

Animal Biological Safety Level 2 (ABSL-2)

BIOSAFETY MANUAL



PROTECT
PEOPLE



PROTECT
ANIMALS



ADVANCE
SCIENCE

Safe Science = Good Science





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Purpose and Scope

Purpose

The purpose of this Animal Biosafety Level 2 (ABSL-2) Biosafety Manual is to establish minimum biosafety practices, containment procedures, training expectations, personal protective equipment (PPE) requirements, decontamination procedures, emergency response expectations, and waste management practices for research involving small laboratory rodents (e.g. mice, rats) housed or manipulated under ABSL-2 conditions.

This manual is intended to protect research personnel, animal care staff, veterinary staff, facility support personnel, the animal colony, the surrounding research environment, the public, and the environment from exposure to biohazardous materials associated with ABSL-2 laboratory animal research.

ABSL-2 practices are required for work involving small laboratory rodents infected with, exposed to, or potentially shedding biological agents associated with human disease, or other biohazardous materials that present moderate risk to personnel or the environment. ABSL-2 builds on ABSL-1 practices and includes additional procedures, containment equipment, PPE, training, access restrictions, and occupational health considerations.

Scope

This manual applies to research personnel, including animal care personnel, who enter, work in, support, perform, or supervise ABSL-2 small laboratory rodent (e.g. mice, rats) work at the University of Kentucky (UK). This manual does not apply to work with large animals, including livestock.

This manual applies to ABSL-2 activities involving:

- Administration of infectious agents, viral vectors, recombinant or synthetic nucleic acid molecules, genetically modified microorganisms, or other biohazardous materials to animals requiring ABSL-2 containment.
- Housing of small laboratory rodents experimentally exposed to agents or materials requiring ABSL-2 containment.
- Handling of contaminated cages, bedding, enrichment, feed, water bottles, equipment, or waste.
- Animal manipulations, sample collection, imaging, surgery, euthanasia, and necropsy requiring ABSL-2 containment.
- Transport of animals, tissues, carcasses, samples, or contaminated materials from ABSL-2 spaces.
- Decontamination, disinfection, spill response, and waste disposal associated with ABSL-2 animal work.

ABSL-2 Practices and Procedures

Personnel must use work practices that minimize exposure to infectious materials, animal waste, contaminated bedding, sharps, droplets, splashes, and aerosols.

The following practices apply to all ABSL-2 animal work:

1. Perform procedures in a manner that minimizes aerosol and splash generation.
2. Long hair is restrained so that it cannot contact hands, animals, specimens, containers, or equipment.
3. Use a certified biological safety cabinet or other approved containment device as specified in the corresponding IBC protocol(s) when manipulating infectious materials or animals or when aerosols or splashes may be generated.
4. Use mechanical pipetting devices; mouth pipetting is prohibited.
5. Minimize sharps use whenever possible.
6. Use safety-engineered sharps whenever feasible.
7. Dispose of sharps immediately into hard plastic medical sharps containers that are marked with the universal biohazard symbol or red in color.



8. Do not recap, bend, shear, or manipulate needles unless justified by procedure and approved by risk assessment as specified in the corresponding IBC protocol(s). Recapping of needles must be performed using the one-handed scoop technique or safe recapping devices.
9. Decontaminate work surfaces after procedures and after spills or contamination with an appropriate disinfectant. Disinfectants are not one-size-fits-all and will be specific to the biohazardous materials in use. Refer to the corresponding IBC protocol(s).
10. Keep doors closed while ABSL-2 work is in progress.
11. Gloves are worn to protect hands from exposure to hazardous materials and when handling animals.
 - a. Double (2x) nitrile gloves should be used unless otherwise specified by risk assessment.
 - b. Bite and/or scratch-resistant gloves may be utilized.
 - c. Gloves worn inside the animal facility are not worn outside of the animal facility.
 - d. Change gloves whenever contaminated, glove integrity is compromised, or when otherwise necessary.
 - e. Do not wash or reuse disposable gloves and dispose of used gloves in designated biowaste bins.
12. Wash hands after removing gloves and before leaving the containment area.
13. Do not eat, drink, apply cosmetics, handle contact lenses, or store food or drink in ABSL-2 areas.
14. Animals and plants not associated with the work being performed are not permitted in areas where infectious materials and/or animals are housed or manipulated.

All ABSL-2 work must be conducted in accordance with an approved animal use protocol, an approved biosafety protocol, and any additional conditions established by the Institutional Biosafety Committee (IBC), Institutional Animal Care and Use Committee (IACUC), Attending Veterinarian (AV), Biosafety Officer (BSO), or facility leadership.

Before initiating ABSL-2 work, the Principal Investigator must ensure that:

1. The biological hazards have been reviewed and approved by the UK IBC.
2. The animal procedures have been reviewed and approved by the UK IACUC.
3. The ABSL-2 animal room, procedure room, or containment area have been approved by the UK IBC.
4. Personnel have completed required biosafety, animal care, species-specific, facility-specific, and protocol-specific training.
5. Required PPE, disinfectants, spill response supplies, sharps containers, biohazard waste containers, and transport containers are available.
6. Signage has been posted to communicate hazards, PPE requirements, entry restrictions, emergency contacts, and other required precautions.
7. An occupational health review has been completed when required by institutional policy or risk assessment.

Access to ABSL-2 animal rooms should be limited to individuals required for experimental, husbandry, veterinary, facility support, or emergency purposes that have been trained in the hazards, precautions, exposure evaluation procedures, and emergency practices applicable to the work.

Roles and Responsibilities

Principal Investigator

The Principal Investigator (PI) is responsible for the safe conduct of ABSL-2 research conducted under their direction. The PI must ensure that all work is performed in accordance with approved IBC and IACUC protocols, institutional policies, and applicable biosafety requirements.

The PI is responsible for:

- Ensuring all applicable approvals, including IBC and IACUC protocol approval, have been obtained before initiating work
- Ensuring that hazards are identified and communicated to research personnel
- Developing laboratory- and protocol-specific SOPs
- Ensuring research personnel complete required training before performing ABSL-2 work



- Ensuring research personnel understand exposure risks, clinical signs or symptoms of concern, spill response procedures, and reporting requirements
- Ensuring appropriate PPE is available and used correctly by research personnel
- Ensuring work is performed only in approved spaces, as specified in the corresponding IBC protocol(s)
- Ensuring disinfectants and decontamination procedures are appropriate for the agent and materials in use as specified in corresponding IBC protocol(s)
- Reporting incidents, exposures, spills, containment failures, animal escapes, or unexpected morbidity/mortality according to institutional procedures

Research Personnel

Research personnel are responsible for following approved practices as described on corresponding IBC and IACUC protocols, using required PPE, completing required training, reporting incidents promptly, and performing work in a manner that minimizes exposure to biohazardous materials, animals, contaminated equipment, bedding, waste, and sharps.

Personnel must not conduct ABSL-2 work until they have been authorized by the PI and animal facility management and have completed all required training and occupational health requirements.

Animal Care Personnel

Animal care personnel are responsible for performing husbandry procedures according to approved ABSL-2 practices, including cage changing, feeding, watering, animal observations, waste handling, room sanitation, PPE use, and reporting animal health or containment concerns.

Animal care staff must be informed of the hazards associated with the animals, cages, bedding, and waste they handle.

Biosafety Officer

The Biosafety Officer provides biosafety guidance, reviews ABSL-2 practices, assists with project-specific risk assessments, evaluates containment and work practices, supports incident response, and provides or coordinates biosafety training.

Veterinary Staff

Veterinary staff provide oversight of animal health and welfare, review clinical signs, support humane endpoints, respond to animal health concerns, and assist in evaluating zoonotic or occupational risks associated with animal research.

Access Control and Signage

Access to ABSL-2 animal rooms and procedure areas is restricted to authorized personnel. Entry is limited to individuals who have completed required training, understand the hazards present, and have a legitimate need to enter the space.

ABSL-2 rooms must be posted with appropriate hazard signage before animals or biohazardous materials are introduced. Signage should include:

- Universal biohazard symbol (when infectious agents are present)
- Animal Biosafety Level (ex. ABSL-2)
- DLAR Supervisor or other responsible personnel's name and telephone contact phone number(s)
- Personal Protective Equipment (PPE) Requirements
- Occupational health requirements, if applicable
- Any additional procedures required for entering/exiting the room

ABSL-2 Door Signage Template is available in Appendix A.

