

Center for Comparative Medicine

Procedures Manual for Animal Biosafety Level 2 Areas Within the Vivaria

Version 2.1

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Purpose

This ABSL2 laboratory manual proscribes practices and procedures to be followed in all University vivaria where approved animal research protocols involving the use of infectious agents in animals at animal biosafety level 2 (ABSL2) agents are conducted. This manual will be reviewed annually and revised if appropriate.

Background

Animal research protocols that involve the administration or use of ABSL2 agents whether they are microbes, biotoxins, viral gene vectors or human cells require special procedures to protect the health of personnel performing the experiments as well as the health of those persons caring for the animals. The procedures described in this manual represent the necessary practices required for animal protocols involving ABSL2 agents to ensure compliance with the *NIH Guide for the Care and Use of Laboratory Animals* (National Academy Press, 1996), *Occupational Health and Safety in the Care and Use of Research Animals* (National Research Council, 1997), and the CDC-NIH Guideline *Biosafety in the Microbiological and Biomedical Laboratories* (5th Edition, 2007).

In accordance with University [Biosafety Policy SEC-012](#), personnel performing ABSL2 experiments at the University of Virginia will comply with all applicable federal, state and local regulations that apply to the possession, manipulation and disposal of biological agents. Additionally, consensus biosafety guidelines such as those from the National Institutes of Health, Centers for Disease Control, and other organizations will be applied as appropriate to protect personnel and the environment from potentially adverse exposures to biological agents.

All animal handlers, animal care staff and veterinary staff working in an ABSL2 environment are required to adhere to the procedures presented in this document, participate in formal ABSL2 training developed by both the Biosafety staff as well as vivarium supervisor, and are required to *read and understand* all protocol-specific requirements *prior to* beginning any ABSL2 work.

Anyone who observes conditions or work practices that are not in compliance with this manual, or that pose a potential biohazard risk to personnel, should notify the vivarium supervisor immediately or the Director of CCM. Supervisors should take appropriate corrective action if within their purview and should also communicate their observations to the CCM Director and/or the OEHS for investigation.

Procedures

1. Approvals

In order to conduct an animal protocol involving any of the following types of biological agents, protocols require [Institutional Biosafety Committee](#) (IBC) approval as well as approval from the Institutional Animal Care and Use Committee (IACUC). IBC approval must be obtained before the IACUC can approve the use of BSL2 agents in research animals.

- A. Risk Group 2 (and above) microorganisms. These are agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available. Examples include: *Entamoeba* sp., *Salmonella* sp., Adenovirus, Herpesvirus, *Helicobacter pylori*.

- B. Viral vectors (e.g. Herpesvirus, Adenovirus, Vaccinia, Sindbis, Lentivirus, etc.).
- C. Materials of human origin including blood, fluids, tissues, primary and established human cell lines.
- D. Recombinant DNA activities required to be conducted at BSL2 containment as described in the [NIH Guidelines for Research Involving Recombinant DNA Molecules](#).
- E. Biotoxins with an LD50 of less than 100 micrograms per kilogram of body weight in vertebrates (e.g. Tetrodotoxin, Ricin, SEB, Diphtheria Toxin, Botulinum toxin)

Refer to the IBC webpage or contact the [Office of Environmental Health and Safety](#) (OEHS) at 982-4911 for assistance in determining appropriate biosafety levels.

Prior to the designation of any vivarium rooms for ABSL2 procedures, the CCM Director and/or vivarium supervisor will consult with the OEHS Biosafety Officer who will provide the CCM with an assessment of the suitability an ABSL2 room.

Special approval for protocols involving “select agents and toxins”: Animal protocols involving agents and toxins known as [Select Agents and Toxins](#) as defined by 42 CFR 73 must be approved by the Responsible Official (RO) prior to IACUC approval. Contact the OEHS for additional communication with the RO.

2. Occupational Health

All animal handlers are required to participate in the Occupational Health and Safety Program for animal research at University of Virginia. Personnel must comply with risk assessment, medical clearance and surveillance program requirements as determined by the IBC and IACUC. Medical clearance for employees is performed by WorkMed <http://www.healthsystem.virginia.edu/internet/occupational-health/animal.cfm> and for students by Student Health Services (<http://www.virginia.edu/studenthealth/>). Depending upon the animal species and/or agents involved, there could be additional or special requirements to work with these animals such as vaccination(s) (e.g. for vaccinia use), serology for antibody titers, serum banking, tuberculosis testing, the use of an N95 respirator requiring pulmonary function testing and fit testing, etc.

3. Training

Personnel must have specific training blood borne pathogens by the Biosafety Group as well as vivarium-specific training in ABSL2 facility procedures. There is information that is unique to each vivarium, necessitating vivarium-specific training manuals; however the routine ABSL2 practices and procedures are the same for all vivaria. Therefore, each vivarium supervisor will conduct training of vivarium and research personnel and document training has been completed prior to providing personnel access to the ABSL2 space. This training will be documented using a checklist that is signed and dated upon completion (see Appendices). Principal Investigators and laboratory supervisors are responsible for ensuring that all research personnel under their supervision are adequately trained to perform ABSL2 protocols in a safe and compliant manner. For further guidance on ABSL2 vivarium training, see Appendix 1: Center for Comparative Medicine Animal Biosafety Level-2 Training Manual for Vivaria.

4. Hazard Notification and Communication

Principal Investigators and research staff are required to notify the vivarium supervisor prior to introducing BSL2 agents into any animal facility. The research staff is also required to affix a sticker with the universal biohazard symbol on each cage containers animals housed at ABSL2. Once informed the vivarium supervisor will post the ABSL2 housing area with biohazard signage where ABSL2 animals will be housed. The sign must include the animal biosafety level, general occupational health requirements (e.g. vaccination), personal protective equipment (PPE) requirements, the supervisor's name (or other responsible personnel), contact telephone numbers both during work and after hours, and required procedures for entering and exiting the ABSL2 area. In satellite animal housing areas and/or procedure rooms that are under the direct supervision of the principal investigator (PI), the PI is responsible for posting biohazard signage at the immediate entry to the room. A copy of the ABSL2 biohazard sign is in Appendix 2 and contained in the attached *Center for Comparative Medicine Animal Biosafety Level-2 Training Manual for Vivaria*.

