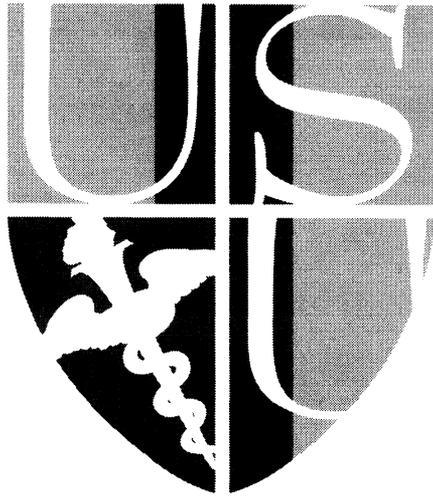


**USUHS  
INSTRUCTION  
6403**





# UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES



## SUBJECT: Biohazard Suite Management

### Instruction 6403

JUN 15 2004

(EHS)

#### ABSTRACT

This Instruction prescribes the safety practices, equipment and biohazard suite requirements for conducting research at the Uniformed Services University of the Health Sciences (USUHS) involving agents assigned to Biosafety Level 3 (BSL-3).

#### A. Reissuance and Purpose.

This Instruction reissues USUHS Instruction 6403 to establish guidelines for the safe operation of the Biosafety Level-3 suite at the USUHS.

#### B. References. See *Enclosure 1*.

#### C. Applicability.

The provisions of this Instruction apply to all research personnel conducting work in the USUHS BSL-3 suite.

#### D. Responsibilities.

1. The President, USUHS is responsible for prescribing guidelines for safe operations in the USUHS BSL-3 suite to protect all personnel and the environment.

2. BSL-3 Oversight Committee is responsible for:

- a. Reviewing all requests to use the BSL-3 suite.
- b. For developing uniform guidelines for safe working practices within the BSL-3 suite.

c. Considering the safety needs of "all" workers in the suite when diverse agents are concurrently used.

d. For establishing special training requirements for Principal Investigators and the staff, as needed.

e. Maintaining a list of the etiological agents under study in the suite.

3. The BioSafety Committee (BSC) is responsible for:

a. Reviewing protocols that utilize recombinant DNA, genetic materials, controlled substances, and Centers for Disease Control and Prevention (CDC) Select Agents, or other hazardous biological or chemical agents to ensure USUHS can adequately and safely support the proposed research.

b. Forwarding all protocols requesting use of the BSL-3 suite to the BSL-3 Oversight Committee.

c. Forwarding all protocols requesting the use of CDC Select Agents to the CDC Select Agent Responsible Official (EHS).

4. The Chair, Department of Microbiology and Immunology is responsible for the physical integrity and maintenance of the BSL-3 suite. The Chair:

- a. Acts as Chairman of the BSL-3 Oversight Committee.
- b. Ensures that safety guidelines are adhered to, and responds to requests and concerns raised by Principal Investigators and others regarding the work environment or work practices in the BSL-3 suite.
- c. Reviews individual requests for unescorted access to the BSL-3 suite. The Chair, Microbiology and Immunology Department is the initial approval authority for issuing access cards to the BSL-3 suite; the request form is sent to Security through Environmental Health and Occupational Safety (EHS).

5. The EHS Department shall:

- a. Respond to, and evaluate emergency situations.
- b. Provide a Biological Safety Officer and a CDC Select Agent Responsible Official.
- c. Ensure the annual certification and any required repair of the biosafety cabinets are completed in accordance with Occupational Safety and Health Administration's (OSHA) 29 CFR Part 1910.1030.
- d. Using Biosafety in Microbiological and Biomedical Laboratories as a reference, inspect the suite to ensure effective operation of UV lights in pass-boxes, airlocks, biological safety cabinets, and over linen containers (29 CFR 1910.1450, App. A, Ch. 6.C.7.1).
- e. Make recommendations to PIs and the Department of Microbiology and Immunology concerning safety equipment maintenance or replacement.
- f. Maintain programs to comply with requirements of the CDC Select Agents (42 CFR Part 73), OSHA standards for Hazard

Communication (29 CFR 1910.1200), Bloodborne Pathogens (29 CFR Part 1910.1030), and Occupational Exposure to Hazardous Chemicals in Laboratories (29 CFR 1910.1450). The EHS Department shall also ensure Material Safety Data Sheets (MSDs) are available to USUHS researchers in the BSL-3 suite.