Cages housing small laboratory rodents infected with or exposed to biological hazards requiring ABSL-2 containment that may pose a risk to research personnel must be clearly marked with an Orange Hazardous Agent Awareness Card (Appendix B) and have an associated Special Animal Safety Protocol (SASP) document and DLAR Hazardous Agent Use Request Form (Appendix C), located in the Service Book of each ABSL-2 housing room.

More information regarding the Special Animal Safety Protocol (SASP) Program is available online:

<https://researchsafety.uky.edu/animal-research-compliance/sasp-program>

Personal Protective Equipment (PPE)

Personnel entering ABSL-2 animal areas must wear PPE appropriate for the hazards, animal species, and procedures being performed. PPE requirements are determined by risk assessment and may vary by room, procedure, agent, and animal model.

Minimum PPE for Researchers Performing Animal Procedures in Conjunction with Biohazardous Materials Requiring ABSL-2 Containment with a SASP:

- Disposable wrap-around gown
- Double (2x) disposable nitrile gloves
- Eye protection (ex. safety glasses, goggles, or face shield)
- Fluid-resistant surgical mask

Additional PPE may be required and will be specified in the corresponding IBC protocol(s).

PPE must be donned and doffed in such a manner as to minimize exposure to biohazardous materials and animal allergens, prevent cross-contamination, and maintain containment during work in ABSL-2 environments.

The order in which PPE is doffed (removed) is more important than the order in which PPE is donned (put on). When you put on fresh PPE, the PPE is clean and there is no risk of contamination. Users are most likely to contaminate themselves or spread contamination when doffing PPE after working with biological hazards or animals.

Donning PPE Upon Entry

The following minimum PPE should be donned by research personnel prior to performing animal procedures in conjunction with biohazardous materials requiring ABSL-2 containment with a SASP:

- Disposable wrap-around gown
- Fluid-resistant surgical mask
- Double (2x) pair of disposable nitrile gloves
 - Gloves should go over the wrist cuffs of your disposable gown such that no skin is visible
 - Consider using two (2) different color gloves to more easily see cuts, rips, or tears in outer gloves
- Eye protection (ex. safety glasses, goggles, or face shield)

Doffing PPE Upon Exit

PPE should be removed when you exit ABSL2 housing and/or procedure rooms to minimize cross-contamination and maintain containment. PPE should be removed in the following sequence:

- Remove outer gloves and discard in biowaste bin.
- Remove eye protection. Be careful not to touch your face, hair, or neck.
 - If reusable, eye protection should be wiped with an appropriate disinfectant and stored safely for future use.
 - If disposable, discard in biowaste bin.
- Remove surgical mask and discard in biowaste bin. Be careful not to touch your face, hair, or neck.
- Carefully remove wrap-around gown, roll the gown away from your body as you go, and discard in biowaste bin.



- Remove inner gloves and discard in biowaste bin.
- Wash your hands in nearby handwashing sink.
 - If handwashing sink is not available, use hand sanitizer.

ABSL-2 Cage Changing

Small laboratory rodent ABSL-2 caging must be changed in such a manner to ensure safe operating procedures and prevent exposure or contamination. Static microisolator caging used for ABSL-2 small laboratory rodents should be changed every 7 days, whenever more than 20% of the bedding material is damp (due to excess urine or water leakage), whenever cages become visibly compromised, or more often as needed. Individually Ventilated Cage (IVC) caging used for ABSL-2 small laboratory rodents should be changed every 14 days, whenever more than 20% of the bedding material is damp (due to excess urine or water leakage), whenever cages become visibly compromised, or more often as needed. ABSL-2 small laboratory rodent caging may be changed by animal husbandry staff or research personnel. When cage changing is to be performed exclusively by research personnel, the "PI Staff Only: DLAR Personnel DO NOT TOUCH" option should be circled on the Orange Hazardous Agent Awareness Card (Appendix B).

Materials Required

- Clean, freshly bedded cages + water bottles
- Certified Biological Safety Cabinet (BSC)
- Disinfectant¹
- Paper towels
- Plastic-backed, absorbent towels (ex. lab diaper)²
- Rubber tipped forceps³
- Dedicated "Dirty" rolling cage rack
- Dedicated "Dirty" water bottle basket

Procedure

1. Don minimum PPE for ABSL-2 procedures – Disposable wrap-around gown, double (2x) disposable nitrile gloves, fluid-resistant surgical mask, and eye protection.
2. Ensure Biological Safety Cabinet (BSC) is turned on and functioning properly prior to the start of cage changing by observing the Magnehelic gauges and/or digital readouts. If BSC has been shut down, BSC blower should be allowed to operate for at least 5-minutes to purge the cabinet prior to use.
3. Place a clean cage, disinfectant, and a tray of disinfectant solution + rubber tipped forceps (if applicable) inside the BSC on the "clean" side. Place plastic-backed, absorbent toweling (ex. lab diaper) soaked in disinfectant in the BSC for dirty cages on the "dirty" side. Maintain unidirectional "clean-to-dirty" workflow to prevent cross-contamination.
4. Carefully remove a single cage of animals, spray/wipe the exterior of the cage with disinfectant and place inside the BSC on the disinfectant soaked lab diaper located on the "dirty" side of the BSC.
5. Using slow, careful movements, remove the lid from the clean cage.
6. Using slow, careful movements, remove the lid from the occupied, dirty cage.
7. Transfer animals to the clean cage, using the rubber tipped forceps (if desired).
8. Replace the cage lid on the now occupied cage.
9. Remove the water bottle from the now empty, dirty cage.
10. Replace the cage lid on the now empty, dirty cage.
11. Thoroughly spray/wipe the exterior of the empty, dirty cage with disinfectant and ensure that all surfaces are thoroughly wet. Place it on dirty cage rack for transport to autoclave. Do not stack cages on top of one another. Ensure cage lid is not latched, if applicable.

¹ Appropriate disinfectant will be specified on corresponding IBC protocol(s).

² If not utilized, care must be taken to ensure the bottom of cages and work surface remain thoroughly wetted with disinfectant.

³ Optional

12. Thoroughly spray/wipe the exterior of the dirty water bottle with disinfectant and ensure that all surfaces are thoroughly wet. Place it on dirty water bottle basket for transport to autoclave.
13. Continue changing cages until finished.
14. Once all cages have been changed, spray the interior surfaces of the BSC with disinfectant. After appropriate contact time, wipe surfaces with clean paper towels and place used paper towels in biowaste bin.
15. Doff PPE upon exit. Wash hands at nearest handwashing sink OR utilize hand sanitizer if no handwashing sink is available.

ABSL-2 Cage Handling

Dirty caging used for ABSL-2 small laboratory rodents must be handled, transported, autoclaved, and washed appropriately to minimize the risk of exposure to personnel, the public, and the environment, and maintain biocontainment throughout animal facilities. Each ABSL-2 housing location must have a dedicated rolling cage rack and water bottle basket for temporary storage and transport of dirty ABSL-2 caging and water bottles, respectively. If dedicated rolling water bottle basket is unavailable, a sturdy, autoclave proof bin (i.e. stainless steel, polypropylene) lined with an orange or opaque autoclave bag marked with the universal biohazard symbol may be utilized as an alternative.

Materials Required

- Disinfectant⁴
- Dedicated “Dirty” rolling cage rack
- Disposable cage rack cover
- Dedicated “Dirty” rolling water bottle basket-cart
- Disposable water bottle basket cover
- Chemical integrator strip⁵
- Autoclave
- Cage Wash

Procedure

1. Don minimum PPE for ABSL-2 procedures – Disposable wrap-around gown, double (2x) nitrile gloves, fluid-resistant surgical mask, and eye protection.
2. Locate ABSL-2 dirty cage rack and cage rack cover.
3. Carefully cover the ABSL-2 dirty cage rack with cage rack cover. Ensure cages and rack are fully covered.
4. Thoroughly spray/wipe the exterior of the ABSL-2 dirty cage rack, including wheels, and cage rack cover with disinfectant. Ensure all surfaces are thoroughly wet.
5. Thoroughly spray/wipe the exterior of the ABSL-2 water bottle basket, including wheels, and water bottle basket cover with disinfectant and ensure that all surfaces are thoroughly wet.
 - a. If autoclave bag lined bin is used in lieu of water bottle basket, ensure water bottles within bag are unstoppered or loosely secured, loosely close the autoclave bag (use a zip tie, autoclave tape, etc. to secure) and thoroughly spray the exterior of the autoclave bag, bin, and cart, including wheels, with disinfectant and ensure all surfaces are thoroughly wet.
6. Doff PPE upon exit. Wash hands at nearest handwashing sink OR utilize hand sanitizer if no handwashing sink is available.
7. Carefully transport covered ABSL-2 dirty cage rack and water bottle basket-cart directly to autoclave. Avoid heavily trafficked areas.
8. Attach a chemical integrator strip and autoclave ABSL-2 dirty cages and water bottles.
9. Verify cycle conditions were met by visually examining chemical integrator strip and/or printed readout. Once verified, cages and water bottles may be transported to cage wash. Mechanical cage wash must have a final rinse temperature of at least 180°F.