Regardless of the species of animal inoculated with a BSL2 agent, the investigators are responsible for ensuring that animal cage cards properly identify the biological agent and are marked with the universal biohazard symbol for the duration in which biological agents may be present in the cage. When ABSL2 animals are removed from the vivarium they must be in a cage that is sealed in a paper or plastic bag obscuring the view of the animals, and the universal biohazard symbol affixed to the outer bag containing the cage(s).

When soiled ABSL2 caging is returned to the vivarium soiled cage wash area for processing, the caging must be appropriately contained in a paper or plastic bag and identified as containing a biohazard. If the soiled ABSL2 caging is steam sterilized by the laboratory prior to return to the vivarium, it should be marked clearly that it does NOT contain a biohazard. For details, see attached *Center for Comparative Medicine Animal Biosafety Level-2 Training Manual for Vivaria*.

5. Access to Vivaria

Access to each vivarium and animal room(s) is limited to those persons authorized to conduct research protocols, animal care or support services. It is the overall responsibility of the CCM Director to restrict access to the vivaria although this responsibility has been delegated to each vivarium supervisor. The vivaria supervisors are also responsible for insuring that all personnel, including research staff, vivarium personnel, service workers, and visitors are advised of the potential hazards in the vivaria as well as any required safeguards such as the use of personal protective equipment (PPE). Access to all vivaria and to many barrier rooms, including ABSL2 rooms, is by electronic key card and is centrally-controlled by University ID Services. Other rooms are conventionally-keyed for security. Approval for access to vivarium and any room key(s) is granted only after research personnel obtain the signature of their supervisor (Principal Investigator) and the vivarium/vivaria supervisor(s) where the animals are housed. Research staff must be assigned to an approved protocol before they are allowed to enter a vivarium.

6. Personal Protective Equipment (PPE)

PPE to be worn in all ABSL2 areas include, but is not limited to, disposable gowns, surgical mask, gloves, bonnets and booties must be worn when handling animals used in ABSL2 protocols. The sleeve of the disposable gown should be covered by the gloves so that no skin of your wrist is shown.

The vivarium provides this PPE wither just outside or just inside the ABSL2 space. Additional PPE for particular rooms, biological agents, or animal species may be required as defined by the Biosafety personnel of OEHS or the IBC. Additional PPE may be required of the animal care staff by the CCM Director.

Double gloving is required to handle animals with microorganisms and viral gene vectors that can replicate see Appendix 3 for a list of agents requiring double gloving. When there is a high probability of aerosolizing the BSL2 agent mucous membrane protection and/or a respirator may be required for all personnel entering ABSL2 housing.

REMEMBER personnel using respirators must be enrolled in the University [Respiratory Protection Program](#), which includes an initial physical examination, pulmonary function testing, respirator fit testing, respirator use training and completion of an annual OSHA questionnaire. Additional information may be found on the OEHS website or through WorkMed.

PPE must not be worn outside the boundary of the ABSL2 areas for any reason. Remove all PPE removing your single pair of gloves last. Please all PPE in a contaminated materials container (CMC), which is located in the ABSL2 space near the exit door.

7. Work Practices

- Each vivarium supervisor designates one individual, usually him/herself or the assistant supervisor, to be a member of the CCM Safety Committee. This committee meets bi-monthly to oversee safety in the workplace, identify and mitigate risks, review procedures, discuss any vivarium accident reports, etc. A biosafety officer from the OEHS also sits on this committee to provide input to the CCM and its vivarium supervisors on ABSL2 and related topics. The CCM Associate Director chairs this committee and maintains its minutes at the CCM office for review by safety officers from the University. Semi-annual and annual reports of committee activity are submitted through the CCM Director to the IACUC and Vice President for Research, respectively.
- Within the vivarium and the ABSL2 area eating, drinking, handling contact lenses, applying cosmetics and storing food for human consumption is not permitted in any animal or procedure rooms. There is a designated lunchroom break area in each vivarium where eating and drinking is permitted.
- Consideration should be given to the use of restraint practices and devices, and the use of drugs to immobilize research animals, in order to reduce risk of exposure during ABSL2 animal manipulations.
- The use of double gloves is required for all replication competent BSL2 agents. When working with the concentrated agent, animals inoculated with the agent, or any other potential source of high concentration of the BSL2 agent the outer glove is removed and appropriately disposed of immediately after the work is performed. This is intended to preclude the transfer of high concentration of the agent to surfaces such as door knobs, drawer handles, etc. If more work with the BSL2 agent is required, don a new outer double glove. If work is completed spray the inner glove with disinfectant prior to leaving the space and doffing PPE.
- When moving rodents between cages, use forceps with tips covered in silicone tubing to grasp the rodent by the tail or the nape of the neck in order to avoid being bitten.
- All procedures must be performed in a manner that minimizes the creation of aerosols, sprays or splatters of infectious materials and waste. Therefore, whenever possible procedures that may produce aerosols should be performed in a properly maintained and annually-certified Biological Safety Cabinet (BSC). Aerosol generating procedures include necropsy, harvesting

of tissues or fluids, intranasal inoculation of animals, gavage and use of hypodermic syringes and needles for administration of BSL2 agent or body fluid collection. In certain cases where physical containment devices are inappropriate for performance of the work, or the BSC is unavailable, research personnel can use appropriate PPE (e.g. respirators and face shields) and work practices may provide satisfactory protection. Such instances must be defined in the IACUC protocol and approved by the IBC and IACUC prior to initiating any animal work.

