

Biohazard Containment Level 3 Facility (ABSL3) Safety and Operations Manual

Northwest Animal Facility (NAF)
University of California, Berkeley



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**NORTHWEST ANIMAL FACILITY
BIOHAZARD CONTAINMENT LEVEL 3 FACILITY SAFETY MANUAL**

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Definitions/Acronyms

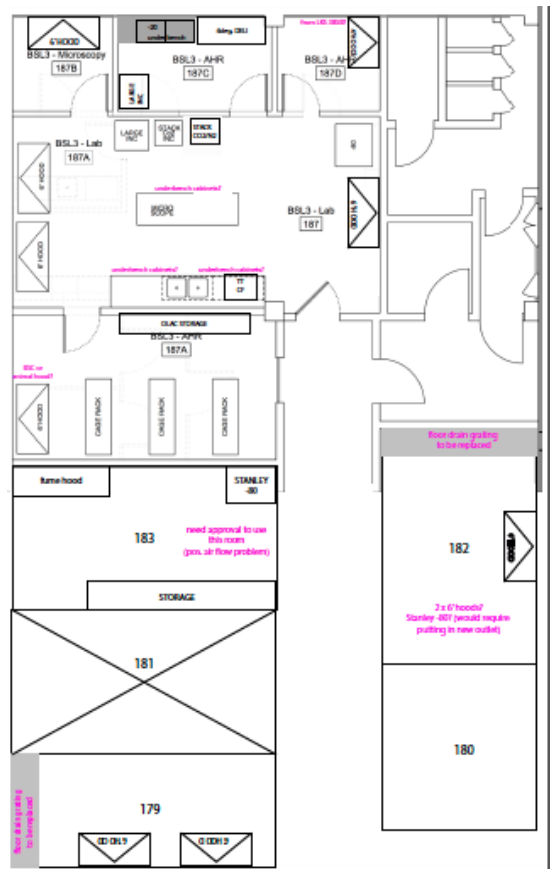
- NAF- Northwest Animal Facility
- HCDL- High Containment Lab Director
- BCF- Biohazard Containment Facility
- ABSL2- Animal Biosafety Level Two
- ABSL3- Animal Biosafety Level Three
- MMP- Mitigation Monitoring Program
- PPE- Personal Protective Equipment
- OLAC- Office of Laboratory Animal Care
- ACUC- Animal Care and Use Committee
- CLEB- Committee on Laboratory Environmental Biosafety
- BSO- Biosafety Officer
- BUA- Biological Use Authorization
- PI- Principal Investigator
- RUA- Radioisotope Use Authorization
- LUA- Laser Use Authorization
- MSDS- Material Safety Data Sheets

INTRODUCTION

The Northwest Animal Facility (NAF) contains an area that has been specially constructed to support the study of infectious disease agents in research animal models. This area is identified as the **Biohazard Containment Facility (BCF)** and can safely support work with infectious agents classified by the Centers for Disease Control/National Institute of Health (CDC/NIH) as “biosafety level two (BSL2) & three (BSL3)” as described in their publication: Biosafety in Microbiological and Biomedical Laboratories, 5th edition.

The Animal BCF is divided into Animal-BSL2 (ABSL2) and Animal-BSL3 (ABSL3) areas.

This manual refers to the ABSL3 section that comprises the following rooms: 179, 180, 181, 182, 183, 185, 187 (including 187b, 187c, 187d) as well as the ante-room, bathroom and airlock rooms 189, 189a, 191 and 193.



Floor Plan. NAF-BSL3 floor plan as of 03-2016.

Full access to ABSL3 requires compliance with the NAF Mitigation Monitoring Program (NAF-MMP), which addresses community concerns regarding the BCF. It entails that each person using the BCF must undergo initial training regarding the use of the facility. They must also complete an annual refresher training.

It is the responsibility of all the researchers using the ABSL3 to have completed and follow the Li Ka Shing (LKS)-BSL3 training requirements. All personnel entering the ABSL3 area must use ABSL3 practices and procedures at all times.

Because this is a “shared-use” facility, courtesy and cooperation are a required part of normal operations.

GENERAL SAFETY PRINCIPLES

The purpose of an animal use ABSL3 is three-fold:

- 1) To protect humans from the agents being used in the facility
- 2) To protect the animals from agents other than those used intentionally
- 3) To protect the rest of the facility and the outside community from the accidental release of infectious agents or infected animals.

ABSL3 practices include the use of personal protective equipment (PPE), such as Tyvek, gloves, respiratory protection, eye protection, and other specialized equipment and procedures designed to prevent the spread of infection or contamination by aerosolizing or vaporization, as well as by accidental self-inoculation or exposure through open wounds or mucous membranes. Training in the proper use of the equipment and procedures is an integral part of the requirements for using the ABSL3. Safe laboratory practices in the facility include the safe handling of infectious agents and careful adherence to animal handling protocols as provided by the Office of Laboratory Animal Care (OLAC), the Animal Care and Use Committee (ACUC), and the Committee on Laboratory and Environmental Biosafety (CLEB). Additionally, safe practices should adhere to principles of general laboratory safety and other special use hazards, such as controlled substances, radioactive materials and machines, or lasers. The specific guidelines and procedures outlined below are to be followed in addition to general safe laboratory practice. Good judgment and proper training should allow each worker in the facility to recognize potentially hazardous situations and to react accordingly to avoid or correct them.

ROOM DOOR SIGNAGE

Each room within the facility that contains hazardous or infectious material must be posted with appropriate information.

- 1) Main entry door signs generated by the campus biosafety officer (BSO) from approved biological use authorizations (BUAs) must indicate the approved principal investigators (PIs), infectious agents, biosafety level and any unique precautions on each door. If your work is not included on the door sign, immediately contact the High Containment Lab director (HCLD).
- 2) General guidance including a list of PPE required for entry and emergency contact information should be posted on the main entrance doors to the ABSL3.

- 3) Radioisotope Use Authorizations (RUAs), Laser Use Authorizations (LUAs) and chemical inventory door signs must be posted on the door to each room to which they apply.
Contact the HCLD for additional signage, as needed.
- 4) Other information should be posted as appropriate, as determined by the PI, HCLD, BSO, UCPD and OLAC personnel.