⁴ Appropriate disinfectant will be specific on corresponding IBC protocol(s).

⁵ Available upon request from UK Research Safety – email biosafety@uky.edu



ABSL-2 Animal Transport

IBC and IACUC approval must be obtained prior to removal of any animals from the ABSL-2 facility. ABSL-2 animals may require transport to and from housing locations, procedure rooms, laboratory spaces, imaging facilities, or other locations, and the safe and compliant transport of small laboratory rodents minimizes the risk of exposure to personnel, the public, and the environment.

Materials Required

- Disinfectant⁶
- Cage Cart
- Cage Cover (if applicable)

Procedure

1. Verify IBC and IACUC approval for transport. Live animals may not be removed from the animal facility without prior approval.
2. Don minimum PPE for ABSL2 procedures—Disposable wrap-around gown, double (2x) disposable nitrile gloves, fluid-resistant surgical mask, and eye protection.
3. Identify animals for transport and visually verify cage information.
4. Carefully remove cage(s) for transport, one at a time.
5. Wipe the exterior of the cage with an appropriate disinfectant prior to loading onto cage cart. Ensure cages are properly secured/latched.
6. Repeat the process until all cages for transport have been wiped with disinfectant and loaded onto cage cart.
7. Animal cages must be completely covered such that no part of the cage is visible.
 - a. If transporting animals between buildings on campus, you must use an enclosed transport cart. Ensure doors are closed and latched prior to transport. Spray the exterior of the cart and wheels with disinfectant prior to transporting cart. Ensure surfaces are thoroughly wet and allow to air dry.
 - b. If transporting animals within the same building, you may use drapes, towels, or other material to obscure cages from view. Cages must be completely covered such that no part of the cage is visible during transport. Spray the exterior surfaces of the cart and wheels with disinfectant prior to transporting cart. Ensure surfaces are thoroughly wet and allow to air dry.
8. Doff PPE upon exit in the following order:
 - a. Remove outer gloves and dispose in biowaste bin.
 - b. Remove eye protection.
 - i. If reusable, eye protection should be wiped with an appropriate disinfectant and stored safely for future use.
 - ii. If disposable, dispose in biowaste bin.
 - c. Remove surgical mask and dispose in biowaste bin.
 - d. Carefully remove the gown, rolling the gown away from your body as you go, and dispose in biowaste bin.
 - e. Remove inner gloves. Wash your hands if handwashing sink is available. Otherwise, use hand sanitizer.
9. Carefully transport cage cart(s) using service/freight elevators. Avoid heavily trafficked areas and all patient areas such as waiting rooms or clinic spaces.

NOTE – Cages must never be carried by hand between locations. Even a single cage must be placed in or on a cart for transport. This ensures animal safety and prevents potential for escape should you drop the cage.

ABSL-2 Sample Transport

Samples obtained from small laboratory rodents housed under ABSL-2 containment must be transported in a safe manner to minimize the risk of exposure to personnel, the public, and the environment. Samples obtained from ABSL-2 containment may need to be transported to and from animal facility locations, laboratory spaces, imaging facilities, or other locations.

⁶ Appropriate disinfectant will be specified on corresponding IBC protocol(s).



Materials Required

- Disinfectant⁶
- Secondary transport container

Procedure

1. Within the Biological Safety Cabinet (BSC), biological samples must be placed in a sealed primary container (e.g. conical centrifuge tube, microcentrifuge tube, Vacutainer).
2. Wipe the exterior of the primary container with an appropriate disinfectant.
3. Place the primary container in a leak-proof, shatter-proof, secure-lidded secondary container with absorbent material in case of spill.
4. Continue filling secondary container. Do not overfill.
5. Once full and ready for transport, close the secondary container lid and spray/wipe the exterior with an appropriate disinfectant. Ensure all surfaces are thoroughly wet.
6. Doff PPE and wash your hands prior to exiting the laboratory/animal facility.

NOTE - Pay special attention to the path you will travel, taking care to avoid public elevators, hallways, and otherwise crowded areas of campus.

ABSL-2 Animal Procedures

Laboratory animal procedures requiring ABSL-2 containment must be performed using approved containment practices as described in the corresponding approved IBC and IACUC protocol(s). All personnel must be trained in the specific animal handling practices and procedures prior to starting work. ABSL-2 animal procedures must be performed in such a way as to minimize the risk of: bites and scratches, needlestick injuries, aerosol generation, splashes or droplet exposure, environmental contamination, escape of animals, and cross-contamination between animals. A biological safety cabinet (BSC) or other approved containment device must be used whenever procedures may generate aerosols, droplets, splashes, or involve manipulations of infectious or potentially infectious materials, unless otherwise specified in corresponding approved IBC protocol(s).

ABSL-2 Animal Inoculations

Inoculation of laboratory animals with biohazardous materials requiring ABSL-2 containment often involves the use of sharps and presents an increased risk of personnel exposure via accidental parenteral inoculation. To minimize the risk of accidental needlesticks or other parenteral exposures, restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g. physical restraint, chemical restraint) are used whenever possible.

Hand-held restraint of conscious, non-anesthetized animals during sharps-based inoculation of biohazardous materials requiring ABSL-2 containment is prohibited unless scientifically justified or required for animal welfare reasons. Any such exception must be clearly described and approved in the corresponding IBC protocol(s). Additional training in appropriate hand-held restraint methods must be provided to all personnel.

ABSL-2 Necropsies

Class II biological safety cabinets (BSCs) are the preferred engineering control for ABSL-2 laboratory animal necropsies. Ventilated downdraft necropsy tables may be used when a BSC is impractical due to animal size, procedural requirements, or equipment limitations. Procedures conducted on a downdraft table must be performed within the airflow capture zone and supported by a protocol-specific risk assessment as detailed in corresponding approved IBC protocol(s). Additional PPE may be required.

Downdraft necropsy tables are specialized dissection tables equipped with built-in ventilation systems that pull air through the work surface and away from a person's breathing zone. It is designed to reduce exposure to aerosols, droplets, odors, allergens, and other airborne contaminants generated during animal necropsies. It is important to note that a downdraft necropsy table is NOT equivalent to a Class II Biological Safety Cabinet (BSC). Unlike a BSC, a downdraft necropsy table

does not provide product protection, it may not completely contain aerosols generated above the capture zone, does not provide any physical barrier between personnel and materials, and can lose efficiency if airflow is obstructed.

ABSL-2 Decontamination and Disinfection

An appropriate chemical disinfectant must be utilized for decontamination of work surfaces, equipment, cages, etc. within ABSL-2 facilities. Disinfectants are not “one-size-fits-all” and must be suited to the specific biological agent(s) in use, organic load present, surface(s) to be disinfected, and equipment utilized. Never mix disinfectants with one another, as hazardous chemical reactions may occur. Always follow the manufacturer’s instructions when diluting and using disinfectants. Appropriate disinfectant(s) will be specified on corresponding approved IBC protocol(s).

Work surfaces in ABSL-2 facilities must be disinfected:

- Before and after ABSL-2 procedures
- After spills or any visible contamination
- At the end of the workday

Equipment in ABSL-2 facilities must be disinfected:

- Before being removed from ABSL-2 facilities
- Prior to any maintenance, service, or repair
- Whenever visibly contaminated or suspected of being contaminated
- After each use

ABSL-2 Waste Management

ABSL-2 work may generate various types of waste, including sharps, small animal carcasses, liquid biohazardous waste, solid biohazardous waste, etc. Care must be taken to ensure waste generated during ABSL-2 work is properly and safely disposed of to minimize risk of exposure to personnel, the public, and the environment.

Sharps

Sharps Waste can be divided into four categories: biohazardous sharps waste, biohazardous plastic sharps waste, non-biohazardous sharps waste, and non-biohazardous plastic sharps waste.