- Cage changing of ABSL2 rodents and rabbits should be in accordance with CCM SOP#222 and 804, respectively (Appendix 4 and 5).
- All personnel must wash hands after handling animals or animal tissues and prior to leaving animal care and use areas, even if gloves are used. If a sink is unavailable for hand washing, an alcohol-based hand sanitizer (or other sanitizer effective against the agent) may be used but hands should be washed with soap and running water as soon as possible prior to exiting the vivarium.
- Equipment and work surfaces must be decontaminated with an effective disinfectant (e.g. SporKlenz, 5-10% bleach, 6% hydrogen peroxide) after work with an ABSL2 agent, and after any spill or splash of viable materials. A minimum contact time of 15 minutes is required for each disinfectant to effect microbial kill.
- Disinfection of solutions containing BSL2 agents is accomplished by adding bleach to a final concentration of 10% and allowing 15 minutes for disinfection before disposing of the liquid in a sanitary line.
- Disinfectant solutions should be poured into floor drains at least twice weekly to prevent the migration of vermin and gases into ABSL2 room. Document this floor drain disinfection on each ABSL2 room sheet. A comprehensive list of [suitable disinfectants](#) is maintained on the OEHS Biosafety website.
- Spills and accidents that result in overt exposure to infectious materials must be immediately reported to the vivaria and/or individual supervisor. Supervisors are responsible for reporting incidents to the Institutional Biosafety Officer at 982-4911 (see 11.C. below).
- All infectious materials must be collected, labeled, transported, and processed in a manner that contains and prevents transmission of the agent(s). The outer surfaces of potentially contaminated containers must be disinfected prior to moving the material out of the ABSL2 area.

8. Use of Sharps

Use of needles and syringes or other sharp instruments when handling BSL2 agents is discouraged, however when there is no alternative they can be used for procedures such as for parenteral injection, blood collection, or aspiration of fluids from animals and diaphragm bottles. Syringes that re-sheathe or retract the needle, needle-less systems, and other safe devices should be used whenever possible.

Plastic ware should be substituted for glassware whenever possible to minimize the risk of breakage. Broken glassware must not be handled directly; it should be removed using a brush and dustpan, tongs, or forceps and should be decontaminated with SporKlenz, 10% bleach or 6% hydrogen peroxide prior to disposal in a broken glass or sharps container.

Hypodermic needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated before disposal. Used hypodermic needles must be carefully disposed of directly into appropriately designated puncture-resistant sharps container located in the animal room or as close to the work site as possible.

9. Animal Transport

Animals must be transported in a manner that contains and prevents transmission of infectious agents during transport, as well as conceals the animal from view by others. The outer surfaces of potentially contaminated containers must be disinfected prior to transport.

If the animals are to be transported from a vivarium to another area in the same building, they must be caged and the cage contained in a sealed paper or plastic bag. Each outer bags must be labeled with a biohazard sticker.

If the animals are to be transported from one vivarium to another building or vivarium, the investigator will submit a delivery request form to the CCM office at least 24 hours in advance of the requested delivery in order to obtain permission for the transfer from the Director or his designee in a timely manner. The investigator will note on the delivery request form that the animals are biohazardous. If any biohazardous animals will be returning to the original vivarium, the investigator will specify the date and time of pickup and insure that the cages are properly packaged and marked with a biohazard sticker.

Delivery request forms and instructions for use can be obtained at:

<http://www.healthsystem.virginia.edu/internet/ccm/docs/PTpdf1.pdf>

For more details on the transport of animals, see the attached *Center for Comparative Medicine Animal Biosafety Level-2 Training Manual for Vivaria*.

10. Waste Disposal

Animal carcasses and tissues generated from ABSL2 protocols must be placed into a red plastic bag or opaque bag marked with the biohazard symbol and returned to the vivaria carcass freezer or cold room for disposal. For rodent carcass disposal, the bodies can be placed in a small black bag labeled with the biohazard symbol, and the black bag placed in a larger red bag in the vivarium carcass disposal area. Bags for disposal may be obtained from vivaria supervisors or the OEHS.

ABSL2 contaminated bedding and caging must be steam sterilized prior to cage cleaning. Since the caging is sealed in a plastic autoclave bag to sterilize, it is prudent to add a cup of water inside the autoclave bag to internally generate steam, prior to sealing the bag and placing in the autoclave. Investigators should autoclave soiled ABSL2 cages before returning them to the vivarium. In instances where cage autoclaving cannot be performed by the investigator, cages must be placed into an autoclave bag clearly labeled with the universal biohazard symbol, and returned to soiled cagewash area of the vivarium for CCM processing. The movement of soiled ABSL2 cages within a vivarium must be in a manner that the cage and/or autoclave bag containing the cage is clearly identified with the universal biohazard symbol and the potential for spillage is minimized.

All infectious wastes from an ABSL2 animal room (including animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse) must be transported in leak-proof, covered containers in a manner that contains and prevents transmission of the agents. The outer surfaces of the potentially contaminated containers must be disinfected prior to moving the material.

Sharp items (e.g. syringes, needles, scalpels, razors, etc.) must be disposed of in puncture-resistant sharps containers.

Refer to the attached *Center for Comparative Medicine Animal Biosafety Level-2 Training Manual for Vivaria* and the OEHS, [Waste Management Decision Tree](#) for more information regarding the proper disposal of ABSL2 waste.

11. Emergencies

A. Emergency Phone Numbers

Fire and medical: 4-2012

Police: 9 + 911

Hospital Emergency Room: 924-2231

WorkMed: 243-0075

Student Health Services: 924-5362, 972-7004 (after hours)

OEHS: 982-4911

UVA Biosafety Officer, Dr. Thomas Leonard: 982-4911 or 982-4928

CCM Director, Dr. Sanford Feldman: 924-5058 (office), PIC # 2165, 973-2248 (home)

CCM Assoc. Director, Dr. Matt Kessler, 924-2090 (office), PIC# 2233, 964-0877 (home)

Vivarium Supervisors and/or Assistant Supervisors

Aurbach (Fontaine Research Park): 243-6606

ETC-1 Modular (North Fork Research Park): 924-8126

Gilmer: 982-5410

Jordan Annex: 243-6695

Jordan Basement: 982-6991

Life Sciences Annex (LISA; Fontaine Research Park): 243-8038

MR4: 924-5293

MR5: 243-9388

MR6 Vivarium:

Old Medical School (Hospital): 924-0151 or 924-2552

Snyder (Fontaine Research Park): 243-8038

B. Fire and/or Personal Injuries

In the case of fire, push the closest fire alarm button and evacuate the vivarium immediately to the designated assembly point or proceed to the Hospital Emergency Room if an injury occurs.