- g. Conduct annual safety audits of the suite to help identify potential problems using the safety survey at Enclosure 3.
- h. Ensure appropriate monitoring for safe work practices regarding the use of biological safety cabinets.
- i. Acquire, develop and update training programs (including self-paced training programs) to meet the general safety requirements of personnel working with etiological agents and chemical hazards in the bio-containment laboratory.
- j. Ensure appropriate decontamination procedures are being implemented.
- k. Investigate the cause of every BSL-3 accident and forward findings to the BSL-3 Oversight Committee and the CDC, if appropriate.
- l. Ensure appropriate use, selection, maintenance, training, and cleaning of respirators.
- m. Provide medical clearance for respirator use.
- n. Coordinate with U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, Office of Research and Technology Applications, to provide immunization to researchers based on etiological agent in use. (*See Enclosure 8 for selection criteria for individuals who might be exposed to Venezuelan Equine Encephalitis (VEE) Virus.*)
- o. Posting and maintaining emergency numbers (*see Enclosure 2*), and ensuring that appropriate biohazard warning signs are posted.

6. The Laboratory Animal Medicine Department shall:

- a. Provide overall responsibility for the care and use of laboratory animals and associated training.
- b. Maintain animal care and use in the BSL-3 suite to comply with the Animal Welfare Regulation (PL.99-198), the “Guide for the Care and Use of Laboratory Animals,” AR 70-18, DOD Policy for the Protection of Animals in DOD Sponsored Programs, and DOD Directive 3216.1.
- c. Approve the use of animals within the BSL-3 suite.
- d. Conduct inspections of animals and animal areas in accordance with laws, regulations, guidelines and DLAM SOPs.

7. The Facilities Division shall:

- a. Ensure facilities personnel are properly escorted and wear appropriate Personal Protective Equipment (PPE) before entering the BSL-3 space to perform required maintenance.
- b. Assist in re-verifying the complete BSL-3 suite design and operational parameters at least annually.

8. The Security Department shall:

- a. Maintain a complete listing of all individuals who have access to the BSL-3 suite; a copy should be provided to EHS.
- b. Manage the electronic key card access system to include:
  - (1) Issuing new cards to individuals who have been approved through the Department of Microbiology and Immunology and EHS.
  - (2) Deactivating lost, stolen or terminated employee’s cards.
  - (3) Providing EHS with an access log.
- c. Advising PIs in matters pertaining to the physical security of the suite and the biological security of CDC select agents.
- d. Conducting annual security

reviews.

e. Notify EHS as soon as any incident or condition is noticed which may involve the biohazard suite, to include water leakage into the following areas:

- (1) Corridor by B4083.
- (2) Rooms B4085, B4077.
- (3) Utility chase, B4087.
- (4) Rooms B-3083, B-3081, B-3079, B-3075.
- (5) Utility chase, B-3087.
- (6) Rooms B-2027, B-2023, B-2019.
- (7) Utility chase, B-2029.
- (8) Cafeteria Dining Room.

In the event of a water leak as described above, or any other malfunction in the biohazard suite, the Director, Facilities Division, or his representative, will also be notified by the Security Guard. Cleanup or repair operations, to include removal of water from the above areas, shall not be attempted or started until specifically approved by EHS after consultation with the appropriate users of the suite.

9. The Principal Investigator (PI) is responsible for:

- a. Implementing safe working practices for researchers working under his/her supervision.
- b. Ensuring the microbiological safety of personnel.
- c. Ensuring that appropriate safety orientation and training is conducted and documented for each new employee whose duties require entry into the BSL-3 suite. The PI will ensure that the employee attends the laboratory safety operations training conducted by EHS and any other training, which may be deemed appropriate before they enter the BSL-3 suite. Training will include mandatory reading of this Instruction and an orientation by the PI (or his designated representative) in the general practices, procedures, and techniques

specific to the USUHS BSL-3 suite. Orientation on agent-specific techniques must be accomplished before performing work involving an etiological agent in the BSL-3 suite. Documentation of the training for each employee will be maintained by the PI, and should be available for audit by EHS or extra-mural inspection teams. (*For a checklist of site-specific training, see Enclosure 4.*) Persons performing daily animal husbandry will receive documented training in proper husbandry procedures.

d. Complying with appropriate safety procedures for etiological agents used within the suite. (*See Enclosure 7 for VEE Virus.*)

e. Cooperating with the Center for Environmental Health/Occupational Safety during safety inspections and for implementing recommendations.

f. Ensuring that etiological agents, biological products, or diagnostic specimens shipped from the suite are correctly processed and packaged.

g. Ensuring that all individuals entering or working in the BSL-3 suite receive the required vaccination or have sufficient Personal Protection Equipment (PPE).

h. Notifying the Director of the Center for Laboratory Animal Medicine (LAM) whenever animals are housed in the BSL-3 suite and when they are no longer used. Maintaining a LAM animal room and monitoring sheet whenever animals are present, and turning completed sheets to the LAM Animal Husbandry Chief within 3 working days.

i. Appointing a supervisor to act for the PI when the PI is not physically present in the suite. Whenever an etiological agent is being used in the suite, a designated “supervisor” or “lead” among the trained staff shall be present if the PI is not present in the BSL-3 suite.