CONTAINMENT

The containment concept of the ABSL3 is for the hazard to be primarily contained within the animal cages (micro-isolator type), secondarily in a biosafety cabinet (BSC) and thirdly by maintaining proper airflow through the ABSL3.

To achieve this containment:

- All work involving infectious agents and infected animals must be done, exclusively, inside a BSC.
 - a. Aerosol infections require special training and only certified researchers can perform this work.
- The directionality of the airflow must be maintained; moving from outside the facility into the corridors and then into the rooms.
- Transfer infectious materials in properly labelled and sealed secondary containers.
- Follow all disinfection procedures and PPE removal requirements.

The [Cal/OSHA Aerosol Transmissible Disease Standard](#) applies to the ABSL3. This standard emphasizes that all work with infectious agents that could generate an aerosol or splash must be performed within a functioning BSC (or similar contained device - approval by HCLD needed). All animal manipulations and cage changes within the ABSL3 areas must also be performed within a BSC and used cages and water bottles must be bagged, disinfected, and autoclaved prior to final disposal.

ABSL3 USERS GROUPS DESCRIPTION

Access to the ABSL3 is limited to authorized individuals only. Authorized individuals or users fall into three categories:

1) RESEARCHERS

Authorized researchers are those persons trained in the necessary experimental and safety protocols and authorized by their PI, HCLD and the NAF facility supervisor to perform the protocols and handle the animals housed within the ABSL3.

All researchers using biohazardous agents **must be listed on a current** BUA. All researchers using radioisotopes will be similarly listed on posted RUAs. Undergraduates may not access the NAF ABSL3.

2) STAFF

Authorized OLAC staff are those persons trained in the necessary safety protocols and ABSL3 husbandry. Authorization by both the NAF facility supervisor and HCLD are necessary for authorization and access.

Authorization for researchers and staff requires medical clearance and extensive training; it is critical that users consider this lead-time prior to entry. At the completion of training, the HCLD will communicate with the OLAC supervisor and/or OLAC trainer to activate the user's card ID. These cards must be used exclusively by the authorized user.

3) VISITORS

The third category includes individuals who are either guests of PIs, UC maintenance personnel or non-UC contractors who may be granted provisional or visitor status:

- a) Guests or short-term visiting scientists sponsored by a PI using the facility may be granted provisional entrance status if their visit is approved by the HCLD and they are continuously accompanied by an authorized experienced user during each use of the facility. See visitor policy on the [ACUC website](#) for further information.
- b) Temporary entrance status may be granted to maintenance personnel (e.g., Facility Services) or contractors who are not UC employees if their visit is approved by the HCLD and they are continuously accompanied by an authorized experienced user during each use of the facility.

The designated experienced user escorting the visitor should explain the general contents of this manual, go over a short informative introduction on the infectious agents handled in this facility as well as the methods used for containment. The visitor shall also confirm that they have no condition that would place them at added risk of illness or injury resulting from possible exposure to an infectious agent used in the BCF. The visitor then should sign the ACKNOWLEDGEMENT OF RISK form found in **Appendix I**, and hand it to the HCLD.

Failure to comply with biosafety and safety guidelines can lead to the immediate withdrawal of authorization to enter the facility. The HCLD, the OLAC director, NAF facility supervisor, CLEB, or the PI may withdraw authorization.

It is the responsibility of anyone who works in this facility, to notify their supervisor of any personal condition that might place them at greater risk while using this facility. Users may also consult the Occupational Health doctor at the Tang Center (510-642-6891).

It is not recommended to work after-hours as outside assistance is limited. Thus, evening or weekend work should be avoided. If it is necessary, you will be advised not to work alone. If you plan on working after-hours, it is recommended to call or text a fellow researcher or the HCLD to let them know you will be working during after hours and what time you may be getting out. Please pay special attention to sign in and out during this time.

ABSL3 USERS ACCESS and TRAINING PROGRAM

All individuals authorized to enter the ABSL3, whether researchers, staff or visitors, are required to review and follow the practices described in this manual and to be familiar with all information contained within. Before beginning training to work in the ABSL3, each user must have their medical clearance and respirator training and fitting completed.

All users should meet the basic requirements for working in either the LKS-BSL3 laboratory or OLAC vivarium, as applicable to their job status. These trainings include all applicable Environment, Health and Safety (EH&S), OACU and OLAC required trainings. In addition to meeting these requirements, anyone gaining access to the ABSL3 must:

1) RESEARCHERS

- a) Be added to your laboratory BUA, biosafety fundamentals training, bloodborne pathogens training, and special trainings listed below.
- b) Attend the OLAC Orientation and Basic Safety Training, including an orientation to the BCF-ABSL2. For specific information about these orientations, follow the instructions on the [OLAC website](#) or contact OLAC Research Staff Trainer at olactraining@berkeley.edu.
- c) Complete full training for the LKS-BSL3.
- d) Complete NAF-ABSL3 specific training:
 - i Introduction to ABSL3 work
 - ii Entry and exiting procedures: Donning and Doffing
 - iii Emergency procedures
 - iv Handling Innocage®
 - v Animal husbandry
 - vi Aerosol infection
- e) For each PI working in the ABSL3 there must be:
 - i An approved BUA
 - ii An approved Animal Use Protocol (AUP)
 - iii A copy of the Materials Safety Data Sheet (MSDS) for each hazardous material provided along with a standard operating procedure (SOP) for proper storage, handling and disposal of this material.

Ultimately the PI and the HCLD will authorize further training and key card access for the ABSL3 facility.

Any new hazardous material, any deviation of research protocols or introduction of new infectious agents must be submitted through a BUA amendment for approval by the CLEB. The trainee must complete the RESEARCHERS TRAINING RECORD found on **Appendix IIA** in this manual and hand it to the HCLD, then their ID access card will be activated.

- e) Completion of annual refresher sessions is also required for continued authorization. The HCLD and PI will determine the topics given during the refresher training sessions.