If an accident involves a biohazard move the injured person out of the ABSL2 area if possible and inactive any biohazard spill after attending to the injured person's needs. Remove PPE and wash any contaminated skin with a disinfectant such as providone iodine-based scrub or solution. If the exposure involves the eyes, the individual should proceed to, or be led to, an eye wash station and flush his/her eyes out for a minimum of 10 minutes. If the exposure involves other parts of the body such as the head and face, the individual should proceed to the nearest emergency shower for decontamination. However, if the injuries are serious, do not move the victim but instead wait for emergency medical professionals to arrive on the scene.

All exposures, injuries, illnesses, animal bites and scratches, needle sticks, allergic reactions, accidents, etc. must be immediately reported to your supervisor. [UVA-WorkMed](#) (243-0075) or

[Student Health Services](#) (982-3915) should be notified to determine if medical attention is warranted. After hours incidents should be referred to the Hospital Emergency Room.

Any injuries should be reported immediately to your supervisor and the vivarium supervisor or assistant supervisor. The supervisory staff will take appropriate action sending the employee to WorkMed or the Hospital Emergency Room (after normal working hours and for serious injury during working hours). If the injury is life-threatening or serious, call 4-2012 or proceed directly to the Hospital Emergency Room. CCM vivarium employee injuries should be reported to the CCM office when convenient using an Accident Investigation Report. ([\lccmserver\penny\MASTER FORMS\Accidentreport.doc](#)).

C. ABSL2 Spills

Spills involving biohazardous materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with these agents. Cleaning must be done with a disinfectant that is effective against the ABSL2 agent of concern and allow sufficient contact time for the disinfectant to work. Follow the directions on the disinfectant bottle. Most disinfectants require about 15 minutes of contact time to be effective.

Cleaning procedures vary with location depending upon if inside or outside of a containment device.

Cleaning ABSL2 spills requires PPE including disposable gown, gloves, surgical mask or N95 respirator (if aerosol is generated), hair cover and shoe covers. Goggles, a face shield and powered air purifying respirator (PAPR) may also be prudent depending on the amount of aerosol generated by the cleaning activity.

All spills must be reported to the Vivarium Supervisor who will notify the OEHS Biosafety Officer at 982-4911 or 982-4928 and the CCM Director.

Spills outside of a Biosafety Cabinet (BSC)

1. Close off spill area to traffic making certain not to contaminate the area as you move about, and notify workers in the area that a biohazard spill has occurred.
2. **If the spill may involve an aerosol**, (e.g. event involving dropping material onto floor, high mechanical force, a forceful expulsion of liquid) **leave the room for 30 minutes** to allow aerosols to settle.
3. Remove contaminated PPE, if gloves are severely contaminated spray them with SporKlenz before removing PPE.
4. Wash any exposed skin with soapy water for at least five minutes.
5. Put on clean set of PPE.
6. Prepare enough volume of a 1:10 dilution of chlorine bleach or other approved disinfectant to saturate the contaminated area. If dilution is not possible, undiluted household bleach can be used (be careful, wear eye protection must be worn and take care to avoid splashing the bleach onto skin or clothing).
7. Contain the spill with paper towels or other absorbent material (e.g. "bench kote", blue diaper pads).
8. Flood the spill area with disinfectant. Leave on for at least 10 minutes.

9. Push the absorbent material at the edge of the spill into the spill's center. Add more paper towels as needed. If glass is present, do not use bare hands! Use tongs (large pieces) forceps (small pieces) followed by a dustpan to remove pieces.
10. Discard the paper towels into a contaminated material container (CMC). If contact with bleach occurs with skin, mucous membranes or eyes, flush area with copious amounts of water.
11. Discard gloves into CMC. Wash hands with soapy water thoroughly
12. Report incident to vivarium supervisor and Principal Investigator.

Spills Inside of a Biological Safety Cabinet (BSC)

1. Leave BSC running.
2. Put on gloves and lab coat and gather paper towels for cleaning.
3. Prepare a fresh dilution of 1:10 bleach or approved disinfectant. Prepare enough solution to cover the entire contaminated area. Always follow manufacturer's directions. Do not use 70% ethanol as it is not a high level disinfectant and evaporates too quickly to allow adequate surface contact time.
4. Flood the area with disinfectant solution and allow the solution to remain in contact for required time: SporKlenz or 10% Bleach for 10 minutes.
5. Spray and or wipe down the cabinet interior and any items inside the BSC with a towel dampened with disinfectant.
6. Discard all contaminated disposable items and towels used for cleaning into a CMC or autoclave according to guidelines
7. Spills large enough to result in liquids flowing through the front or rear grilles require more extensive decontamination; contact the OEHS immediately at 982-4911, for guidance. If a spill such as this has occurred, do not turn the BSC off.
8. Report incident to vivarium supervisor and Principal Investigator.

12. Additional Information

For additional information regarding precautions for handling ABSL2 agents, contact the Institutional Biosafety Officer, Dr. Thomas Leonard, at 982-4911 or 982-4928.

Further details and complementary information on ABSL2 vivarium procedures can be found in the attached appendices.

Appendix 1

Training Manual for Animal Biosafety Level 2 Within the Vivaria

Center for Comparative Medicine

Version 2.1

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Introduction

The USPHS Centers for Disease Control and National Institutes of Health require the following of facilities that conduct Animal Biosafety Level-2 (ABSL2) research as detailed in the 2007 5th edition of *Biosafety in Microbiological and Biomedical Laboratories*. Note: at this time, the BMBL 5th edition is only available on-line. See http://www.cdc.gov/od/ohs/biosfty/bmb15/BMBL_5th_Edition.pdf

General Requirements

1. Access to the animal facility is restricted;
2. Personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of pathogenic agents;
3. Personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and
4. Procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, should be conducted in biosafety cabinets (BSCs) or by use of other physical containment equipment.

Appropriate personal protective equipment (PPE) must be utilized to reduce exposure to biohazardous agents, animals, and contaminated equipment.

In addition, there should be an employee occupational health program.

