment, facility design)

- See BSL1 Overview.
- Basic requirements for animal facility design are in the “Guide for the Care and Use of Laboratory Animals” by the National Research Council, including sealing utility penetrations. In addition, sink traps and floor drains are filled so they don’t dry out, fixture design and installation facilitates cleaning/minimizes debris or fomites, any external windows are resistant to breakage, and equipment and furnishings minimize pinch points, sharp edges and corners for personnel.
- As provided in the ‘IBC Approval/UCAR Biohazards, NIH Guidelines’ letter, containment equipment is required for transporting biohazards, animal tissues, and animals administered materials containing recombinant or synthetic nucleic acid molecules so that cages do not spill, or spill soiled bedding in the corridors.
 - Animals transported from a housing room to a laboratory outside the Vivarium must be done in such a manner that soiled bedding and cages will not be spilled in the corridors. Follow Vivarium procedure, use a rubber band around the cage and ensure the biohazard card or symbol is on the cage.
 - Empty soiled cages must be returned to the Vivarium in a timely manner. Place the biohazard card inside the cage, then follow Vivarium policy. Once in the cage wash area, remove the rubber band.

3. Work practice controls (Standard Microbiological Practices)

- BSL1 requirements apply.
- The Vivarium labels animal room doors.

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- As provided in the “IBC Approval/UCAR Biohazards, NIH Guidelines” letter, Biohazard labelling for cages is required so that animal care staff know an IBC-approved biological was administered: red ‘biohazard’ card behind cage card and visible (preferred), or biohazard sticker on cage card with room for agent name. Both are available at the Animal Resource/Vivarium Office.
 - For transgenic mice from UR’s Mouse Genome Editing Resource, cages do not require the biohazard symbol unless required above.
 - At least one (1) week prior to using materials in vivo, arrange BSL housing:
 - Submit a “[Notification of Intended Use of ABSL1 or 2 Agents in the Vivarium](#)” form.
 - Personnel administering hazards - attend a brief orientation with Animal Resources.
 - Follow Vivarium procedures for animal remains, and include the name of the biological or biohazard on the tag (tags available at the Animal Resource Office).
4. Personal Protective Equipment (PPE)

Follow standard Vivarium procedure – varies by species and procedure (e.g. handling animals vs. performing routine animal checks)

E. Biosafety Level 2 (BSL2)

1. Overview

Biosafety Level 2 (BSL2) is assigned to work with agents associated with human disease and that pose moderate hazards to personnel and the environment. This does include agents that can cause fatal disease, but many times, vaccines or treatments are available.

BSL2 builds upon BSL1. All BSL1 requirements apply.

Lab access is more restricted, personnel demonstrate proficiency in Standard Microbiological Practices prior to handling BSL2 materials, and all procedures that may generate infectious aerosols, splashes or sprays are conducted in biological safety cabinets or using other containment equipment.

Most published laboratory acquired infections (e.g. Pike RM. [Annu Rev Microbiol.](#) 1979;33:41-66) don’t have a known exposure incident, and at BSL2 are thought to result from the inhalation of infectious aerosols (for respiratory pathogens), or direct contact of broken skin or mucous membranes with infectious droplets (or surfaces contaminated by those droplets). As a result, aerosol containment is required when:

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- Applying high energy to a liquid: pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, etc.
- Manipulating “high concentrations or large volumes” of infectious agents or organisms containing recombinant or synthetic nucleic acid molecules

Benchtop work is acceptable for non-aerosol-generating procedures at BSL2 (other than respiratory pathogens).

2. Engineering controls (containment equipment)

CDC and NIH follow OSHA’s hierarchy of controls, and add the “box within a box” concept of Primary and Secondary Barriers. Engineering controls are Primary Barriers that protect lab personnel. Primary barriers/containment equipment generally rely on HEPA filters and secure closures (o-ring), and include biological safety cabinets and other aerosol containment.

- The Sharps Safety Plan must address sharps safety devices. See Lab-Specific Biosafety Manual and/or LAB form.
- Splash shields may be used for benchtop work, if bench top work has been approved by the IBC.
- Biological Safety Cabinets (BSCs)
 - Use a biological safety cabinet (or other aerosol containment devices), when performing procedures that involve:
 - A “potential for creating infectious aerosols or splashes” include “pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials...” (CDC/NIH’s BMBL)
 - A “high potential” for creating aerosols (NIH Guidelines); in comparison to CDC:
 - Pipetting is not included.
 - Qualifiers are given for “vigorous” shaking or mixing (e.g. vortexing), and opening containers of materials “whose internal pressures may be different from ambient pressures”.
 - “High concentrations or large volumes” of infectious agents or organisms containing recombinant or synthetic nucleic acid molecules
 - Opening centrifuge safety cups and rotors