2) **STAFF**

- a) Complete OLAC specific training, as assigned by the facility supervisor and OLAC trainer.
- b) Attend the OLAC Orientation and Basic Safety Training, including an orientation to the BCF-ABSL2.
- c) Complete training specific for the NAF-BSL3:
 - i Class - Introduction to ABSL3 and Aerosol Transmissible Disease training
 - ii Introduction to ABSL3 safety practices
 - iii Entry and exiting procedures: Donning/Doffing and PAPR use
 - iv Cage change protocols with observation and hands-on training
 - v Health check on animal care issues related to ABSL3 work
 - vi Waste disposal and autoclave use

The trainee must complete the STAFF TRAINING RECORD found on **Appendix IIB** in this manual and hand it to the HCLD. Ultimately their supervisor, OLAC trainer and the HCLD will authorize training completion and key card access for the ABSL3 facility.

- c) Completion of annual refresher sessions is also required for continued authorization. The HCLD, NAF supervisor, and OLAC trainer will determine the topics given during the refresher training sessions.

ENTRY AND EXITING PROCEDURES

The BCF is divided into the ABSL3 and ABSL2 areas by a door in the main corridor. This is considered an emergency exit and must remain closed, but cannot be locked. It is prohibited to move through this door except in case of an emergency. Any time this door is open, an alarm will go off advising the OLAC supervisor of an emergency.

DONNING AND DOFFING SOP

DONNING

- 1) Sign in on the door to room 193.
- 2) Enter through the locker room (room 193), which is adjacent to the north airlock's outer door. From the shelves in the locker room entry hallway take, primary layer of PPE:
 - a) Tyvek suit or other approved tyvek lab coat
 - b) Respirator; either fit-tested N95 disposable mask (for researchers only) or PAPR (which are found in the locker room itself) with full hood (no mask)
 - c) Inner and outer gloves, and
 - d) Two layers of shoe coversProceed into the locker room.
- 3) Don this PPE following hands-on training instructions. Refer to Figure 1. All users must test and wear the PAPR properly. Refer to the Occupational Health Program where more details on PAPRs usage are stated.



Figure 1. Full ABSL3 attire: Tyvek suit, second layer of shoe covers, PAPR and double set of gloves.

If you need a locker for storing clothes, you may use any empty locker in the locker room; Please request a lock from area supervisor if you require items to be secured.

OLAC will provide Tyvek suits or other approved tyvek lab coat, gloves, and shoe covers and. If any items of protective equipment appear to be in short supply, please notify the OLAC facility staff for restocking.

Only researchers may wear an N95 mask after proper annual fit testing and only for specific procedures. All other people entering the facility must wear PAPRs. All respirator users **must be medically cleared and trained by EH&S and PAPR disinfection procedures must be followed.**

Entering the ABSL3 facility without PPE is strictly prohibited.

DOFFING

Everyone must exit the ABSL3 through the airlock, except during an emergency.

1. When you are ready to leave the ABSL3, follow hands-on training instructions to remove PPE.
 - a) Remove outer layer of shoe covers, step into clean area by the exit door.
 - b) Spray outer gloves and front of your gown with disinfectant
 - c) Remove tape and outer gloves
 - d) Spray inner gloves and wipe the front of your PAPR hood with approved disinfectant.
2. Dispose of items in red bin right before entering the airlock. Wipe or spray the front of your Tyvek suit and PAPR belt with approved disinfectant prior to entering airlock. Refer to Figure 2.



Figure 2. Doffing outer PPE layers prior to exit.

3. Enter the airlock and take off the Tyvek and place it in the waste bins if you were performing a “first cage change,” conducting an aerosol infection, or believe you may have been exposed to TB. Otherwise, Tyvek suits may be taken back to the locker room and reused for subsequent visits to the facility. Discard tyvek suits that show signs of wear.
4. Wipe your PAPR hood, PAPR unit, and battery/filter pack with Accel TB wipes. Take off inner-gloves last.
5. Wash hands in airlock bathroom sink, room 189A.
6. Enter locker room with the PAPR battery/filter pack and hood. Plug the battery/filter pack back into the outlet. Keep the PAPR hood in your locker.
7. Shower if desired or if needed due to gross contamination from a spill.
8. Wash your hands before leaving the locker room.
9. Exit locker room through room 193.
10. Sign out

TRANSPORT PROCEDURES

OLAC and ACUC must approve any animal used in the ABSL3 before entry. If necessary, these animals may be held in a quarantine area until the veterinary staff determines they carry no infectious agents that would threaten the existing animal populations.

Consult with the HCLD about any intentions to bring a piece of equipment into the ABSL3. Any equipment brought into the ABSL3 must be capable of withstanding disinfection procedures; it must be chemically disinfected before bringing in and before removal from the facility. You may bring in clean cages and other new supplies, but everything must be thoroughly disinfected by chemicals or autoclave prior to removal from the facility.

It is forbidden to bring porous materials such as wooden furniture and cardboard boxes into the facility.

REMOVING WASTE, RESEARCH MATERIALS AND ANIMALS

Full red bags will be placed into an empty lined barrel in the autoclave room. The red bins should be able to contain all the red trash. No trash can be left on top of the barrels or on the floor. Leave the bag in the bins for autoclaving. OLAC will get the PPE waste from the ante-room and all the waste generated in the animal room. Follow proper red-bag disposal rules.

The autoclave will be run in a schedule as determined by the facility supervisor and lab users.

For items too large to autoclave, please contact the HCLD for special procedures.

Volatile chemicals and flammable materials like formalin and ethanol should never be put into the red barrel waste and autoclave. Please contact the HCLD to coordinate disposal as chemical waste.

In the NAF Mitigation Monitoring Plan, all waste generated in the ABSL3 is considered hazardous, even after autoclaving. Follow the specific SOP and in-person training for red-bag waste disposal. Only trained personnel should operate the autoclave. Authorized personnel will include a chemical indicator strip or tape in each autoclave run, properly set up the trash and set the appropriate cycle.

OLAC personnel will perform a monthly operations function using a biological indicator and will keep an updated list of the results obtained.

