

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



**Northwest Animal Facility (NAF)
University of California, Berkeley**

Table of Contents

Introduction	4
NAF-BSL ₃ floor plan as of 03-2016	4
General Safety Principles	6
Room Door Signage	6
Containment	7
ABSL ₃ Users Groups Description	8
Researchers	8
Staff	8
Visitors	8
ABSL ₃ Users Access and Training Program	10
Researchers	10
Staff	11
Entry and Exiting Procedures	12
Donning and Doffing SOP	12
Donning	12
Figure 1. Full ABSL ₃ attire	13
Doffing	13
Figure 2. Doffing outer PPE layers prior to exit	14
Transport Procedures	15
Removing Waste, Research Materials and Animals	16
Waste Removal Instructions	16
ABSL ₃ Autoclave Operation	18
Biological Indicator (BI) Instructions	18
Maintenance and Facility Services Guide	19
Definition of “Break in Containment”	19
Work Not Requiring a “Break in Containment”	19
Providing Access for Service Personnel	20
Animal Housing and Handling	21
Animal Husbandry of Infected Mice	22
Biosafety Considerations	22
Infected Mice Cage Change - OLAC SOP	23
First Cage Change Technique	24
Figure 3. First cage change technique	25
Second and Subsequent Cage Change Technique	28
Animals/Materials Room-to-Room Transport Procedure	29
Hazardous Materials	30
General Maintenance Responsibilities	31
General Housekeeping	31

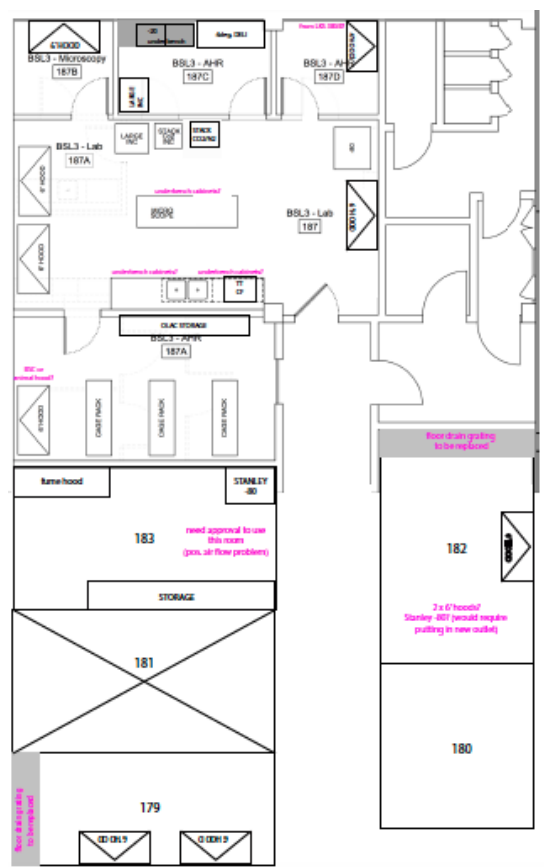
Fume Hoods and BSCs	31
Negative Air Pressure Monitoring	31
Emergency Procedures	33
Spill	33
Sudden Illness or Injury	33
Fire	33
Major Earthquake	34
Contact Information	35
Emergency Contacts	35
Appendix I - Acknowledgement of Risk Form	36
Appendix IIA - Researchers Training Record	37
Appendix IIB - Staff Training Record	39

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, and 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) 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.
- 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. 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 to activate the user's card ID. These cards must be used exclusively by the authorized user.

3. Visitors

The third category include 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 are 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 afterhours and what time you may be leaving.

If you use the facility outside of normal working hours (7:00 AM - 4:00 PM, Monday-Friday), you must sign in AND out at the security guard desk in the building entry hall.
Please indicate that you will be in the ABSL₃ in case of emergency response.

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 instructions on the [OLAC website](#).
- 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 Autoclave and 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 Research Protocol (AUP)
 - iii A copy of the Safety Data Sheet (SDS) for each hazardous material provided along with an 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.

- f) 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 NAF facility supervisor and training coordinator.
- b) 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 powered air purifying respirator (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 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 and NAF supervisor 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
 - b) Respirator; either fit-tested N95 disposable mask (for researchers only) or PAPR (which are found in the locker room itself)
 - c) Hair cover
 - d) Inner and outer gloves, and
 - e) A pair of shoe covers to go over the Tyvek suit booties

Proceed into the locker room.

- 3) Don this PPE following hands-on training instructions. Refer to Figure 1. Remember to 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; you will need to provide your own lock and place your name and contact information on each locker used.

OLAC will provide Tyvek suits, gloves, shoe covers and hair covers. If any items of protective equipment appear to be in short supply, please notify the OLAC facility staff for restocking. If you wish to use the shower, there is adequate space within the shower room to both shower and change. You may wish to bring your own toiletries, as the only soap provided by OLAC is liquid hand soap.

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 should always exit the BSL₃ through the airlock, except during an emergency.