This manual establishes vivarium procedures in compliance with the above federal requirements and University policies of the Institutional Animal Care and Use Committee (IACUC) and Institutional Biosafety Committee (IBC).

Procedures

Be sure to read and understand all instructions below, and applicable approved IACUC and IBC protocols, *before* beginning an animal BSL-2 (ABSL2) protocol. These procedures must be followed when conducting any ABSL2 protocols.

Note also that prior to performing any work with ABSL2 agents all animal handlers must fulfill any special requirements (health screening, vaccinations, serology, serum banking, tuberculosis testing, etc.) required by WorkMed or Student Health Services (components of UVa's occupational health program) related to the specific agent(s) or animal species involved in the protocol.

If you have any questions, do not hesitate to ask the vivarium supervisor or contact the CCM Director, Dr. Sanford Feldman at telephone 4-5058 or email shf2b@virginia.edu A list of vivarium supervisors is located at the end of this document.

1. Initial Notification of ABSL2 Experiments

- a. Research personnel must consult with the vivarium supervisor (or assistant supervisor) when planning on housing BSL-2 animals containing biohazardous microbes, biotoxins, viral gene vectors or human cells.
- b. Research personnel will inform the vivarium supervisor what species and how many cages and/or numbers of biohazardous animals will be housed in the vivarium.
- c. Research personnel will provide the vivarium supervisor with the name of the agent (genus and species for microbes, biotoxin, gene vector parent virus and construct, or human cell type).
- d. The vivarium supervisor will assign a room where the investigator's animals will be housed. This room might be exclusively for this investigator or it might be shared with another investigator.

2. Biohazard Signage for Rooms Used to House ABSL2 Animals

- a. A biohazard sign (see below) must be on the door whenever biohazardous animals are in the room.
- b. The vivarium supervisor (or assistant supervisor) will post a biohazard sign on the door of the room housing the biohazardous animals (see attached biohazard door poster).
- c. Research personnel will help the vivarium supervisor fill out the door poster so that it is accurate and complete.
- d. Research personnel will remove the sign and give it to the vivarium supervisor when biohazardous animals have left the room whether permanently or temporarily.
- e. If the biohazardous animals return to a room in the vivarium for housing, the room will be re-posted by the vivarium supervisor.

3. Personnel Protective Equipment (PPE) Required for ABSL2 Rooms

The following universal PPE must be donned prior to entering an ABSL2 posted room:

- a. Gloves: single pair latex or nitrile. Double gloving is required for replication competent bacterial or viral agents (e.g. vaccinia, Salmonella sp., etc. For a more comprehensive list see Appendix)
- b. Disposable gown
- c. Mask: surgical
- d. Hair bonnet
- e. Shoe covers

Additional PPE might be required depending on specific circumstances e.g. N95 respirator or PAPR, eye/face protection (safety goggles/disposable face shield), leather gloves for dealing with ferrets infected with influenza.

4. Biohazard Labeling of Cages

- a. The investigator must place a biohazard sticker on each cage containing animals that might shed biological agents (the shedding period, if any, is stated in the IACUC protocol).
 - b. The sticker should only be put on the cage card. Do not put stickers on the cage, filter top or directly on the cage cardholder.
 - c. The identity of the agent (provide genus and species for microbes, biotoxin, gene vector parent virus and construct, or human cell type) must be clearly written on the sticker. Do not use unusual abbreviations or other identifiers that may not be easily understood by caretakers—particularly for new staff and those unfamiliar with your research.
5. Transport of Biohazardous Animal Cages from the Vivarium to Other Areas in Building
- a. All ABSL2 animals being transported from the vivarium to another area of the same building must be caged and the cage contained in a sealed paper bag.
 - b. Each of these paper bags must be labeled with a biohazard sticker.
6. Transport of Biohazardous Animal Cages from the Vivarium to Another Building
- a. If animals are moving to another building, the investigator will submit a delivery request to CCM and CCM will transport them as per the request.
 - b. If animals are moving to another vivarium, the investigator will submit a delivery request form to the CCM office at least 24 hours in advance of the requested delivery in order to obtain permission for the transfer from the Director or his designee in a timely manner.
 - c. The investigator will note on the delivery request form that the animals are biohazardous.
 - d. If any biohazardous animals will be returning to the original vivarium, the investigator will specify the date and time of pickup and insure that the cages are properly packaged and marked with a biohazard sticker.
 - e. Delivery request forms and instructions for use can be obtained at:
<http://www.healthsystem.virginia.edu/internet/ccm/docs/PTpdf1.pdf>
7. Use of ABSL2 Procedure Rooms within ABSL2 Vivarium Suites (if provided)
- a. A biohazard sign must be posted on the procedure room door whenever biohazardous animals or biohazard-labeled cages are in the procedure room.
 - b. Double gloves are required for handling of ABSL2 animals or materials in ABSL2 procedure rooms located within ABSL2 suites if the agent is capable of replicating e.g. vaccinia, Salmonella. Outer gloves should be replaced with fresh gloves after working with contaminated material or animals, or when entering ABSL2 suites (e.g. to return cages to housing rooms).
 - c. Biohazard-labeled cages can only be opened in an ABSL2 procedure room or in a biosafety cabinet (BSC). Any exceptions to this rule must be obtained in writing from CCM Director.
 - d. The BSC is used whenever conducting procedures with a potential for creating aerosols or splashes. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal and oral inoculation of animals. Alternatively, when indicated in the IACUC protocol, a combination mucous membrane (respirator or surgical mask) and eye protection (goggles or face shield) in lieu of primary BSC containment can be used. When procedures involving biological agents

are performed outside of the BSC, room access must be restricted. Restrictions are to be noted on room biohazard sign.

- e. The BSC and bench tops must be cleaned and decontaminated with an appropriate disinfectant (e.g. SporKlenz, 10% bleach, 6% hydrogen peroxide) after each use.
- f. Exiting these procedure rooms must occur by way of re-entry into the ABSL2 suite and then into the anteroom. Direct exit to the anterooms is restricted to emergency situations only.