WASTE REMOVAL INSTRUCTIONS

- 1) Transport all autoclave waste in appropriately closed, red biohazard bags using tightly closed barrels. If the trash is too big to fit into a barrel, inspect for punctures or holes and disinfect the bag. Then carry it carefully on a cart to the autoclave and run the cycle immediately. If the autoclave is in use, place the bag inside the gray holding bin next to the autoclave, and schedule for its sterilization as soon as possible.
- 2) Deposit waste into lined red barrels and close the lids. OLAC staff will collect this trash and load the autoclave. Only trained OLAC personnel shall run the autoclave following instructions and training.
- 3) All autoclaved materials shall be removed through the autoclave “clean side.” This is the door in the corridor OUTSIDE the facility. OLAC personnel will treat as waste all items

retrieved from the autoclave “clean side.” OLAC staff should only use the freight elevator near the autoclave for transporting biohazardous waste barrels to the cold room. Return the barrels to the corridor by the autoclave.

- 4) Notify OLAC if you do not want items discarded after the autoclave cycle. Use **clear autoclave bags** for this purpose. Clear autoclave bags should only contain the items to be autoclaved, washed and returned in to the ABSL3 suite.
 - a. After autoclaving, do not wash plate cylinders. Bring sterilized plate cylinders back into the ABSL3.
- 5) Supplies and equipment must also be disinfected before removal from any area of the ABSL3.
- 6) Decontaminate large equipment or materials that cannot be autoclaved by gas or chemical disinfection. The HCLD must approve the removal of these type of materials and equipment.
- 7) Spray the equipment with appropriate and approved disinfectant, to completely decontaminate every surface. Wipe up any spray that reached the floor to prevent a slip hazard.
- 8) Some equipment may require decontamination performed by an outside vendor. Small equipment can be set up inside a BSC for gas decontamination. Consult with the HCLD, and consider that this procedure may take a few days, and may disrupt work in the facility.
- 9) Infectious materials or animals being taken from any area of the ABSL3 to other CLEB-approved laboratories must be contained in a primary, leak proof container and carried in a secondary leak-proof container. This secondary container must be made of hard opaque material and leak proof (usually a specific category A shipment container). The secondary container should display a biohazard sign label and the responsible user’s contact information. This container must always be kept under your supervision or the supervision of other researchers.
- 10) Any unwanted chemicals or waste from the ABSL3 requires special handling. Do not put them in bio-hazardous barrels as autoclaving volatile chemicals like formalin or ethanol can be dangerous. Safely store this waste and call the HCLD for help.
- 11) Work that differs from permitted research with infectious agents in the ABSL3 needs special approval. Submit a BUA amendment including materials handling, storage and waste disposal procedures in the form of an SOP.

ABSL3 AUTOCLAVE OPERATION

Only authorized personnel may operate the autoclave.

- 1) Remove all debris from the bottom of the autoclave.
- 2) Inspect and clean the chamber drain screen near the door.
- 3) Inspect the autoclave for leaks during startup and shutdown.
- 4) For every autoclave run, check that the indicator strip indicator ink has correctly changed color. If the indicator fails to change color properly, notify the OLAC supervisor immediately. Re-autoclave the materials from the failed run.
- 5) OLAC personnel will place a biological indicator test ampoule deep within a full load once monthly to monitor autoclave function. The result will be recorded in an autoclave log sheet.

BIOLOGICAL INDICATOR (BI) INSTRUCTIONS – refer to OLAC SOP 615 “Autoclave validation testing”

MAINTENANCE AND FACILITY SERVICES GUIDE

This section describes the procedures to follow when service or maintenance is required within the NAF BCF. The foremost goal of these procedures is the safety of service/maintenance personnel, followed by the dual goals of minimal interruption of normal facility operations and successful completion of the service or maintenance work. Because of the primary goal, it is imperative that all facility users understand and agree to these procedures. If at any time there are concerns or questions about these procedures, please contact the NAF supervisor or HCLD for assistance. Because there may be time or materials costs associated with arranging access to the ABSL3, those costs are the responsibility of the investigator or department requesting the service.

There are two basic kinds of work that need to be performed: facility and equipment. Facility work is usually done by campus employees who have been trained in the safety concerns of the facility. Less often, outside contractors will need to do work, and providing them access requires special procedures. Because of the nature of the work done in the facility, every precaution possible must be taken to protect these outside contractors and service people from accidental contact with hazardous materials or agents. You must contact the HCLD or NAF supervisor to receive permission before allowing these persons to enter.

Facility maintenance can often be done in corridors or common areas, in which case no PPE is needed for the BL2 portion of the BCF. The most serious concern is when a “break in containment” is required. Facility Services personnel have been trained NOT to enter the ABSL3 without the HCLD, the BSO, or a fully trained OLAC staff member present to provide guidance.

DEFINITION OF “BREAK IN CONTAINMENT”

A “break in containment” is defined as any penetration of a wall, ceiling or floor that opens an area outside the epoxy surface of the room to the contaminants otherwise contained by that surface. In simple terms, if a hole is drilled, a fixture (water or electrical) is removed, or a ceiling access panel is opened, containment is broken. Special decontamination procedures are required before this kind of work is done.

WORK NOT REQUIRING A “BREAK IN CONTAINMENT”

Whenever possible, the item requiring service should be decontaminated, removed, and serviced outside the facility. All service-related items (tools, parts, etc.) must be decontaminated before they can be removed from the containment room. Service personnel must be made aware of this requirement before they start work.

PROVIDING ACCESS FOR SERVICE PERSONNEL

Because the ABSL3 is a secured area, service personnel cannot leave and re-enter at will. Do not loan your keycard to service personnel. If the service work can be done quickly and cannot be done outside of containment, the person requesting the service must stay with the service personnel until the work is done. This will require that the service person acknowledges their understanding of the restrictions and that their health status complies with the requirements for working in the facility. These requirements are for the protection of the service personnel; to prevent accidental contamination of other research work, and to prevent the release of hazardous or biohazardous materials outside the facility. ***ABSOLUTELY NO EXCEPTIONS TO THIS POLICY CAN BE ALLOWED.***

Service personnel who are campus employees are required to attend training sessions on BCF. Contact the HCLD for the schedule.

ANIMAL HOUSING AND HANDLING

All animals housed within the ABSL3 must be kept in disposable micro-isolator caging Innovive®. This serves as the first barrier to contain the hazardous agent. Currently, only mice are approved in this facility. Any requirement for new species must receive special clearance from OLAC and CLEB prior to being brought into the facility.

Red tape is used to document recent infection, include date of infection and “TB”. White cage cards include the project name and PI. Researchers may include other information such as personal contact information.