- Biohazardous sharps waste consists of objects that have the high potential to produce punctures or lacerations and have been used in conjunction with biohazards. This includes needles/syringes, lancets, unfixed glass slides, scalpel blades, glass vials, glass Pasteur pipets, razor blades, and disposable surgical instruments. Biohazardous sharps waste must be collected in a hard plastic medical sharps container that is marked with the universal biohazardous symbol or red in color.
- Biohazardous plastic sharps waste typically consists of plastic serological pipettes, micropipette tips, plastic Pasteur pipets, plastic inoculating loops, plastic plate spreaders, and any other pointy plastic items that have been utilized in conjunction with biohazardous materials that may be likely to puncture an autoclave bag. While these items may not be considered as sharp as needles or glass, they do still pose a risk of puncture or laceration. Loose biohazardous plastic sharps, such as pipettes or tips, are prohibited in autoclave bags.
- Small biohazardous plastic sharps waste (micropipette tips, etc.) may be collected in small benchtop biohazardous waste bags. Small benchtop biohazardous waste bags must be loosely closed, placed into a larger biohazardous waste bag with other solid biohazardous waste, and autoclaved before final disposal. Alternatively, small biohazardous plastic sharps waste may be collected as described directly below.
- Large biohazardous plastic sharps waste (ex. plastic serological pipettes and plastic Pasteur pipets) must be collected in a plastic lined box (ex. a cardboard box lined with a biohazardous waste bag on the interior). Once full, close the cardboard box loosely with tape, and put the closed box into a biohazardous waste bag. Boxes should not be overfilled such that they cannot be safely closed. The bagged box of biohazardous plastic sharps may then be autoclaved as solid biohazardous waste.
- Used needles, syringes, lancets, razor blades, and other sharp objects which have NOT been used in conjunction with biohazardous or hazardous chemicals shall be placed in a hard plastic or metal container with a screw-on lid.

When full, reinforce the lid with heavy-duty tape, label the container “Not recyclable trash,” and place with regular trash for collection by housekeeping. If unsure as to whether these items have been used in conjunction with biohazardous materials, it is advisable to dispose of them in a medical sharps container as outlined above in Biohazardous Sharps Waste.

- Non-biohazardous plastic sharps waste often consists of plastic micropipette tips, plastic transfer pipettes, plastic Pasteur pipets, and plastic serological pipettes not used with biohazardous materials. Pipettes and pipette tips which have not been utilized in conjunction with biohazardous materials or hazardous chemicals shall be disposed of in a hard plastic or metal container with a screw-on lid. Once full, the container’s lid shall be taped securely shut, labeled with “Not recyclable trash,” and placed with the regular trash for removal by housekeeping. Larger plastic sharps (ex. Serological pipets and plastic Pasteur pipets) not used with biohazardous materials or hazardous chemicals shall be disposed of in a plastic lined cardboard box. Once the box is filled to a capacity where it can be safely closed (not filled above the top flap seam), its opening must be secured with tape and “Trash” written on the outside. It can then be placed with regular trash for disposal. Containers and boxes should not be overfilled such that they cannot be safely closed.

Solid Biohazardous Waste

Solid biohazardous waste consists of any non-sharp items that may be contaminated with human or animal research material (ex. bodily fluids, tissue, etc.), pathogenic organisms, or recombinant and/or synthetic nucleic acid materials. Solid biohazardous waste may consist of used gloves and other disposable PPE (Personal Protective Equipment), culture plates, specimen vials, lab bench paper, etc., and must be collected in *orange* or *clear/opaque* autoclave bags clearly marked with a biohazard symbol. Red bags are NEVER to be used for collection and autoclaving of solid biohazardous waste. Bags containing solid biohazardous waste must be held in a non-porous, leak-proof container, preferably with a foot operated lid.

Liquid Biohazardous Waste

Liquid biohazardous waste may consist of blood, blood products, and body fluids from human or animal research and culture media contaminated with pathogenic or recombinant microbiological materials which may be hazardous to humans, animals, plants, or the environment. Liquid biohazardous waste can come in many forms in the laboratory. These include, but are not limited to liquid bacterial cultures, non-adherent human tissue cultures, supernatants of tissue cultures infected with viral vectors, human blood samples and blood products (serum, plasma, and other blood components), and human tissue culture media that is aspirated into a vacuum line trap.

To disinfect liquid biohazardous waste prior to disposal:

1. Add unexpired household bleach to achieve a final concentration of 10% bleach.
2. Allow a contact time of at least 20 minutes.
3. Once the appropriate concentration and contact time have been met, test the pH of the liquid using a pH meter or pH test strip (available free upon request - biosafety@uky.edu) to ensure it is within acceptable limits for drain disposal (5.5-11.5 SU).
4. Once the mixture has met all requirements (10% bleach, 20 minutes, pH 5.5-11.5), it may be poured carefully and slowly down the laboratory sink drain with copious amounts of water.

Liquid biohazardous waste must not be left to sit overnight and should be promptly decontaminated as described and disposed of at the end of each workday or when the container is 2/3 full, whichever comes first.

NOTE – Once bleach has been added to liquid biohazardous waste, it cannot be autoclaved.

Small Animal Carcasses

Small Animal carcasses (intact or partial) must be disposed of according to how the animal/carcass was treated and where it was originally obtained. If the animal/carcass was acquired from a Department of Laboratory Animal Resources (DLAR) facility, and the animal/carcass was exposed to biohazardous materials, it must be collected in a red bag and placed in designated morgue location in DLAR. Do not place carcasses in with other biohazardous waste to be autoclaved. For small animal carcasses not originally obtained in DLAR, contact the Office of Biological Safety to



determine a suitable disposal method. Infected animal carcasses or tissue that are also contaminated with hazardous chemicals or radioactive materials is a type of mixed waste, please contact UK Biosafety at biosafety@uky.edu for guidance regarding mixed waste.

Emergency Response

All workplace incidents, and workplace acquired injuries or illness sustained by UK personnel, shall be reported by the Principal Investigator/Supervisor to Worker's Care by calling (800) 440-6285.

Exposure

If exposure to biohazardous materials occurs, keep the following steps in mind:

1. Stay calm. Secure any animals.
2. Doff PPE and follow first aid procedures.
3. If an animal was involved, make note of its cage card, PI owner, and status.
4. Report your injury to your supervisor and, if needed, proceed to UHS for medical attention.⁷
5. Supervisor should inform the Biological Safety Officer (BSO) of an exposure to biohazardous materials by emailing biosafety@uky.edu or calling (859) 257-1073.

First Aid Procedures

In case of laceration, cut, or puncture (including animal bites):

- Stop bleeding by applying gentle pressure to the wound
- Clean the wound with water
- Do not attempt to squeeze blood from the wound
- Apply antibiotic and cover wound with dressing

In case of needlestick:

- Rinse area with soap and water
- Do not attempt to squeeze blood from the wound
- Apply antibiotic and cover wound with dressing

In case of splash or other contact:

- Wash area with warm water and soap
- If there is a splash to eyes, flush area with tepid water in emergency eyewash and/or shower
- Remove contaminated clothing or equipment
- Exit area of exposure

Biological Spills

In case of biological spill outside of the BSC (including contaminated bedding):

1. Alert personnel nearby to leave the area. Post signage warning personnel of spill & DO NOT ENTER (Appendix D). Doff PPE upon exit.
2. Allow 30 minutes for aerosols to settle.
3. Don appropriate PPE (disposable gown, double (2x) gloves, fluid-resistant surgical mask, eye protection).
4. Cover the spill with paper towels or other absorbent materials.
5. Carefully pour a freshly prepared 1:10 dilution of household bleach around the edges of the spill and into the spill. Avoid splashing. Allow 20-minute contact period.
6. Use paper towels to wipe up the spill, working from the edges into the center.

⁷ Call 911 or proceed immediately to the UK Chandler Emergency Department for life threatening injuries.

7. Clean spill area with fresh paper towels soaked in disinfectant.
8. Place paper towels into orange/clear biohazard bag for autoclaving & disposal.

ABSL-2 Safety Equipment (Primary Barriers and Personal Protective Equipment)

Biological Safety Cabinets (BSCs)

Engineering controls, such as biological safety cabinets (BSCs), reduce the risk of employee exposure by removing or isolating the worker from the hazard. Biological safety cabinets (BSC) are the primary means of containment for personnel working with infectious agents and other biohazards. BSCs are only one part of an overall biosafety program that requires consistent use of good microbiological practices. The effectiveness of BSCs depends upon the behavior of the operator, the unit's orientation in the facility, and the movement of personnel in the laboratory. Personnel must use appropriate practices and procedures while working in a BSC for the cabinet to contain potentially infectious splashes and aerosols, which are generated by many experimental procedures. Personnel must be adequately trained in the use of biological safety cabinets prior to use. BSC exhaust air is passed through a certified high efficiency particulate air (HEPA) filter, which is effective at trapping particulates and infectious agents. The exhaust air from the BSC is either re-circulated back into the laboratory or exhausted out of the building. BSCs that recirculate air into the room shall not be used for work with volatile or toxic chemicals as health and safety hazards can result from the buildup of chemical vapors in the cabinet and laboratory.