8. Use of a Procedure Room for ABSL2 Procedures

- a. A biohazard sign must be posted on the procedure room door whenever biohazardous animals or biohazard-labeled cages are in the procedure room.
- b. In addition to regular PPE, double gloves are required for handling of ABSL2 animals or materials in ABSL2 procedure rooms located within ABSL2 suites if the agent is capable of replicating e.g. vaccinia, Salmonella. Outer gloves should be replaced with fresh gloves when contaminated.
- c. A biosafety cabinet (BSC) must be used whenever conducting procedures with a potential for creating aerosols or splashes. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal and oral inoculation of animals. Alternatively, when indicated in the IACUC protocol, a combination mucous membrane (respirator or surgical mask) and eye protection (goggles or face shield) in lieu of primary BSC containment can be used. When procedures involving biological agents are performed outside of the BSC, room access must be restricted. Restrictions are to be noted on room biohazard sign.
- d. The BSC and bench tops must be cleaned and decontaminated with an appropriate disinfectant (e.g. SporKlenz, 10% bleach, 6% hydrogen peroxide) after each use.

9. Exiting the ABSL2 Suite or a Procedure Room Used for ABSL2 Procedures

- a. When exiting ABSL2 suites, PPE must be removed and placed in a CMC prior to entering the anteroom.
- b. When exiting a procedure room used for ABSL2 procedures, PPE should be removed and placed in a CMC prior to exiting the procedure room into the vivarium corridor.
- c. Inner gloves, if you are double gloved, or gloves should be removed last, followed by hand washing. If a sink is not present in the anteroom or procedure room, alcohol-based hand sanitizer should be used and hands washed with soap and water as soon as possible after exiting the anteroom or procedure room.

10. Disposal of Biohazardous Caging and Animal Carcasses/Tissues

- a. Any dead biohazardous animals (or tissues there from) must be placed into a red biohazard bag and then into a red biohazard container in the vivarium's carcass cold room (walk-in carcass refrigerator).
- b. If an animal carcass needs to be saved for any reason, it should be put in a bag labeled with a biohazard sticker and then placed on a shelf in the cold room.
- c. To protect personnel, vivarium staff autoclave all soiled ABSL2 cages prior to dumping the bedding. Therefore, it is imperative that all soiled cages from ABSL2 animals be placed in an autoclavable bag and marked with a biohazard label. If soiled cages are being returned to a vivarium from an outside ABSL2 laboratory, the cages must be transported in biohazard-labeled autoclave bag(s) as per IACUC Policy.

11. Emergency Procedures

- a. In case of a laboratory accident in an ABSL2 area, refer to the Biosafety Manual for instructions on how to proceed.
- b. In case of an accident involving an animal bite or scratch, immediately stop work and contact the vivarium supervisor or investigator. During working hours and depending upon the extent of the injury or exposure, employees should proceed to WorkMed or the hospital emergency room while students should proceed to Student Health Services or the hospital emergency room.

For additional information or assistance, contact the respective Vivarium Supervisor or Assistant Supervisor, or CCM Director, Dr. Sanford Feldman (4-5058, shf2b@virginia.edu)

Aurbach Vivarium (Fontaine Research Park)
Supervisor – Marty White 3-6606 marty.white@crl.com
Assistant Supervisor – Joy Gunter joy.gunter@crl.com

ETC-1 Modular Vivarium (North Fork Research Park)
Supervisor – Linda Rummery 4-8126 linda.rumery@crl.com

Jordan Annex Vivarium
Supervisor - Shelly Verling 3-6695 smv3p@virginia.edu
Assistant Supervisor – Ayoub Tambire 3-6696 ast2a@Virginia.EDU

Jordan Basement Vivarium
Supervisor - Kim Dean 2-6991 kdd2z@virginia.edu

MR4 Vivarium
Supervisor - Alice Kenney 4-5293 ask5g@virginia.edu
Assistant Supervisor - Laurie Edwards 4-5293 lrc8r@virginia.edu

Snyder and Life Sciences Annex Vivarium (LISA Vivarium; Fontaine Research Park)
Supervisor – James Weirich 3-8038 jweirich@themccgroup.com
Assistant Supervisor – Merritt Newman mnewman@themccgroup.com

MR5 Vivarium
Supervisor – Karen Oehrli 3-9388 karen.oehrli@crl.com
Assistant Supervisor – Vivian Hoeffler vivian.hoeffler@crl.com

Old Medical School (Hospital)
Supervisor - Audrey Martin 4-0151 abm8h@virginia.edu
Assistant Supervisor – Shawn Anderson 4-2552 sma7d@virginia.edu

Appendix 2



ABSL2 BIOHAZARD

AGENT: _____

DATE IN EFFECT: _____

INVESTIGATOR: _____

OFFICE/EMERG PHONES: _____

VIVIARIUM SUPERVISOR: _____

OFFICE/EMERG PHONES: _____

ENTRY REQUIREMENTS:

- ✓ **CCM ABSL2 TRAINING**
- ✓ **ANIMAL HANDLER MEDICAL CLEARANCE** (OCC. HEALTH) *Special:*
Recommended vaccinations (e.g. HBV, vaccinia, n/a) _____
- ✓ **PPE: GOWN, GLOVES, BOOTIES, HEADCOVER & SURGICAL MASK**
Special: Respiratory (N-95) & Eye/Face Protection as indicated by ACUC Protocol

EXIT REQUIREMENTS:

- ✓ **REMOVE PPE & WASH HANDS** (or hand sanitizer then hand wash)

Appendix 3: Animal Protocol Transfer and Delivery Request

BASIC INFORMATION SECTION

Date Form Completed: **Principal Investigator:**

Principal Investigator's e-mail: Phone number:

Species: Strain:

IDs: (Sex, pen #, tag # etc.) Biohazardous (ABSL2): Yes No

No. of animals to be moved or transferred: No. of cages to be moved or transferred:

Building animals sent from: **Room Number:**

Protocol Animal is currently under:

Per Diem Fund Code:

Principal Investigator's Signature Date

Section One: PROTOCOL TRANSFER REQUEST

Building Animals to be housed in:

Building: Room Number:

Protocol Animal is being transferred TO:

Principal Investigator:

Per Diem Fund Code:

Section Two: DELIVERY REQUEST

Date Delivery Needed: Time Delivery Needed: AM PM

Deliver to: Building: Room Number:

**Animals must be fully alert in order to be returned to animal housing*

.Return transport to originating facility and room? Yes No. If Yes, Pick-up time:

How will cages be marked for transport?