A piece of red tape will be used to mark recently infected animals; special precautions are taken when OLAC staff performs the first cage change after infection. When the first cage change after infection occurs, the OLAC staff will place a piece of yellow tape over the red one.

1) All procedures that may generate an infectious aerosol must be performed within a BSC. The use of BSCs is required under all ABSL3 operations. ABSL3 BSC disinfection protocols must be followed both before and after each procedure. Disinfection of shared areas is of the utmost importance.

2) All carcasses and animal tissues, bedding, caging, and dry waste used in conjunction with infectious agents must be handled as biohazardous waste. ABSL3 waste and carcasses must be autoclaved before disposal as biohazardous waste. Animal carcasses and tissues that have been frozen are to be moved to a facility refrigerator 48-72 hours before autoclaving to ensure that the material is adequately thawed.

Place the carcass inside a specimen bag, seal it completely, and spray the outside with approved disinfectant. Bring this waste into the designated refrigerator located in the storage room (183), place the bag in the correct tray as they are labeled by day.

3) Dead animals found by OLAC staff will be red-bagged and left in the designated area in the designated refrigerator. If the PI or their staff requests it, the bag may be disinfected

and placed in the refrigerator or freezer in room 183 for the researcher to examine. Label all containers with the PI name and room number where found.

- 4) Autoclave all carcasses in a red autoclavable bag weekly, or more often as needed.

Refer to ANIMAL HUSBANDRY OF INFECTED MICE found in the next section of this manual.

ANIMAL HUSBANDRY OF INFECTED MICE

BIOSAFETY CONSIDERATIONS

Follow all PPE requirements. Only fully trained staff and researchers may perform animal husbandry of infected mice.

ABSL-3 biohazard signage, which must be posted on the animal room door, shall list the PI, contact information, PPE requirements, hazards, and the infectious agent(s) used in the room.

- 1) All dirty cage units should be autoclaved before disposal through the medical waste path.
- 2) Follow all quality assurance procedures for autoclaving containment caging.
- 3) All dirty cage units should be placed in autoclavable red biohazard bags. After filling each bag with no more than **10 nested cages**, the open end should be gathered closed with tape. There should be a small opening in the taped end of each bag comparable in size to the diameter of one finger.
- 4) All other items (eg. Feeders), lids and bottles may be consolidated under the BSC and sprayed with RESCUE® disinfectant and place into the lined barrel.
- 5) Ensure bags are not punctured, always keep them inside red barrels with the lid closed. In the event of a torn bag, double bag the items and ensure the outer bag is not punctured.
- 6) Spray bags and red barrels with RESCUE® disinfectant before exiting the animal room.
- 7) Spray cart handles and cartwheels used in the change-out process with approved disinfectant before exiting the animal room.
- 8) Dirty-bagged cages must be autoclaved at appropriate settings. Autoclave on Vacuum cycle for 60 minutes (cycle #3).

OLAC HEALTH COMMUNICATION

1. Any immediately reportable health concerns found in the ABSL3 must be communicated to vet staff via approved texting application prior to leaving the ABSL3. See SOP 101- Rodent Daily Health for more information. If there is no response, call 3-vets.

INFECTED MICE CAGE CHANGE - OLAC SOP

This SOP specifies the techniques used by OLAC personnel for the husbandry of mice (*Mus musculus*) housed in individually ventilated cage (IVC) racks in the NAF Animal Biosafety Level (ABSL3) Laboratory.

In general, there are three types of cage status in the NAF ABSL3:

- 1) Cages containing recently infected mice. Mice infected with *M. tuberculosis* in cages that have not been changed after the infection date. These cages are labeled with a piece of red tape by the researchers. These cages are also known as “**red cages.**” Red cage mice are considered a higher risk for infection transmission, even though the risk of infection is very small given the fact that mice don’t shed *M. tuberculosis*. A **two-person technique** must be followed when changing these cages; this cage change is known as “**first cage change.**”
- 2) Cages containing mice infected with *M. tuberculosis* that have been changed, at least one time, after the infection date. These cages are labeled with a piece of yellow tape by the OLAC technician that performed the cage change. These cages are also known as “**yellow cages.**”
- 3) Cages containing non-infected mice. These animals have been recently brought into the NAF-ABSL3 for infection or other experimental uses.

The cages will be changed according to an OLAC calendar determined by the NAF supervisor. The cages on each rack will be changed according to their “risk level”; new (non-infected) cages first, then yellow cages and, at the end, red labeled cages.

Rodent cages will be changed using proper safety practices as trained by the HCLD and the trainer or training designee. Always wear all ABSL3 designated PPE, according to the Donning and Doffing SOP, and replace outer gloves if torn. Cages must only be opened within a BSC.

Change rodent cages at the frequency outlined in the ACUC guideline [Guidance on Exceptions Regarding Housing or Husbandry of Laboratory Animals](#) or as instructed by the facility supervisor or veterinarian.

Any technicians performing cage changes **must** complete the training indicated in the ABSL3 users access and training program as indicated on page 12 of this document, prior to performing this activity by themselves.

FIRST CAGE CHANGE TECHNIQUE

Two OLAC assigned technicians are required to perform the task described below for first cage change: the inside technician (IT) will work inside the BSC and the second outside technician (OT) will work outside the BSC.

1. Collect all supplies in preparation to cage changing. If supplies are not available or equipment is not functioning properly, contact the facility supervisor immediately before cage changing begins.
2. Check "Special Service Requests" prior to conducting work in the room for exceptions to standard husbandry practices. "Special Service Requests" are either posted on the back of the door or within the room binder. In the same way, observe all special instructions noted on cage cards such as DO NOT CLEAN or DO NOT CHANGE. Make a note of these instructions.