All users should be trained prior to utilizing a BSC. Online BSC training is available in the SciShield Course Directory - <https://uky.scishield.com/raft/training/courses>. In-person BSC training and demonstration are available upon request. Email biosafety@uky.edu for more information.

Class/Types of BSCs

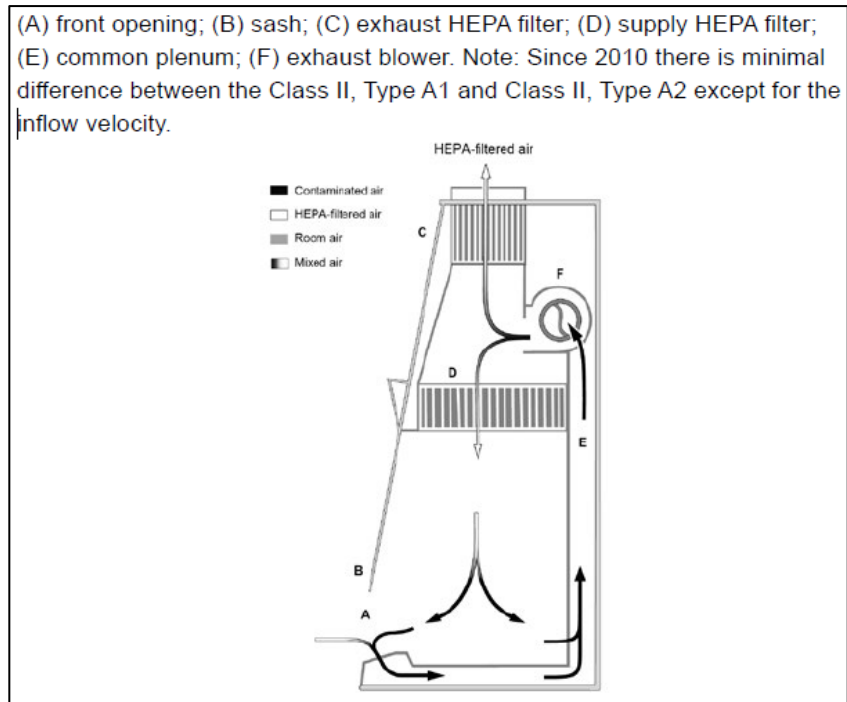
Class II, Type A1

- Internal fan draws room air through the front grille to maintain average inflow velocity of at least 75 lfm (linear feet per minute) at the face opening of the cabinet.
- Supply air flows through a HEPA filter and provides particulate-free air to the work surface.
- Downward air splits as it approaches the work surface and the internal fan draws air to the front grille and the rear grille.
- Approximately 30% of air passes through the exhaust HEPA filter and 70% is recirculated through the supply HEPA back onto the work surface of the cabinet.

Class II, Type A2

- Differs from Class II, Type A1 in the inflow velocity; Class II, Type A2 cabinets have an inflow velocity of 100 lfm.
- Small quantities of volatile toxic chemicals may be used in Class II, Type A2 cabinets **ONLY** if exhausted to the outside via a canop or thimble connection with an exhaust alarm.

Figure 1. Class II, Type A BSC

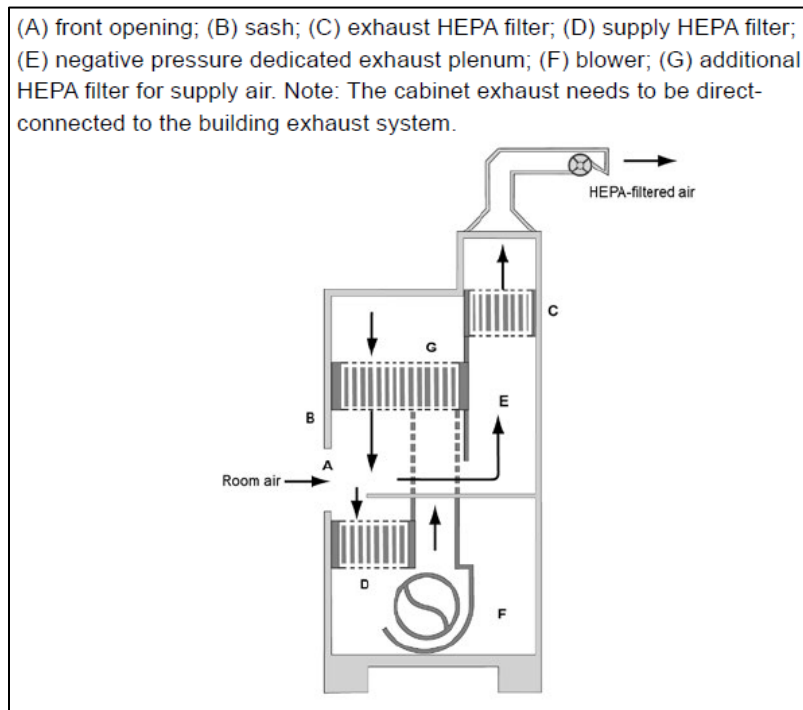


Class II, Type B1

The Class II, Type B1 cabinet is suitable for work with biohazardous materials that also requires the use of small quantities of toxic volatile chemicals, including organic solvents or carcinogens.

- Supply blower draws room air and a portion of the cabinets recirculated air through the front grille and through the supply HEPA filter.
- Particulate-free air flows upward through a plenum on each side then downward to the work area.
- Room air is drawn through the face opening at an inflow velocity of 100 lfm.
- As with Type A cabinets, the downward airflow splits just above the work surface.
- Approximately 70% of the downflow air exits through the rear grille, passes through the exhaust HEPA filter, and is discharged from the building.
 - Because the air flowing to the rear grille is discharged into the building exhaust, work with toxic volatile chemical vapors or gases should be conducted towards the rear of the cabinet.
- The remaining 30% of downflow air is drawn through the front grille, passes through the supply HEPA filter, and is recirculated.

Figure 2. Class II, Type B1 BSC

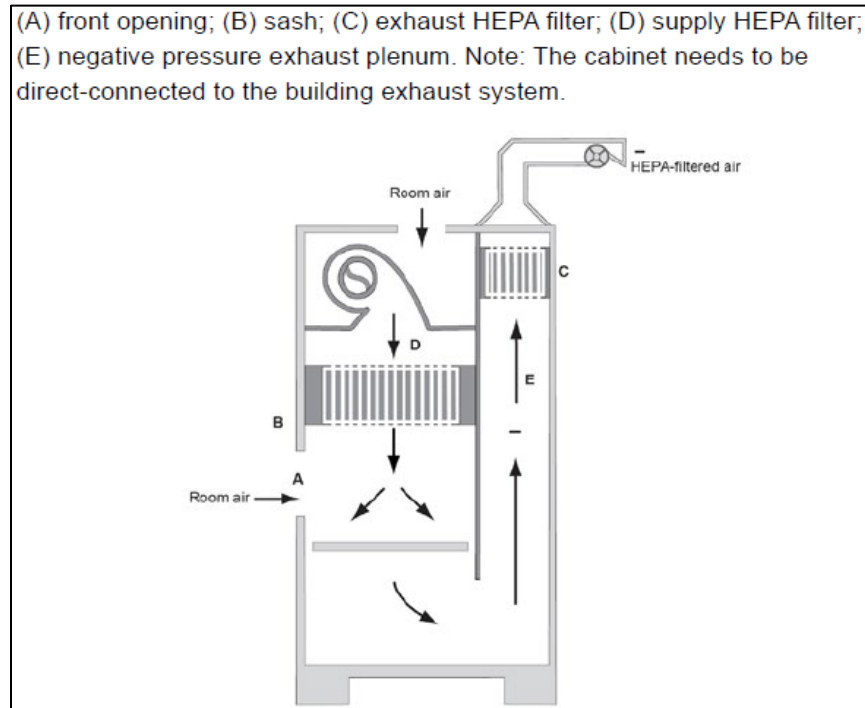


Class II, Type B2

The Class II, Type B2 cabinet provides both biological and (small quantity) chemical containment.