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- See BS010 – Requirements for Biological Safety Cabinets Used in Laboratories for Biohazard Containment for how to select and properly use a biological safety cabinet.
 - Aerosol containment equipment for centrifuges, homogenizers, sonicators, flow cytometers, shaker flasks
 - Centrifuge safety cups, individual tube caps, seals for homogenizers and sonicators all have o-rings and secure closures
 - Per [CDC](#) and the NIH Guidelines, “high concentrations or large volumes” of infectious agents or organisms containing recombinant or synthetic nucleic acid molecules require centrifuge safety cups or sealed rotors if the centrifuge is too large to put in a biological safety cabinet, or it disrupts the airflow in the cabinet.
 - If possible, let centrifuged materials rest first to allow aerosols to settle and open the safety cups in a biological safety cabinet.
 - Shaker flask filter tops are available.
 - Protect house vacuum/maintenance workers - use a disinfectant trap and a 0.2um (or HEPA equivalent) hydrophobic filter to prevent accidental aspiration into the vacuum lines.
 - Waste containers are red, durable and leak-proof. Per New York State’s Department of Environmental Conservation, Regulated Medical Waste (except medical waste sharps) is required to be placed in plastic bags and then:
 - Packaged in containers prior to transport for treatment, either
 - single use (e.g., corrugated boxes) – e.g. for River Campus labs that have individual Stericycle accounts and pickups
 - reusable rigid (e.g., plastic) or semi-rigid, leak proof containers (i.e. red totes)
 - Once packaged, this waste is either transported to
 - a designated secure storage or collection area within the facility for pick-up – determined either by Stericycle or EH&S Environmental Compliance Unit
 - UR’s Medical Waste Treatment Facility (Rotoclave®)
3. Engineering controls (facility design)
Inward directional airflow into the lab is preferable. All labs at UR should have inward directional airflow and be 100% exhaust (no recirculation to other areas). The IBC may require verification of airflow during annual lab inspections.
4. Work practice controls (Standard Microbiological Practices and Special Practices)
- Follow Standard Microbiological Practices (see BSL1).
 - The NIH Guidelines state that “Experiments of lesser biohazard potential can be conducted concurrently in carefully demarcated areas of the same laboratory.”

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- Door signs include the universal biohazard symbol, Name of agent, Biosafety Level, Emergency contacts, PPE requirements, general occupational health requirements (e.g. immunizations, respiratory protection), and if there are any special provisions for entering and exiting the space while sign is posted.
- Medical surveillance/vaccinations, as per IBC. See Lab-Specific Biosafety Manual and/or LAB form.
- Lab-specific Biosafety Manual, including a Sharps Safety Plan
- Personnel demonstrate proficiency in standard and special microbiological practices before working with BSL2 agents.
- Animals and plants, unless part of the work, cannot be in the lab. The UR IBC will consider allowances on a case-by-case basis.
- OSHA's Bloodborne Pathogens standard's [inspection guidance](#), states "gross contamination must be cleaned up first with a soap and water solution, to ensure the disinfectant is completely effective," as disinfectants don't typically penetrate organic material.

5. Personal Protective Equipment (PPE)

See BSL1.

Dispose of disposable PPE as Medical Waste (i.e. red bag waste).

- Lab coats:
 - CDC, the NIH Guidelines, and OSHA's Bloodborne Pathogens standard (for HIV and HBV cultures) all state that lab coats or similar protective clothing shall be used, shall not be worn outside of the work area, and shall be decontaminated before being laundered.
 - Reusable labcoats: Do not take them home. Place them in a plastic bag affixed with a biohazard symbol so that the laundry company knows to decontaminate before laundering. Or, use disposable lab coats.
 - Tyvek sleeves can cover the exposed gap between lab coat and glove cuff. Or, use a glove with a longer cuff.
- Eye and face protection (safety glasses and a surgical mask, or a face shield):
 - CDC (for non-recombinant organisms) states that eye and face protection are worn when the microorganism are handled outside the biological safety cabinet or containment device, for anticipated splashes or sprays of infectious or other hazardous materials.

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- Dispose of eye and face protection (surgical mask, chin-length face shield, other splatter guard) with other contaminated lab wastes or decontaminate before reuse.
- o Gloves:
 - Double-gloving is recommended for long procedures to minimize leaks.
 - Consider how chemicals used (like disinfectants) impact glove integrity.

F. Animal Biosafety Level 2 (ABSL2)

1. Overview

Animal Biosafety Levels (ABSLs) include animal-specific hazards:

- aerosols are generated during animal activities and husbandry,
- biohazards may be shed in excretions,
- infectious diseases may be transmitted in bites/scratches, etc.