SUPPLIES

Caging Supplies

- a. Clean cages; includes bedding & enrichment
- b. Food
- c. Water bottles
- d. Lids
- e. Feeders

In BSC

- a. Absorbent towels
- b. Bucket containing working solution of disinfectant
- c. Transfer tongs in a holder
- d. Food in secondary container w/ lid or sealed closed
- e. Disinfectant spray bottle

At Racks

- a. Step stool
- b. Knee pads (optional)
- c. Biohazard barrels
- d. Biohazard bags
- e. Yellow tape
- f. Autoclave tape

- g. Any additional required items for “Special Services Requests.”
3. Review and sign off for room temperature, humidity and all daily and monthly checkoff items.
 4. Check proper function in negative pressure at the rack’s touch screen display. Report any dysfunction and document the details in “Facility/Comments Reports.”
 5. Set up the BSC, maintain the work surface separated into **clean** and **dirty** sides:
 - a. Make sure the BSC is on.
 - b. Spray and wipe the inside of the BSC with the appropriate dilution of disinfectant.
 - c. Place an absorbent towel or similar material soaked in appropriate disinfectant on the working surfaces.
 - d. Bring in a bucket with disinfectant solution, absorbent towel and forceps and holder.
 - e. Keep the food for filling feeders in a lidded secondary container; label with mill date, expiration date and container sanitation date.



Figure 3. First cage change technique

6. Set up supplies outside the BSC. All clean caging supplies will be stored outside the BSC.
7. Double gloved hands should be inspected prior to beginning work and periodically during work from any possible tears. Outer gloves should be replaced when damaged. Should

inner gloves become compromised with a tear or puncture, stop work and report the incident to the supervisor on duty as a possible exposure and await further instruction.

8. First cage change procedure:

Prepare for a full cage change which includes: lid and cage bottom, water bottle, feeder, wooden block and nesting material. Note: no nesting material will be transferred over.

Once IT's hands have entered the BSC, they cannot be withdrawn until the outer layer of gloves have been thoroughly washed with disinfectant or replaced with new outer gloves. OT will periodically spray hands as they hand off used cages and clean supplies.

- 8.1) OT will slide a clean cage in the BSC.
- 8.2) OT will remove sticker from bottle of water cap and slide it in the BSC.
- 8.3) OT will then retrieve and place a dirty cage of mice inside the BSC. Follow an adequate order technique to make sure all cages have been changed.
- 8.4) IT will position clean and dirty cages in the BSC.
- 8.5) IT will transfer the cage card holder from the dirty cage.
- 8.6) IT will remove the water bottle from the dirty cage and push it aside in the BSC.
- 8.7) OT retrieves the dirty bottle of water and will place it in a dedicated red bin for water bottles.
- 8.8) IT will remove the filter lids from the clean and dirty cages, making sure the interior surface of the lid does not touch the cabinet surface.
- 8.9) IT will remove the feeders from the clean cage and set it beside the clean cage.
- 8.10) IT will utilize the forceps to transfer mice.

Note: Using forceps that have been soaking in an approved disinfectant, pick up the mice by the base of the tail or scruff of the neck and transfer to the clean cage. Use same pair of forceps to move all mice from the same dirty cage to the same clean cage. Dip forceps in approved disinfectant between cages.

- 8.11) After transferring all the mice, IT will re-position the feeder, lid, water bottle, and cage card holder on the clean cage.
- 8.12) IT will wipe the outside of the clean cage with disinfectant.
- 8.13) IT will move the clean cage to the front of the BSC.
- 8.14) OT will retrieve this clean cage and replace it in the proper position on the rack.

- 8.15) IT will stack up to 10 dirty cages inside the BSC before placing a lid on the top cage and moving them to the front of the BSC where OT will retrieve.
- 8.16) OT will place the stacked dirty cage in a bin with an autoclavable biohazard bag.

Repeat steps 8.1 to 8.16 until all cages on each assigned rack have been changed.

After IT hands the last clean cage to the OT, IT will start the wave of cleaning the BSC.

- 8.17) OT will tape shut and then spray with approved disinfectant after each biohazard bag is filled with dirty cages.
- 8.18) IT will stack the last of dirty cages, close the top one and hand it to the OT.
- 8.19) OT will place this last stack into the biohazard bin and close the biohazard bag appropriately.
- 8.20) Extra feeders and water bottles that cannot be consolidated into the cage stack will be sprayed or wiped thoroughly with the appropriate disinfectant prior to being removed from the BCS and placed directly into the secondary disposal container for autoclaving.
- 8.21) IT will close and wipe with disinfectant any item to be kept.
- 8.22) OT will receive these items and place them in their place (food container, forceps, extra card holders, etc.).

At this point the only items inside the BSC should be the bucket with disinfectant, absorbent towel and the spray bottle with disinfectant.

- 8.23) IT will wipe down the entire BSC, moving any remaining items to clean all surfaces. If the rag used to clean the BSC seems full of debris (bedding for example), IT should use a new one.
- 8.24) OT will hand more rags as needed.
- 8.25) IT will spray the inside of the BSC with approved disinfectant, and wipe it down with a rag. **The exhaust area should not be sprayed into as this will damage the HEPA filter.**
- 8.26) When BSC is clean and tidy, OT will open the trash can so IT can throw absorbent towel(s) and outer pair of IT's gloves into it.
- 8.27) In addition, the IT will remove the vents and clean gross matter from underneath.
- 8.28) IT can remove hands from BSC and immediately don another pair of outer gloves.

- 8.29) Now either of the techs will gather all trash, close the biohazard bags, include a chemical steam indicator on each bag, and load the autoclave.
- 8.30) All the remaining items should be put in their place and environmental monitoring duties should be performed.

If a health condition is found, refer to SOP 621 "OLAC Health Communication".

Final exiting procedures should be followed as outlined in ENTRY and EXITING PROCEDURES on page 12.

SECOND AND SUBSEQUENT CAGE CHANGE TECHNIQUE

Only one technician is required to perform the second (and subsequent) cage changes. The technician will follow the same procedures described above and follow standard ABSL2 cage changing techniques. The technician in this case will thoroughly wipe hands before exiting the BSC each time to retrieve dirty cages or any other material outside the BSC.

ANIMAL/MATERIALS ROOM-TO-ROOM TRANSPORT PROCEDURE

These instructions are for transporting mice from the NAF ABSL2 to the NAF ABSL3.

The following materials will be used for transport:

- Proper mice cages.
- Wheeled cart for transporting mice.

FILL OUT THE ANIMAL TRANSFER REQUEST FORM. Only OLAC staff may transfer animals.