- Supply blower draws room or outside air in at the top of the cabinet, passes it through a supply HEPA filter and down over the work surface.
- The building exhaust system pulls air through the front and rear grilles. This captures the supply air plus room air needed to create an inflow velocity of 100 lfm.
- All air entering this cabinet is passed through a exhaust HEPA filter and exhausted via the building exhaust system.
- **BEST** option for working with small quantities of volatile toxic chemicals.
- A Class II, Type B2 cabinet will exhaust as much as 1,200 cubic feet per minute of conditioned room air.
- Expensive to operate and maintain.

Figure 3. Class II, Type B2 BSC



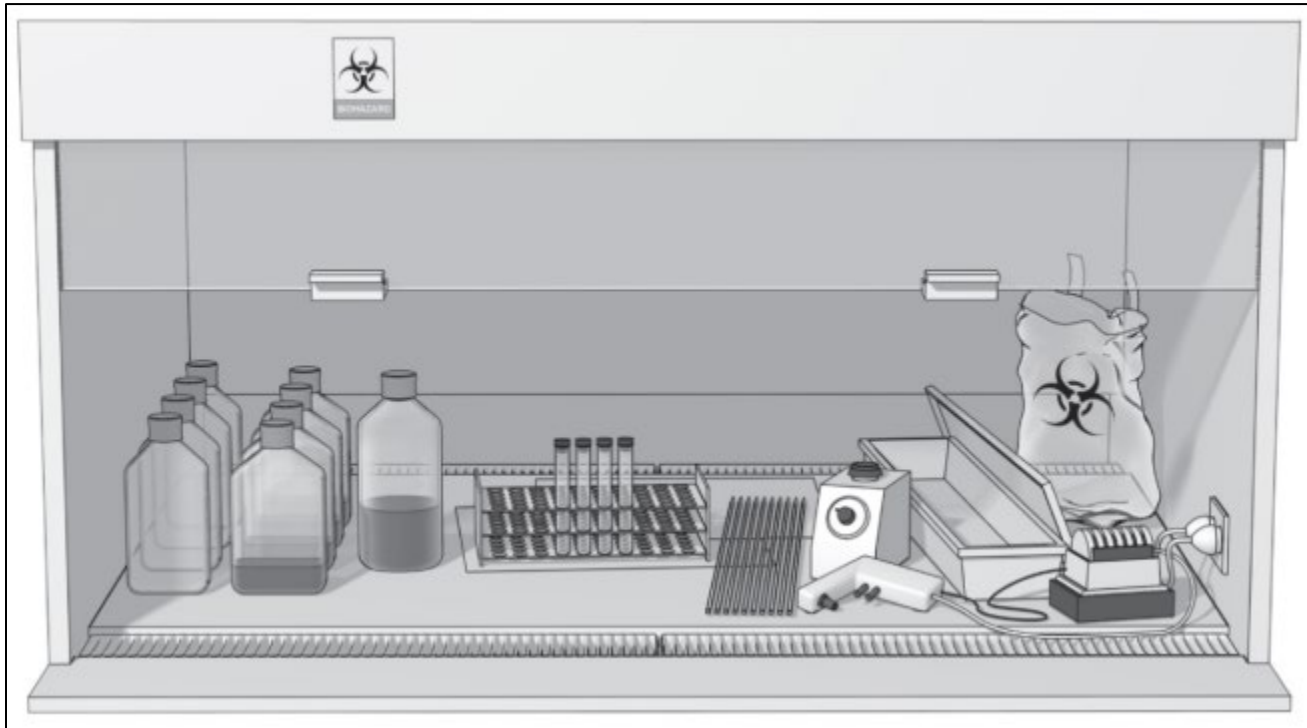
BSC Operation

- Conduct all procedures involving the manipulation of potentially infectious materials, including aerosol-producing activities, when using aerosol-producing equipment, or when using high concentrations/large volumes of organisms, inside a biological safety cabinet.
- Storage of excessive materials or equipment inside a BSC can disrupt airflow, resulting in turbulence, cross-contamination, or breach of containment. Therefore, only materials and equipment necessary for immediate work will be placed in the BSC. Additionally, storing items on top of the BSC can interrupt exhaust airflow and can result in damage to the BSC. Place large items close to the sidewalls, rather than at the back of the cabinet where they will interfere with airflow. Blocking the intake grills in the front and rear of the cabinet will interfere with proper functioning of the cabinet, which can cause a loss of containment of infectious organisms.
- Prior to each use examine the cabinet to ensure it is clean and in good repair.
 - Verify that the cabinet blower is on and functioning properly based upon observance of Magnehelic gauges and/or digital readouts.
 - Ensure that UV lights are disengaged prior to commencing work in the BSC.
- Wipe interior of BSC (work surface, walls & interior surface of window) and items placed in the cabinet with an efficacious and approved disinfectant.
- Ensure the cabinet drain valve is closed prior to starting work, to contain spills.
- Place items necessary for the procedure (including a receptacle for waste) in BSC prior to initiating work. This will minimize the number of arm-movement disruptions to the air barrier, which can compromise the containment of the BSC.
- Verify that the sash is set at the appropriate height. The operating height of a BSC is not variable; follow the manufacturer's recommendations for the BSC that you are using.
- Cabinets are designed for a single operator. More than one person working in the BSC at one time (even in a six-foot BSC) produces enough disturbances in the airflow to breach the containment of the BSC. Never lean into a BSC or place head into a BSC.



- Arrange the materials within the cabinet in a manner to ensure good aseptic technique; e.g., all workflows from one side of the cabinet (clean) to the other side (dirty). Contaminated items never cross over uncontaminated/clean supplies.

Figure 4. Clean-to-Dirty



A typical layout for working from the clean to the dirty side within a Class II BSC. Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded and other contaminated materials placed in the biohazard bag (right). Reverse this arrangement for left-handed persons.

- The BSC must be emptied and decontaminated at the end of each procedure and/or workday. Every individual is responsible for cleaning the BSC when they have finished working.
 - Remove all items from the BSC. The exterior of all potentially contaminated material must be surface decontaminated with an appropriate disinfectant prior to being removed from the unit.
 - Place all biohazardous waste in an autoclave bag in the BSC and seal prior to removing.
 - Wipe the interior surface of the unit (work surface, sides and back, interior of the glass) with an appropriate disinfectant after all items have been removed from the unit. Ensure adequate contact time.
 - DO NOT rely on UV light as a means of decontamination.
- Avoid the following common mistakes:
 - Air currents and drafts can disrupt BSC airflows. Locate BSCs away from doors, vents/diffusers and traffic paths.
 - Do not store equipment or supplies inside the cabinet.
 - Make sure that items necessary for procedures are inside the BSC prior to starting work in the BSC.
 - To prevent disruption of airflow and damage to the HEPA filter, do not store items on the top of the cabinet.
 - Prevent damage to cabinet by keeping all objects (i.e. paper towels, Kim Wipes, work surface diapers, animal bedding, etc.) from being pulled into the back, front, and side grills or slots.
 - Never disengage the alarm. The alarm indicates when the cabinet has improper airflow or reduced performance, which may endanger the researcher and/or the experiment.



- Avoid rapid motions at the front of the unit and minimize movement of arms in and out of cabinet which may disrupt the air curtain.

Animal Transfer Stations/Cage Changing Stations

Animal Transfer Stations (ATS), sometimes referred to as Cage Changing Stations, are a specialized laminar flow device used in vivarium facilities to transfer laboratory animals between cages. They are designed for procedures that may stir up dust, dander, and other allergens, such as changing animal bedding. These units often look very similar to a BSC or other types of “hoods”, however, they are **NOT** designed to protect users from hazardous or infectious agents and should **NEVER** be used for ABSL-2 practices and procedures. There is no regulatory or performance standard for ATS units, so their design and functionality can vary greatly between manufacturers.

Individually Ventilated Caging (IVC) Systems

IVC systems are specialized laboratory animal housing systems commonly used in animal facilities that provide enhanced containment, animal protection, and environmental control. IVC systems consist of individual rodent cages mounted on a ventilated rack. Each cage receives a dedicated supply of HEPA-filtered air and is connected to a controlled exhaust system. Individual cages function as a separate microenvironment, reducing airflow exchange between cages and minimizing the spread of allergens, infectious agents, and other contaminants. They can be operated under negative or positive pressure. IVC systems used for housing of laboratory animals at ABSL-2 containment must be operated under negative pressure. Exhaust plenums must be sealed. Safety mechanisms must be installed such that if the exhaust fan fails, cages and exhaust plenums do not become positively pressurized. IVC systems for ABSL-2 containment must be equipped with an alarm to notify users that system operations are malfunctioning. Exhaust HEPA filters and filter housings must be certified annually.