The Biosafety Officer writes a “IBC Approval/UCAR Biohazards, NIH Guidelines” letter.

2. Engineering controls (containment equipment, facility design)

- o Self-closing doors, never propped open
- o Inward directional airflow
- o Filter top cages or negative, ventilated racks with HEPA-filtered exhaust (UR requirement)
- o Biological safety cabinets (Class II A2, A/B3 or B) for:
 - Preparing or administering agents,
 - Disposing of contaminated sharps,
 - Collecting or handling bodily fluids, tissues, performing necropsy, and
 - Opening cages, all cage changes.
 - Exceptions are made on a case-by-case basis (e.g. for human cells)
- o Downdraft tables may also be used for necropsies provided personnel wear PPE.

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- For transport:
 - Biohazards and animal tissues from inoculated animals: as mentioned previously, snap top or screw-top closure with o-ring
 - Transporting biohazards, animal tissues, and animals administered materials containing recombinant or synthetic nucleic acid molecules so that cages do not spill – same as ABSL1.
- 3. Work practice controls (Standard Microbiological Practices and Special Practices)
 - ABSL1 and BSL2 procedures also apply.
 - Decontamination (in addition to standard practice) - Following injection, any excess virus, viral vector, or human cells present on the fur or skin must be decontaminated with Betadine solution.
 - All injections must be performed on immobilized animals (i.e. anesthetized or tranquilized) to reduce the risk of a needlestick injury or, for intranasal administrations, biohazards being expelled from the nose.
 - If there is a scientific justification for not anesthetizing animals for injections, then mechanical restraints are used.
 - For human cells administered IP (intraperitoneal) or SC (subcutaneous) routes, manual restraint may be considered, but mechanical restraint options must be discussed with one of the veterinarians (+/- the Biosafety Officer) and used if appropriate for the injection type.
 - Additional alternatives are considered on a case-by-case basis by the IBC.
- 4. Personal Protective Equipment (PPE) (in addition to Vivarium requirements):
 - Gloves, clothing cover (gown)
 - Face protection for ABSL2 materials handled outside of a biological safety cabinet or without a shield:
 - Chin-length face shield, or
 - Safety glasses and a surgical mask (mask can be non-fluid resistant)

G. Biosafety Level 2+ (BSL2+)

1. Overview

While the designation of Biosafety Level 2+ (BSL2+) is not used by regulatory agencies, at UR it means ‘BSL2 plus additional precautions’. These additional precautions may include:

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- When manipulating concentrated amounts or conducting procedures that may produce droplets or aerosols, CDC/NIH's [BMBL](#) recommends Biosafety Level 3 practices in a Biosafety Level 2 lab.
- For cultures of HIV or HBV, OSHA's [Bloodborne Pathogens standard](#) prohibits bench top work.
- USDA transport permits may require pathogen-specific requirements. These requirements apply as long as the agent is possessed, even if the transport permit is allowed to expire.
- For viral vectors that could integrate an oncogene or silence a tumor suppressor in the genome of a person, UR's IBC requires Biosafety Level 3 practices in a Biosafety Level 2 lab.

At Biosafety Level 3 (BSL-3), agents may cause serious or potentially lethal disease through the inhalation route of exposure, therefore, BSL-3 practices include using biological safety cabinets or other physical containment devices for all manipulation of infectious materials, and PPE includes solid-front gowns, scrubs or coveralls to protect street clothing.

2. Engineering controls (containment equipment, facility design)

- As BSL2 labs are used, labs have closed door(s), a sink for handwashing, an eyewash, lab finishes that can be easily cleaned (i.e. no cloth chairs or rugs), and windows that are fitted with screens if they can open to the outside.
 - For labs that handle HIV or HBV cultures, OSHA requires that the eyewash is in the work area and provides a 15-minute supply of continuous free-flowing water, hands-free.
- Inward directional airflow (UR requirement)
- Card swipe access at the room level (UR requirement for selected pathogens; generally not for viral vectors assigned BSL2+)

3. Work practice controls (Standard Microbiological Practices and Special Practices)

- BSL1 and BSL2 procedures also apply.
- Personnel demonstrate proficiency in standard and special microbiological practices before working with BSL2 agents. OSHA requires the same for labs handling HIV or HBV cultures.

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- All work at BSL2+ (excluding microscopy) is conducted either in a biological safety cabinet or in other aerosol containment unless approved by the IBC (and then face protection is required).
- Centrifuge safety cups or sealed biological safety rotors are used and are loaded and unloaded inside a biological safety cabinet.
- Disposable sharps are placed directly into a sharps container located with the biological safety cabinet.
- Contaminated reusable sharps are placed immediately into a disinfectant approved on the LAB form inside the biological safety cabinet for at least 10 minutes prior to removal from the BSC.
- Used pipette tips are placed into a disinfectant approved on the LAB form inside the biological safety cabinet for at least 10 minutes prior to red bag waste disposal.