- 1) When you are ready to leave the ABSL₃, following hands-on training instructions take off secondary PPE layer. This includes top shoe-covers and top gloves. Dispose of items in red-bin right before entering the airlock. Wipe the front of your Tyvek suit and PAPR belt with the provided disinfecting wipes. Refer to Figure 2.



Figure 2. Doffing outer PPE layers prior to exit.

- 2) Enter the airlock and take off the Tyvek and place it in the waste bins if you were performing a “first cage change,” doing 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.
- 3) Wipe your PAPR hood and battery/filter pack with Accel TB wipes. Take off inner-gloves last.
- 4) Wash hands in airlock bathroom sink, room 189a.
- 5) 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.
- 6) Shower if desired or if needed due to gross contamination from a spill.
- 7) Wash your hands before leaving the locker room.
- 8) Exit locker room through room 193.
- 9) Sign off! Include the PAPR number you just wore.

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 should be chemically disinfected before bringing in and before removal from the facility. You may bring in clean cages and other new supplies, but everything should 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 biohazard bags will be placed into an empty 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.

The autoclave will be run in a schedule as posted in the autoclave room:

- a) Researchers:
Thursday at 8:00 am for TB-Plates – liquid cycle #3 (1 hour)
- b) OLAC:
Monday, Wednesday and Friday at 4:00 pm, after cages change, the autoclave will be loaded with dirty cages. The autoclave will be run first thing the following morning. All operations on Tuesday, Thursday and Saturday can be done from the “clean side” of the autoclave. Run and collect the autoclaved trash. Bring it down to the collection area.

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 biological integrator strip 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 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 items to be autoclaved, washed and returned in to the ABSL₃ suite.
- 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 appropriate disinfectant (Vesphene Ilse[®]) and wipe with paper towels to completely decontaminate every surface. Follow with ethanol 70%. 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 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) Sweep out 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 integrator strip indicator ink has correctly changed color. If the indicator fails to change color properly, notify the OLAC supervisor immediately. The materials from the failed run shall be re-autoclaved.
- 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

- a) The BI is a self-contained vial for use in monitoring the efficacy of steam sterilization cycles. The vials will be available in autoclave side A (dirty side). Store in dry room temperature conditions.
- b) The culture medium will change from purple to yellow when viable spores are present and proper incubation instructions are followed. If the medium remains purple, the spores did not grow, which means they are not viable. Therefore, the sterilization process was effective. If the autoclave run is not effective, the spores will turn the medium yellow.
- c) At least once a month, include a BI in a small biohazard bag. Place this bag inside the trash to be run. Follow instructions and training procedures to operate the autoclave. When the cycle is completed, and the load is cool and dry, retrieve the BI. The chemical indicator will turn from blue to black when processed. Activate the BI by compressing the plastic vial to break the glass ampule. This will allow the growth media to contact the spore strip. Incubate to 55-60°C for 48 hours. Check for growth every 12 hours. Always include a positive control for the incubation procedure. The positive control media will turn to yellow within 24 hours. The test BI media should remain purple to consider the cycle successful.
- d) If the cycle fails (test BI media turns yellow during incubation) repeat the test. If it fails again, contact the HCLD to assess the problem and modify procedures accordingly.
- e) All procedures will be initially tested with a mock load.
- 6) Report any failure of autoclave function to the OLAC manager or the NAF supervisor immediately.

Maintenance and Facility Services Guide

This section describes 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.

Orange cage cards must be used to document all infection procedures, date, infectious agent and route of administration. Cage cards also 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 NAF 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 (ziplock type), seal it and spray the outside, then put it inside a small autoclave bag and close it appropriately to avoid any spill. Bring this waste into the -20 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 -20 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.

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 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) Make sure the bags are not punctured, always keep them inside red barrels with the lid closed.
- 5) Bags and red barrels should be sprayed with RESCUE[®] disinfectant before exiting the animal room.
- 6) Cart handles and cartwheels used in the change-out process should be sprayed with Vesphene[®] disinfectant before exiting the animal room.
- 7) Dirty-bagged cages should be autoclaved at appropriate settings. Autoclave for Gravity cycle #3.

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 the 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. These cages are labeled with a piece of green tape. These cages are also known as “new mice” or “**green cages.**”

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”; Green 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 facility supervisor 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 10 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
- b. Food
- c. Water bottles or Hydropacs[®] and Lixits[®]
- 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
- 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) Turn on BSC and allow to run for a minimum of five minutes before placing arms or hands inside.
 - 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, a blue rag and tongs 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.

First cage change procedure:

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 constantly spray hands as handling 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 remove 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 dual forceps method 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. Alternate using each forceps between cages. Alternate using each disinfectant bottle to hold the forceps (not in use) between cages.
- 8.11) After transferring all the mice, IT will re-position the feeder, lid, and cage card holder on the clean cage.
- 8.12) IT will place the new water bottle on the clean cage.
- 8.13) IT will wipe the outside of the clean cage with disinfectant.
- 8.14) IT will move the clean cage to the front of the BSC.
- 8.15) OT will retrieve this clean cage and re-place it in the proper position on the rack.
- 8.16) IT will stack up to 10 dirty cages before placing a lid on the top one and moving them to the front of the BSC where OT will retrieve.