1. Ensure each cage containing mice is clean, labelled with a cage card, and recently changed.
2. Use a wheeled cart for transporting.
3. Safely secure each cage on the cart.
4. Push the cart into the anteroom of the NAF ABSL3 facility.
5. Wheel the cart into the ABSL3 and the animal holding room, where the cages will be transferred onto the IVC rack.
6. Spray cart with appropriate disinfectant, allow to sit for contact time, then wheel out of the ABSL3 facility.
7. The cart should then go directly to the cage wash for a thorough washing.

HAZARDOUS MATERIALS

1) Personnel at risk:

It is the responsibility of the PI (for researchers) or department supervisor (for staff) to advise any employee or researcher who will be using the ABSL3 on the hazards they face if accidentally exposed to infectious or toxic materials. Persons who are at increased risk of acquiring infection or suffering increased illness or injury from infection or exposure should be advised about the risks working in the ABSL3. It is the responsibility of the PI or supervisor to ensure that any additional appropriate protective measures and equipment are provided. It is the **responsibility of everyone** who works in this facility to notify their supervisor if they have any personal condition that may reasonably place them at greater risk of infection or adverse effects of infection while using this facility. Such conditions include but are not limited to: pregnancy; some chronic, long-term infections (such as HIV, hepatitis, and others), significant loss of sight or hearing (inability to recognize alarms), immunosuppressant conditions and mobility limitations. Employees or students with particular concerns or conditions should be evaluated for questions regarding risk or exclusion by their personal health care provider/physician. PIs and supervisors with questions about this risk may consult with the Occupational Health Clinic at (510) 642-6891.

- 2) All chemical hazardous materials contained within the ABSL3 must be recorded by the PI into the [chemical inventory](#). Update the quantities annually or when hazardous materials are introduced or removed.
- 3) Hazardous materials storage, use and disposal must conform to building requirements, PI SOPs, and campus regulations. A Chemical Hygiene Plan must be posted in each laboratory where hazardous materials are used. Any material that is volatile or may generate a hazardous aerosol must be used within a fume hood.
- 5) A copy of the MSDS and SOP for each hazardous material stored in the ABSL3 should be in the location where the material is located for ready reference.
- 6) Currently detailed inventories of infectious agents is not required. It is possible that this may change in the future. The HCLD will inform users of any new requirements.

GENERAL MAINTENANCE RESPONSIBILITIES

All authorized personnel are responsible for keeping their laboratory and housing areas clean and safe. Facility maintenance requests are coordinated through OLAC.

1) GENERAL HOUSEKEEPING

Researchers must clean the laboratory area after completion of their day's experiments. The BSC should be thoroughly sprayed with disinfectant, followed by a wipe down with ethanol 70%. The lab bench and computer areas should also remain free of clutter and stains. Decontaminate surfaces with appropriate disinfectant, remove waste using appropriate procedures, keep floors and surfaces free of stains and ensure all hazardous or infectious materials are properly stored.

OLAC is in charge of in-depth cleaning. Sweep all floors daily and mop floors weekly with approved disinfectant in all in use spaces. Sanitize horizontal & vertical surfaces monthly in all rooms, excluding the main lab (room 187).

2) FUME HOODS AND BSCs

All fume hoods and BSCs must be used according to the manufacturer's specifications and hands-on training for effective protection. All fume hoods and BSCs are certified at least yearly by or through EH&S. Report any failure of a hood or cabinet or components to the OLAC manager or the HCLD immediately. They will obtain repair service at once. OLAC staff will vacuum the Fume Hoods & BSCs on OLAC frequency (weekly). Exceptions include the hoods in room 182, and any hoods with equipment or supplies stored inside.

3) NEGATIVE AIR PRESSURE MONITORING

The ABSL3 air pressure gradient is designed to be negative with respect to the outside hallways, and the rooms are to be negative relative to the corridor (air flows from outside the facility through the corridor and into each room.) Air should NOT flow from any room into the corridor, or from the corridor to the outside hallway. All air leaving the ABSL3 is High-Efficiency Particulate Air (HEPA) filtered. The differential pressure created by the air handling system (AHS) is measured by magnehelic gauges located at each door within the facility. A normal readout should be between -0.05 and -0.12.

To test if a room's pressure is negative to the corridor, hold a piece of tissue paper roughly 1/2" x 6" in the narrow gap created by holding the room's door slightly ajar. The paper should be pulled into the room by the airflow. The airflow monitors on the fume hoods should always register in the green zone as indicated by the small colored lights.

Please report any alarms or significant change in facility or room to corridor airflow to the OLAC supervisor or the HCLD at once.

If a fume hood monitor moves into a red zone, CLOSE THE SASH AND DO NOT USE THE HOOD until the problem is corrected. Some BSCs have airflow monitors similar to the fume hoods; others do not. If the monitor alarm sounds or if you think the BSC is malfunctioning, report the problem immediately as above.

EMERGENCY PROCEDURES

1) SPILL

In case of an accidental release of infectious material outside the BSC, researchers must follow spill procedures as indicated in the LKS-BSL3 manual and in-person training. Staff does not handle infectious materials; nevertheless, they will be trained to respond in case of a spill.

Notify all other ABSL3 occupants of the spill, everybody must leave the facility. Report **immediately** to the HCLD and your supervisor. Remove all contaminated PPE before leaving the facility to the locker room. A sign stating “spill do not enter” should be posted at the facility entrance, and disinfection procedures should follow.

2) SUDDEN ILLNESS OR INJURY

Call 911 from a campus phone or call (510) 642-3333 from a cellular phone. There is a telephone located in the ABSL3 area that can be used to reach 9-911 or another campus number. In an emergency, you may go through the door separating the ABSL2 and ABSL3 areas and use the nearest phone. You will need to communicate the nature of the emergency, stating that you are in the NAF ABSL3 facility. If you cannot call, activate the fire alarm. If you pull the fire alarm, the building must be evacuated, and help may be slightly delayed; but this is an effective alternative if you cannot reach a phone.

Give any needed first aid if possible or appropriate.

Because of the isolated nature of the ABSL3, it is very strongly recommended that you **DO NOT work alone** in the facility.

3) FIRE

A fire extinguisher is in the ABSL3 corridor. Only try to put out the fire yourself if you have already called 911, you have been trained and are comfortable with using a fire extinguisher and you will not endanger yourself by doing so. Refer to the general fire safety procedures in the NAF Building Safety Manual.