I. INSTRUCTIONS FOR COMPLETING FORM

- Everyone should complete the BASIC INFORMATION SECTION at the top of the form.
- If you are only **transferring** an animal(s) from one protocol to another, complete **Section One**.
 - **Animals that have had any experimental manipulation may not be transferred between animal protocols.**
 - **Only the signature of the principal investigator will be accepted for transfers between animal protocols.**
 - **ABSL2 cages must be enclosed in a paper or plastic bag and labeled with the biohazard symbol.**
- If you are requesting **delivery only** of an animal(s) from one place to another complete **Section Two**. 24 hours notice and veterinary approval from the Director of Comparative Medicine is required for delivery of an animal(s) from one vivarium to another, e-mail shf2b@virginia.edu and state where they are coming from and going to.
- If you are requesting **transfer** of an animal(s) from one protocol to another **and** also requesting **delivery** to another location **complete both sections one and two**.
- Fax to the completed form to 4-0354.

Appendix 4: Common Replicating Biological (Microbial) Agents Requiring Double Gloving

Double gloving serves as an effective control measure to reduce potential for biological contamination of personnel, animals, and environmental surfaces; Outer gloves may be removed when contaminated, yet risk of personal skin exposure is minimized as the inner pair of gloves remains in place to prevent skin exposure. Double gloving is particularly warranted and expected for procedures involving manipulation of animals that have been inoculated with, and may shed, biological agents capable of replication. The list below gives **examples** of replicating biological agents requiring the use of double gloves.

Note: Double gloving is *not* required for protocols involving the use of established human cell lines as long as basic biosafety measures (e.g. hand washing) are practiced.

- *Bacillus anthracis*
- *Bacillus anthracis* (Ames strain)
- *Clostridium difficile*
- *Cryptosporidium parvum*
- *Entamoeba histolytica*
- Enteropathogenic *Escherichia coli*
- *Francisella tularensis*
- *Helicobacter pylori*
- Herpes viruses
- Influenza virus type A
- Kaposi's sarcoma-associated herpesvirus (HHV8 or KSHV)
- *Klebsiella*
- Poxviruses
- *Pseudomonas aeruginosa*
- Respiratory syncytial virus
- Retrovirus
- *Salmonella*
- *Salmonella typhimurium*
- *Staphylococcus aureus*
- *Streptococcus pneumoniae*
- *Streptococcus pyogenes*
- Vaccinia
- *Yersinia enterocolitica*
- *Yersinia pseudotuberculosis*

**Appendix 6: University of Virginia
Center for Comparative Medicine**

SOP#: 222

APPROVED _____ DATE _____

Issue Date: 1/2003

Revised Date: 5/13/09

Page 1 of 2

TITLE: Cage Change of Rodents Inoculated with Biosafety Level 2 Agents

SCOPE: Animal Care Personnel

RESPONSIBILITY: Attending Veterinarian, Vivarium Supervisor

PURPOSE: To outline proper procedures when changing ABSL2 rodents.

PROCEDURES: Cages and racks are changed twice per week according to the rodent area cage change schedule or more frequently if needed.

A) Personal Protective Equipment/Clothing (PPE)

- 1) Personnel performing this procedure will wear the following protective clothing:
 - a) Hair bonnet
 - b) Surgical mask [or respirator or PAPR (if required)]
 - c) Work scrubs covered with a disposable gown
 - d) Shoe covers
 - e) Latex or nitrile gloves. Double gloves are required for replication competent bacteria and viruses.
 - f) Eye protection (goggles and/or face shield, if required)
- 2) After donning the above clothing and PPE begin this procedure

B) Cage and Rack Changing

- 1) Rodents are housed in static microisolator caging and will be on a normal husbandry schedule:
 - a) Cages changed twice per week or more frequently if needed
- 2) Racks and cage cards for the cages containing adenovirus exposed animals will be labeled with a biohazard symbol.
- 3) Cages will be changed in the Animal Transfer Station or Biosafety Cabinet using Spor-Klenz sprayed, gloved hands. Soiled cages and bottles will be placed in an autoclavable bag for autoclaving. The bags exterior will be sprayed with Spor-Klenz before moving to the sterilizer. The sprayed bags should sit for at least 5 minutes for decontamination or be put directly into a steam sterilizer.
- 4) If the rack is changed, a clean rack of cages will be moved into the area. The cages are changed according to B.3 above. The cages are then placed into a biohazard bag and both the rack and bags are sprayed with Spor-Klenz using a garden sprayer. Depending upon the agent(s) involved, a surgical mask or respirator or PAPR might have to be worn. After 15 minutes the bagged cages and racks can be taken to the cage wash for further decontamination.

C) Protective Clothing Removal

- 1) When the cleaning procedures are completed, the technician will remove the PPE removing the gloves last and place the items in a contaminated materials container (CMC) before leaving the room.
- 2) Wash hands with soap and water in sink prior to leaving the ABSL2 room. If a sink is not available, disinfect hands with the alcohol-based hand cleaner and exit the room. Wash hands as soon as possible prior to exiting

the vivarium.

- 3) When the core carton containing gown, shoe covers, and gloves is full it is sealed. The outside of this container is sprayed with Spor-Klenz and allowed to sit 15 minutes before transport for steam sterilization.

D) Duration of ABSL2 Practices

- 1) Studies involving replication deficient gene therapy vectors (adenovirus, adeno-associated virus and retrovirus) are considered biohazardous for 72 hours. Therefore, animals inoculated with these vectors are placed in a clean cage labeled with the biohazard symbol and treated at ABSL2 for 72 hours (usually the next cage change). Thereafter, the animals are considered ABSL1 and the biohazard symbol is removed, unless otherwise instructed by the Facility Director or Institutional Biosafety Committee.
- 2) Vaccinia vector studies shall be considered ABSL2 for 14 days in immune competent mice and until there are no active skin lesions on the inoculated animals.
- 3) Immune deficient mice with human tumor cells are considered ABSL2 for the duration of the experiment.