4. Personal Protective Equipment (PPE):

- While BSL2+ experiments are in progress, ALL personnel working in the room must wear the same PPE even if not working at BSL2+.
- At a minimum:
 - Eye protection (see BSL2)
 - A gown that ties in back/side OR lab coat buttoned to neck
 - Those persons working at BSL2+ in the biological safety cabinet must ensure arms are completely covered (no exposed skin) and cloth coats are not contaminated (disposable sleeves are used).
- Double gloves are used when working with cultures of BSL2+ materials. The outer gloves are removed inside the biological safety cabinet.

H. Animal Biosafety Level 2+ (ABSL2+)

The Biosafety Officer writes any additional requirements (e.g. double gloves, outer gloves removed inside the biological safety cabinet) in the “IBC Approval/UCAR Biohazards, NIH Guidelines” letter.

At UR, existing Vivarium Animal Biosafety Level 2 practices incorporate the majority of CDC/NIH BMBL’s Animal Biosafety Level 3 practices.

1. All work (excluding microscopy) is conducted either in a biological safety cabinet or in other aerosol containment unless approved by the IBC.

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2. Animals are housed in filter top cages or negative, ventilated racks with HEPA-filtered exhaust.
3. Closed front gowns with elastic cuffs are used to ensure skin is not exposed inside the biological safety cabinet or at the same level as the opening below the biological safety cabinet sash.
4. The only additions relevant to the animal facility are:
 - Contaminated reusable sharps are placed immediately into disinfectant for at least 10 minutes prior to removal from the biological safety cabinet.
 - Double gloves are used when handling cultures (e.g. animal administration). The outer gloves are removed inside the biological safety cabinet.

VI. REFERENCES

A. Applicable Federal Biosafety Regulations

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules -
<https://osp.od.nih.gov/biotechnology/nih-guidelines/>

CDC/NIH "Biosafety in Microbiological and Biomedical Laboratories -
<https://www.cdc.gov/labs/BMBL.html>

OSHA's Bloodborne Pathogens standard 1910.1030
www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1030

OSHA's Eye and Face protection eTool
www.osha.gov/SLTC/etools/eyeandface/employer/requirements.html

B. Applicable University Biosafety Resources

Door signs and lab inspection checklists
www.safety.rochester.edu/labsafety/NewPIinformation.html

Emergency Procedures for Biological Spills in BSL1, BSL2 and BSL2+ Labs
www.safety.rochester.edu/labbiosafe/emergencyprocedures-biohazards.html

UR IBC meeting minutes – 02/03/2016, 05/10/2017, 06/07/2017, 11/15/2017, 02/07/2018, 03/07/2018, 05/29/2019, 06/12/2019, 07/19/2019, 03/04/2020

UR IBC home page www.safety.rochester.edu/homepages/ibchome.html

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UR Incident and Near Miss Report Forms www.safety.rochester.edu/SMH115.html

UR Requirements for Transporting Hazardous Samples within Buildings -
www.safety.rochester.edu/pdf/transportsample.pdf

VII. APPENDICES

BSL1 & ABSL1 regulatory requirements – CDC/NIH BMBL, NIH Guidelines

BSL2 & ABSL2 regulatory requirements – CDC/NIH BMBL, NIH Guidelines, OSHA's
Bloodborne Pathogens

VIII. REVISION HISTORY

| Date | Revision No. | Description |
|------------|--------------|---|
| | New | UR Animal Biosafety Level 1 Requirements |
| 1/9/2012 | 1 | Update policy/link corrections |
| 9/26/2012 | 2 | Links to pdf version of signs added to Appendices/Forms section |
| 6/29/2018 | 3 | Consolidate BS020, BS021, BS022, BS023; incorporate 3/18/2016 updates to BS022/BSL1 for biohazard symbol requirements; add BSL2+; move regulatory language to Appendix; refer to LAB form where appropriate; delete facility requirements for chemicals; updated web links |
| 3/6/2020 | 4 | Updates subsequent to IBC discussions since last update (joint IBC/UCAR bio letter, update ABSL transport procedures and PPE, update ABSL2 injections/anesthesia requirements, delete ABSL2 surgical mask requirement), condense BSC section/refer to BS010, update web links |
| 12/30/2020 | 5 | Biosafety in Microbiological and Biomedical Laboratories 6 th edition published – relevant updates made |
| | | |