Static Microisolator Cages

Static microisolator cages are enclosed laboratory animal housing units that provide a physical barrier between laboratory animals and the surrounding environment. Whereas IVC systems provide active airflow, microisolator cages rely on passive air exchange through a high-efficiency filter located in the cage lid. The filter barrier helps to protect laboratory animals from environmental contaminants and reduces the risk of release of animal allergens, bedding dust, infectious materials, or other contaminants into the surrounding environment. Microisolator cages provide primary containment of biohazardous materials but are generally considered to offer less containment and environmental control as compared to IVC systems.

Laboratory Attire

Proper PPE begins with appropriate lab attire. Long-pants and close-toe shoes are required. Sandals, shorts, etc. are NOT acceptable lab attire.

Disposable Wrap-Around Gowns

Disposable wrap-around gowns must be worn by researchers in animal facilities where infectious materials and/or animals are housed or manipulated. Much like reusable laboratory coats, disposable wrap-around gowns provide a physical barrier against contact with animals, dander, bedding, cages, fluids, and other infectious materials that may be encountered while working with laboratory animals. Disposable wrap-around gowns should be donned prior to entering ABSL-2 animal facilities and removed prior to exiting these spaces. Disposable wrap-around gowns should be fluid resistant, have solid fronts, elastic or knit wrist cuffs, and ensure that shoulders, chest, arms, and legs are fully covered. Disposable wrap-around gowns are single-use and must never be reused.

Gloves

Gloves are worn to protect hands from exposure to laboratory animals, allergens, infectious materials, and other contaminants when handling laboratory animals. Double pairs of disposable nitrile gloves should be donned prior to entering ABSL-2 animal facilities and removed prior to exiting these spaces. Gloves should go over the wrist cuffs of your



disposable wrap-around gown such that no skin is visible. Consider using two (2) different color gloves for first and second pair to more easily see cuts, rips, or tears in outer gloves. Disposable gloves should be replaced as soon as possible when contaminated or compromised. Disposable gloves should NEVER be washed or decontaminated for re-use. Bite and/or scratch resistant gloves may be considered (worn underneath disposable nitrile gloves), where necessary.

Fluid-Resistant Surgical Mask

Fluid-resistant surgical masks protect mucous membranes (nose and mouth) from fluid splashes, spray, splatter, or droplets when handling laboratory animals. When worn properly, fluid-resistant surgical masks can minimize contamination and reduce potential exposure to particulates and fluids. Surgical masks are not equivalent to N95s or other forms of respiratory protection and do not provide personnel protection from aerosols.

Eye Protection

Eye protection is designed to protect eyes from fluid splashes, spray, splatter, or droplets when handling laboratory animals. Suitable options for eye protection vary from disposable face shields to reusable safety glasses or goggles. Eye protection should be donned prior to entering ABSL-2 animal facilities. Disposable face shields are designed to be single-use and should be disposed of in biowaste upon exiting ABSL-2 facilities. Reusable eye protection, such as safety glasses or safety goggles, must be removed, thoroughly wiped with an appropriate disinfectant, and stored for future reuse upon exiting ABSL-2 facilities.

Respiratory Protection

Respiratory protection may be required as determined by risk assessment of the biohazardous materials in use and planned work. If respiratory protection is required, this will be specified in corresponding approved IBC protocol(s). If respiratory protection is required, users must be enrolled in a Respiratory Protection Program (RPP), complete a departmental written RPP, complete online Respirator Use training, submit a completed medical questionnaire to UK Employee Health, and complete respirator fit testing, if applicable.

<https://ohs.uky.edu/industrial-hygiene/respiratory-protection>

ABSL-2 Animal Facilities

Facilities

ABSL-2 facilities should be separated from general traffic patterns and restricted to authorized personnel. Animal areas should be placed away from exterior walls of buildings to minimize impact from outside environment temperatures. External facility doors must be self-closing and self-locking. ABSL-2 housing and procedure room doors should open inward, be self-closing, and are kept closed whenever experimental animals are present. ABSL-2 housing and procedure room doors should never be propped open.

Handwashing Sinks

Handwashing sink(s) must be located near the exit of areas where infectious materials and/or animals are housed or manipulated and be stocked with liquid hand soap and paper towels to facilitate proper handwashing. Emergency eyewash and shower are readily available, easily accessible, and maintained, as appropriate. Sink and floor drain traps must be filled with water and/or appropriate disinfectant to prevent migration of vermin and gases.

Facility Design

ABSL-2 animal facilities are designed, constructed, and maintained to facilitate cleaning and housekeeping. Interior surfaces (e.g. walls, floors, ceilings) are water-resistant. Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Floor drains are sloped to facilitate cleaning. Penetrations must be sealed, including openings around ducts,



doors, door frames, outlets, and switch plates to facilitate pest control and proper cleaning. Internal facility features (e.g. light fixtures, air ducts, utility pipes) are designed and installed to minimize horizontal surface areas to facilitate cleaning and minimize accumulation of dust, debris, and fomites. External windows are strongly discouraged; if present, external windows must be sealed and resistant to breakage. Illumination must be adequate for all activities and avoid reflections and glare that may impede vision.

Furniture

Furniture in ABSL-2 facilities must be minimized to the extent possible and designed to support anticipated loads and uses. Benchtops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Chairs utilized in ABSL-2 facilities must be covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant and sealed to prevent harboring insects/vermin. All equipment and other furnishings in ABSL-2 facilities must be evaluated to minimize exposure of personnel to pinch points, sharp edges, and corners.

Ventilation

Ventilation must be provided in accordance with the [*Guide for the Care and Use of Laboratory Animals*](#). Ventilation system design considers the heat and high moisture load produced during cleaning of animal rooms and cage wash. The direction of airflow into animal facilities is inward; animal rooms maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system must be provided. Exhaust air must be discharged to the outside without being recirculated to other rooms.

Cage Wash

Mechanical cage wash must have a final rinse temperature of at least 180°F. The cage wash area is designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants, and 180°F water temperatures during cage/equipment cleaning process.

BSCs & Other Primary Containment

BSCs and other primary containment systems must be installed and operated in accordance with UK IBC policy. BSCs are installed such that fluctuations in room air supply and/or exhaust do not interfere with proper BSC operations. BSCs should be located away from doors, windows, heavily trafficked areas, and other sources of possible airflow disruption.

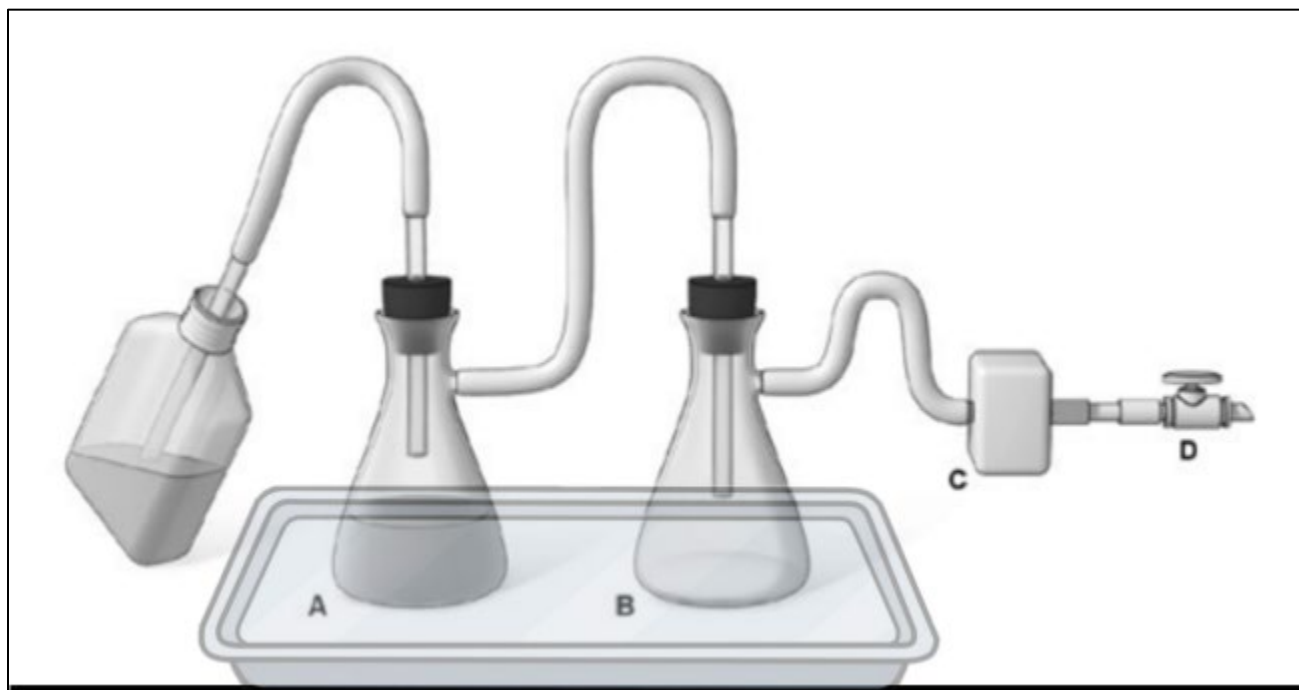
Class II, Type A2 BSCs may be connected to the building exhaust system via a canopy/thimble connection or recirculate HEPA-filtered exhaust air back into the room. Class II, Type B1 and Type B2 BSCs must be hard ducted to the building exhaust system and exhausted to the outside. Class II, Type B2 BSCs are the best choice for use of small quantities of volatile toxic chemicals. No volatile toxic chemicals of any quantity are allowed in recirculating BSCs that exhaust into the room (e.g. Class II, Type A2 BSCs NOT canopy/thimble ducted).