If you have not been trained to use a fire extinguisher, or it is unsafe to fight the fire yourself, leave the room, close the door, and pull the fire alarm. Leave the ABSL3 as

usual. You must identify yourself to the emergency coordinator (the person in the orange hat outside the NAF) as being the activator of the alarm and offer whatever information you can to firefighters when they arrive.

4) MAJOR EARTHQUAKE

PLAN AHEAD. For each room, know where you will go during an earthquake. Remember that you will probably lose power at least temporarily and be in **total** darkness (carry a working penlight), so know where your protected area is and go to it at once.

When the building stops shaking, if you or someone else is injured, give first aid if possible, and either transport the victim or send others for help. **It may be impossible to tell clearly whether it is better to move an injured person (which could result in more severe injury) or leave them where they are. All you can do is use your best judgment and decide.** The most important things to accomplish are to safely evacuate as many people as possible from the building and to know who, where, and in what condition, any remaining people are, so that help can reach them quickly. If you are alone and injured, use whatever means available to get assistance (i.e., yell for help, use a telephone or pull the fire alarm).

Your primary responsibility is your own safety. If your safety is threatened, leave the ABSL3 at once, removing PPE as you go. Maintaining containment is only of secondary importance. If it is safe to remain long enough to do so, secure all your work, check to see if any infectious or toxic material has spilled and if so, quickly decontaminate or neutralize it. If inactivation is not possible, try to contain the spill so that it doesn't get to a floor or sink drain, and then leave the area and remove your PPE as you normally would.

Never remove any animals from the facility during an emergency situation.

ABSL3 PHONE NUMBER: (510) 642-0652

Dial 9 + 1 + area code and number. Use the speaker button for hands-free mode.

EMERGENCY CONTACTS

High Containment Lab Director	Laura L Flores	floresll@berkeley.edu	(510) 495-9084
OLAC Manager	Vincent Flournoy	supervisor@berkeley.edu	
Occupational Health	Erika Schwilk MD	eschwilk@berkeley.edu	(510) 642-6891
Biosafety Officer	Chips Hoai	chips@berkeley.edu	(510) 849-7142
ABSL3			(510) 642-0652

PI	Sarah Stanley	sastanely@berkeley.edu	(415) 845-7214
	Stanley lab		(510) 666-3729
PI	Jeff Cox	jeff.cox@berkeley.edu	(510) 984-4720
	Cox Lab		(510) 664-7799
PI	Russell Vance	rvance@berkeley.edu	(510) 735-7173
	Vance Lab		(510) 642-4264

EH&S Biological Spill	Main Line	Business hours	(510) 642-3073
Emergency Response	UC Police For police, fire or medical emergencies		911 or (510) 642-3333
Veterinarian After Hours		(510) 643-8387	3-VETS

APPENDIX I - ACKNOWLEDGEMENT OF RISK FORM

I agree as follows:

The responsible staff of the NAF BSL3 Laboratory has taken reasonable steps to provide me with appropriate personal protective equipment, disinfection protocols, and ventilation and maintenance information according to mandated standards so that I can perform my work inside this facility, the matter expert _____ has informed me that this activity is not without risk. Certain risks are inherent with each type of activity performed inside the BSL3 facility and cannot be eliminated due to the nature of the work.

I recognize that it is important to be aware in advance what to expect and to be informed of the inherent risks. The BSL3 facility is used to perform research with the airborne pathogen, *M. tuberculosis*. *M. tuberculosis* causes tuberculosis, a disease that affects primarily the lungs.

I acknowledge that engaging in this activity requires proper training and that as a VISITOR I am aware of the risks.

DATE

VISITOR'S name and signature

APPENDIX IIA - RESEARCHERS TRAINING RECORD:

User name: _____

Lab and department affiliation:

Status (circle one): Grad student Postdoc Visiting scholar Other:

LKS-BSL3 training Date completed: _____

ABSL3 initial training:

Introduction and emergency response ☐

Donning/doffing demonstration and guidance ☐

Given by: _____ Date(s): _____

ABSL3 training by OLAC:

Inno-vive cage handling and disposal ☐

Carcass disposal ☐

Given by: _____ Date(s): _____

BSL3 use of aerosolizer training:

Observation of proficient user

Wire cages, mice and materials ☐

Aerosolizer setup ☐

Aerosolizer breakdown ☐

Given by: _____ Date(s): _____

BSL3 use of Aerosolizer supervised:

Supervised by: _____ Date(s): _____

NOTES:

I certify that I have completed all the above training for work in the Hildebrand BSL3 Laboratory. I have read the Safety Manual and understand that I am responsible for all the information therein. I am also responsible for discussing and **clearing** with the director any **new** procedures that I plan to perform in the BSL3.

User signature: _____ Date: _____

Name (print):

E-mail address:

Phone number:

Emergency contact name:

Emergency contact phone number:

"Exit" protocol completed and USER INACTIVE AS OF

(date): _____

Appendix IIB - STAFF TRAINING RECORD:

Completed form must be kept on file by the HCLD before you are permitted to do unsupervised ABSL3 work.

User name: _____

Enrollment to OHSS (date) _____

ABSL3 Donning/Doffing and Orientation:

Introduction/ Orientation ☐

Donning/doffing demonstration and guidance ☐

Emergency response ☐

Given by: _____ Dates: _____

Signature of director:

ABSL3 training: Performed by OLAC Trainer or Training Designee. Refer to OLAC ABSL3 training documents.

Given by: _____ Dates: _____

ABSL3 Proficiency:

User demonstrates proficiency in all ABSL-3 procedures..

Supervised by: _____ Dates: _____

NOTES (include date and initials):

Signature of HCLD:

Signature of NAF-ABSL3 supervisor:

I certify that I have completed all the above training for work in the Hildebrand BSL3 Laboratory. I have read the Safety Manual and understand that I am responsible for all the

information therein. I am also responsible for discussing and **clearing** with the director any **new** procedures that I plan to perform in the BSL3.

User signature: _____

Date: _____

Name (print):

E-mail address:

Phone number:

Emergency contact name:

Emergency contact phone number:

<p>"Exit" protocol completed and USER INACTIVE AS OF (date): _____</p>
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