Appendix 7: University of Virginia
Center for Comparative Medicine
STANDARD OPERATING PROCEDURE

SOP#: 804

APPROVED _____ DATE _____

REPLACES SOP#:

Attending Veterinarian

EFFECTIVE DATE:

Date Revised: 5/13/09

Page 1 of 2

TITLE: Cage Change and Removal of Rabbits Inoculated with Animal Biosafety Level 2 (ABSL2) Agents

SCOPE: Animal Care Personnel

RESPONSIBILITY: Vivarium Supervisor, Attending Veterinarian

PURPOSE: To outline proper procedures when dealing with ABSL2 rabbits.

PROCEDURES:**A) Personal Protective Equipment (PPE) and Clothing**

- 1) Personnel performing this procedure will wear the following PPE and clothing:
 - a) Hair bonnet
 - b) Surgical mask [or respirator or PAPR (if required)]
 - c) Disposable gown over work scrubs
 - d) Shoe covers
 - e) Latex or nitrile gloves. Double gloves are required for replication competent bacteria and viruses.
 - f) Eye protection (goggles and/or face shield) if required
- 2) After donning the above clothing and PPE, begin the following procedure:

B) Pan, Cage, and Rack Changing

- 1) Rabbits will be on a normal husbandry schedule:
 - a) Pans changed three times per week.
 - b) Cages and rack changed once every other week.
- 2) Pans and liners will be removed from the bottom of the cage. Liners are placed in a red biohazard bag and the pan sprayed with SporKlenz, wait 10 minutes and remove to soiled cagewash for processing. New pans and liners will be placed under the cage. Dirty pans will be taken to the dirty side of the cage wash area for sanitizing.
- 3) On the day when the rack is changed, a clean rack of cages will be moved into the room. The rabbits are then placed into a clean cage, on the clean rack. The absorbent plastic backed pan liner in the cage pan is folded and placed in a biohazard bag. The inside of the soiled rack, including the cage and pan, are sprayed with SporKlenz. The sprayed solution will remain for a minimum of five minutes for decontamination to occur. The rack with cages will then be taken to the dirty side of the cage wash area for sanitizing. The red bag with the pan liners is sealed and put on the dirty side of cage wash, adjacent to the large autoclave.

C) Animal Removal

- 1) Animals being removed from room will be placed in a filtered animal transport box prepared with plastic backed absorbent paper in the bottom.
- 2) The box will be labeled with the proper information:

- a) ABSL2
 - b) Protocol number
 - c) Investigator's name
 - d) Type of animal
 - e) Emergency contact number on BL sign at door.
 - f) Universal biohazard symbol
- 3) The box will then be taped securely and the outside sprayed with the SporKlenz solution.
 - 4) Place outside the room door.

D) Protective Clothing Removal

- 1) When the cleaning procedures are completed, the technician will remove the tyvek suit, shoe covers, and lastly the gloves. These items will be placed in a contaminated medical waste box before leaving the room.

E) Duration of ABSL2 Practices

- 1) Studies involving replication deficient gene therapy vectors (adenovirus, adeno-associated virus, and retrovirus) are considered biohazardous for 72 hours. Therefore, animals inoculated with these vectors are placed in a clean cage labeled with the biohazard symbol and treated at ABSL2 for 72 hours. At the next cage change the animals are considered ABSL-1 and the biohazard symbol is removed.
- 2) Studies involving vaccinia virus are considered biohazardous for 14 day, as long as an active skin lesion exists.
- 3) Studies using human cells or tissue, and replication competent bacteria will be considered ABSL2 for the duration of the experiment.

Appendix 8: University of Virginia
Center for Comparative Medicine
STANDARD OPERATING PROCEDURE

SOP#: 252 APPROVED _____ DATE _____
REPLACES SOP#: Attending Veterinarian
EFFECTIVE DATE: 5/26/09
Date Revised:
Page 1 of 2

TITLE: Decontamination of ABSL2 Waste by Autoclaving
SCOPE: Animal Care Personnel
RESPONSIBILITY: Vivarium Supervisor, Director CCM
PURPOSE: To outline proper procedures when dealing with ABSL2 rabbits.

INTRODUCTION:

Steam sterilization (autoclaving) is one of the most effective methods for decontaminating biohazardous material. Autoclaves use saturated steam under high pressure to decontaminate infectious materials (i.e., ABSL2 waste, etc.) and to sterilize items like surgical instruments. Biohazardous material and full sharps containers generated within the vivarium are placed in Contaminated Materials Containers (CMCs) and treated at the hospital autoclave. However, vivarium ABSL2 rooms that generate biohazardous material may use a dedicated vivarium autoclaves. When using vivarium autoclaves to decontaminate biohazardous materials, **the Vivarium Supervisor (or Director, CCM) is responsible for meeting the following mandatory requirements:**

1. Maintaining a logbook in accordance with Section 4a below.
2. Autoclaving for a minimum of 30 minutes (see Section 4b) unless biological indicator validation data demonstrates that less time is sufficient for decontamination.
3. Documenting proper validation techniques using biological indicators when decontaminating materials (see Section 8).

The procedures below serve as guidelines to help autoclave operators ensure a safe and effective process. For questions concerning autoclave requirements or guidelines and safety, contact the Office of Environmental Health and Safety Office at 982-4911.

PROCEDURES:

- A) Select appropriate container/bag for autoclaving
- 1) For biohazardous dry materials
 - i. Use polypropylene bags or reusable containers. Make sure your plastic container is suitable for sterilizer temperatures (polypropylene and polycarbonate).
 - ii. Ensure that contaminated materials are free of sharp objects that may puncture bags. Autoclave bags are tear resistant but can be punctured or burst in the autoclave,
 - iii. Fill autoclave bags only 2/3rds full. Ensure adequate steam penetration by closing the bags loosely, leaving a one-inch opening and closing with autoclave indicator tape.
 - iv. Autoclavable sharps containers must be autoclaved and placed in a CMC for pick up.