<https://researchsafety.uky.edu/biological-safety/laboratory-equipment/biological-safety-cabinets-bscs>

Vacuum Lines

Vacuum lines present in ABSL-2 facilities must be protected with liquid disinfectant traps and in-line hydrophobic/HEPA filters.

Figure 5. Vacuum-Line Trap Setup



The diagram in Figure 5 details the correct setup of two vacuum flasks and an in-line hydrophobic/HEPA filter in series connected by vacuum lines to a port for house vacuum. Alternatively, a vacuum pump may be used if house vacuum is not available.

Liquid waste material is drawn into the catch flask A which is pre-filled with an appropriate disinfectant*. Flask A is connected to the overflow flask B. Note that flask B is empty. Flask B provides overflow protection for flask A. Care must be taken to prevent overflow of flask A. An in-line hydrophobic/HEPA filter C is located between overflow flask B and the vacuum port D. Both flasks should be seated in a spill tray to contain any spill should the flasks be knocked over. The spill tray must be non-porous and autoclavable.

**Household bleach is commonly used for disinfection of liquid biohazardous waste. When utilizing household bleach for disinfection of liquid biohazardous waste, the catch flask A should be pre-filled with household bleach such that when the flask is 2/3 full, the final concentration is 10% bleach. Ensure appropriate contact time (20 minutes for 10% bleach). Once the appropriate concentration and contact time have been met, test the pH of the liquid using a pH meter or pH test strip (available upon request from Research Safety) to ensure it is within acceptable limits for drain disposal (5.5-11.5 SU). Once the mixture has met all requirements (10% bleach, 20 minutes, pH 5.5-11.5), it may be poured carefully and slowly down the laboratory sink drain with copious amounts of water.*

<https://researchsafety.uky.edu/biological-safety/laboratory-equipment/vacuum-line-traps>

Autoclave

An autoclave should be present in the facility to facilitate decontamination of infectious materials and waste. All autoclaves used in UK animal facilities for the decontamination of biohazardous waste must be enrolled in the UK Autoclave Verification Program (AVP) to ensure that biohazardous waste is appropriately decontaminated prior to final landfill disposal. Alternative methods of decontamination (e.g. alkaline digestions, incineration) may be considered for decontamination and disposal of laboratory animal carcasses.

<https://researchsafety.uky.edu/biological-safety/laboratory-equipment/autoclaves>



Appendix A: ABSL-2 Door Sign Templates

ABSL-2 Housing Room

This sign should be posted on entry door of all ABSL-2 Housing rooms.

Animal Biological Safety Level 2 (ABSL2)		
Building: Medical Science - 0298		
Room Number:		
Emergency Contact Information:		
Name	Office	Phone
Minimum PPE Required for Entry:		
Disposable Wrap-Around Gown	Disposable Nitrile Gloves	Surgical Mask
		
CAUTION		
ABSL2 housing may have multiple biological hazards in various cages at any time.		
Cages housing animals infected with or exposed to biological hazards that may pose a risk to personnel must be marked with an Orange Hazard Awareness Card with the agent name and have a corresponding Special Animal Safety Protocol (SASP) document in the Service Book.		
Refer to the Service Book located in this ABSL-2 housing room for agent specific information.		
Special Notes and Instructions:		
PPE required for ABSL-2 Animal Procedures Requiring a SASP: Disposable wrap-around gown, double (2x) disposable nitrile gloves, fluid-resistant surgical mask, and eye protection (ex. safety glasses, goggles, or face shield).		
Additional PPE may be required. Refer to the approved IBC protocol for more information.		
Doff PPE upon exit and wash hands prior to leaving the animal facility.		

 Research Safety



ABSL-2 Procedure Room

This sign should be posted during active ABSL-2 procedures in animal facilities.

Animal Biological Safety Level 2 (ABSL2)			
Building: Medical Science - 0298			
Room Number:			
Emergency Contact Information			
Name	Office	Phone	
Minimum PPE Required for Animal Procedures:			
Disposable Wrap-Around Gown	Double (2x) Nitrile Gloves	Surgical Mask	Eye Protection
			
		CAUTION	
Biohazardous agents in use			
Do Not Disturb			
Special Notes and Instructions:			
Don minimum PPE prior to performing ABSL2 procedures. Additional PPE may be required. Refer to the approved IBC protocol for more information.			
Doff PPE upon exit and wash hands prior to leaving the animal facility.			
 Research Safety			



ABSL-2 Procedure Flip Sign

This sign may be posted in lieu of the above ABSL-2 Procedure Room sign during active ABSL-2 procedures in animal facilities or laboratories.

Animal Biological Safety Level 2 (ABSL2)			
			
Building:			
Room Number:			
Emergency Contact Information			
Name	Office	Phone	
Minimum PPE Required for Animal Procedures:			
Disposable Wrap-Around Gown	Double (2x) Nitrile Gloves	Surgical Mask	Eye Protection
			
 CAUTION			
Biohazardous agents in use			
Do Not Disturb			
Special Notes and Instructions:			
Don minimum PPE prior to performing ABSL2 procedures. Additional PPE may be required. Refer to the approved IBC protocol for more information.			
Doff PPE upon exit and wash hands prior to leaving the animal facility.			

Room Unoccupied

Safe to Enter
Please flip this sign around when the room is occupied

Appendix B: Orange Hazardous Agent Awareness Card

An orange hazardous agent awareness card should be affixed to each cage housing small laboratory rodents infected with or exposed to biological hazards requiring ABSL-2 containment.

Haz Agents

UK DLAR Hazardous Agent Awareness Card

Cage Card # _____ DLAR Hazardous Agent Service Request # _____

Hazardous Agent Name(s) _____

Risk to Humans: Circle the pictograms that apply.

 Chemical Agent	 Biological Agent	 Radiological Agent	 Other Hazard
---	---	---	---

Circle if animals are to be handled by PI research staff only.



Appendix C: DLAR Hazardous Agent Use Request Form

This form must be completed by the Principal Investigator and submitted to the DLAR Facility Supervisor. The DLAR Facility Supervisor who completes the form is responsible for placing the completed form in the Service Book of the ABSL-2 housing room.



DLAR Hazardous Agent Use Request

INSTRUCTIONS: Complete the form, print, sign date and submit to DLAR Facility Animal Care Supervisor

LABORATORY CONTACT INFORMATION:

Study Director: _____ GLP Study #: _____

Principal Investigator: _____ Protocol #: _____

Person Requesting (if Not PI): _____ Date Submitted: _____

Contact Person: (Last, First)	Daytime	Emergency	Email

HAZARDOUS AGENT USE SUMMARY:

Agent(s) in Use: _____ SASP attached for each agent requested No

Projected Administration Start Date: _____ Projected End Date: _____

SPECIES: _____ **Room #:** _____

BARCODE NUMBERS of CAGES or ANIMALS (Complete Table Below)

1.	11.	21.
2.	12.	22.
3.	13.	23.
4.	14.	24.
5.	15.	25.
6.	16.	26.
7.	17.	27.
8.	18.	28.
9.	19.	29.
10.	20.	30.

Signature of Person Submitting Request: _____

Date: _____

DLAR USE ONLY

Date Received/Supervisor

Service Request Number

Rev: April 2026 F-HUS-1301-ATTACHMENT 1

1

Form Reset



Appendix D: Do Not Enter – Biological Spill

This sign must be posted in the event of biological spill.