- 8.17) OT will place the stacked dirty cage in a bin with an autoclavable biohazard bag. Repeat steps 8.1 to 8.17 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.
- 8.18) OT will tape shut and then spray with approved disinfectant after each biohazard bag is filled with dirty cages.
- 8.19) IT will stack the last of dirty cages, close the top one and hand it to the OT.
- 8.20) OT will place this last stack into the biohazard bin and close the biohazard bag appropriately.
- 8.21) 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.
- 8.22) IT will close and wipe with disinfectant any item to be kept.
- 8.23) 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, a blue rag and the spray bottle with disinfectant.
- 8.24) IT will wipe down the entire BSC, moving any remaining items to clean all surfaces. If the blue rag used to clean the BSC seems full of debris (bedding for example), IT should use a new one.
- 8.25) OT will hand more blue rags as needed.
- 8.26) After wave of cleaning is complete, IT will spray the inside of the BSC with 70% alcohol and wipe down all surfaces with a blue rag, except the exhaust surface area in the ceiling of the BSC. **The exhaust area should not be sprayed into as this will damage the HEPA filter.**
- 8.27) 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.
- 8.28) In addition, the IT will remove the vents and clean gross matter from underneath.
- 8.29) IT can remove hands from BSC and immediately don another pair of outer gloves.

- 8.30) 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.
- 8.31) All the remaining items should be put in their place and environmental monitoring duties should be performed.

The Rodent Daily Checklist should be documented after the completion of cage changing and cleaning.

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.

Animals/Materials Room-to-Room Transport Procedure

These instructions are for transporting mice from the NAF ABSS₂ 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 before moving any mouse cage.

- 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 10 minutes 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.

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.

- 2) 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.
- 3) A copy of the SDS and SOP for each hazardous material stored in the ABSL₃ should be in the location where the material is located for ready reference.
- 4) 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. Surfaces should be decontaminated with appropriate disinfectant, waste should be removed using appropriate procedures, floors and surfaces should be kept free of stains and all hazardous or infectious materials should be checked to see if they are properly stored.

OLAC is in charge of in-depth cleaning. Floors in room 179 (aerosolizer room) and room 187 (main lab) will be swiped and moped with disinfectant on a bi-weekly basis. Floors in all other rooms will be swiped and moped with disinfectant on a monthly basis. This will avoid buildup of dust and stains.

2. Fume Hoods and BSCs

All fume hoods and BSCs must be used according to the manufacturer's specifications and hand-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.

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 should 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 911 or another campus number. In an emergency, you may go through the door separating the ABSL₂ and ABSL₃ areas to use the ABSL₂ 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.

Contact Information

ABSL₃ Phone Number: (510) 642-0652

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

Emergency Contacts

Laura L Flores

High Containment Lab Director

floresll@berkeley.edu

(510) 495-9084

Erika Schwilk MD

Occupational Health

eschwilk@berkeley.edu

(510) 642-6891

Sarah Laraway

LKS/NAF OLAC Supervisor

slaraway@berkeley.edu

(415) 632-7298

Allison Liljedahl

Biosafety Officer

aliljedahl@berkeley.edu

(415) 609-3233

Sarah Stanley

Principal Investigator

sastanely@berkeley.edu

(415) 845-7214

Stanley Lab: (510) 666-3729

Russell Vance

Principal Investigator

rvance@berkeley.edu

(510) 735-7173

Vance Lab: (510) 642-4264

Jeff Cox

Principal Investigator

jeff.cox@berkeley.edu

(510) 984-4720

Cox Lab: (510) 664-7799

Biological Spill Response

EH&S Main Line:

(510) 642-3073

Business Hours

Emergency Response

For police, fire, or medical
emergencies

911 or (510) 642-3333

Veterinarian

3-VETS or (510) 643-8387

After Hours

Appendix I - Acknowledgement of Risk Form

I agree as follows:

Although 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 Facility Director _____ 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

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) _____ Introductory training (date): _____

PAPR training (date and signature): _____

ABSL₃ initial training:

- Walk through
- Autoclave use
- Donning/doffing demonstration and guidance
- Entering the BSL₃
- Emergency response

Given by: _____ Date(s): _____

Signature of director:

BSL₃ training:

Observation of trainee includes donning/doffing without guidance, entering the BSL₃.

- Hands on of mock cage change setup

Questions to trainer

Given by: _____ Date(s): _____

BSL₃ testing:

Trainee shows proficiency on given tasks by the supervisor, using mock up experiments.

Questions are not allowed through the test; at the end, the user is given feedback.

Supervised by: _____ Date(s): _____

BSL₃ supervised trial session 1:

After successful test, user to perform a cage change with companion under supervision.

Supervised by: _____ Date(s): _____

BSL₃ Supervised Trial Session 2:

- Waste collection
- Autoclave
- Emergency response
- Other: _____

Supervised by: _____ Date(s): _____

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:

“Exit” protocol completed and USER INACTIVE AS OF
(date): _____