## **E. Training.**

1. The BSL-3 Oversight Committee ensures that:

a. PIs have satisfactory knowledge, training, and experience with the proposed biological agent, assume full responsibility for the training of researchers working under their protocol, and are mindful of the additional health, safety, and security requirements of concurrent BSL-3 suite users.

b. If PIs require additional training or experience with the agent or in the BSL-3 environment, the BSL-3 Oversight Committee ensures that this training is successfully completed before recommending approval to use the BSL-3 suite in an unescorted status.

### 2. Principal Investigator (PI)

PIs or their designated representatives (supervisors) are primarily responsible for ensuring that all researchers working under their protocols have the requisite training to safely and healthfully perform work with the etiological agent. At a minimum, this should include:

a. Review and comprehension of this Instruction, especially the Biohazard Suite User’s Guide (*see Enclosure 6*).

b. Affirming that researchers have received the general lab safety training provided by EHS, security training provided by Security, and CDC select Agent Training.

c. Provide agent specific training to researchers including a detailed explanation of the SOPs unique to the research protocol.

d. Perform an annual “walk-through” of the BSL-3 suite identifying and remarking on the items listed under the Site Specific Training Guidelines (*see Enclosure 4*).

3. The Center for Environmental Health and Occupational Safety (EHS) shall:

Acquire, develop and update training

programs, to include self-paced training programs, to meet the CDC Select Agent, general safety requirements of personnel working with etiological agents, and chemical hazards in the bio-containment laboratory.

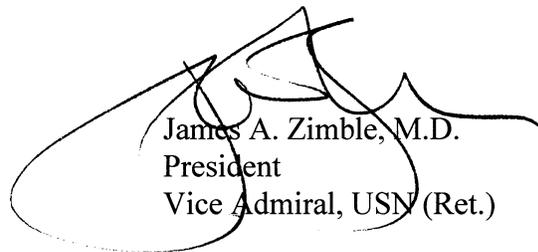
4. The Department of Laboratory Animal Medicine (LAM) shall:

Acquire, develop and update training programs, to include self-paced training programs, to meet the proper animal husbandry procedures in the BSL-3 suite.

5. The Security Department shall acquire, develop and update training programs to meet the security requirements of working with CDC Select Agents and the BSL-3 suite.

**F. Guidelines.** *See Enclosures 3-8.*

**G. Effective Date.** This Instruction is effective immediately.



James A. Zimble, M.D.  
President  
Vice Admiral, USN (Ret.)

Enclosures:

1. References
2. Emergency Telephone Numbers
3. Safety Survey
4. Site Specific Training Checklist
5. BSL-3 Use Application Form
6. Biohazard Suite Users Guide
7. Specific Safety Items Related to VEE
8. VEE (TC-83) Vaccination Selection Criteria
9. USUHS Workplace Hazard Analysis



**REFERENCES**

- (a) USUHS Instruction 6403, "Biohazard Suite Management," 29 November 1985 (hereby cancelled)
- (b) USAMRIID Regulation Number 385-69, March 1995
- (c) 42 CFR Part 73, Select Agents and Toxins
- (d) 29 CFR 1910, OSHA
- (e) *Biosafety in Biomedical and Microbiological Laboratories – A Supplement to the NIH Guidelines for Recombinant DNA Research*, 1999
- (f) *Biosafety in Microbiological and Biomedical Laboratories – 4<sup>th</sup> Edition*, U.S. Department of Health and Human Services, May 1999 (pages 62-69)



**EMERGENCY TELEPHONE NUMBERS**

MEDICAL EMERGENCY	777
FIRE	777
AMBULANCE	777
EHS (EMERGENCY)	295-9443
SECURITY	295-3038
MICROBIOLOGY and IMMUNOLOGY (BSL-3 Property Owner)	295-3419
BSL-3 CHAIR	295-3170
CDC	(404) 498-2259



**SAFETY SURVEY**

**SUBJECT:** Biosafety Level 3 Safety Survey

**REFERENCES:** (a) ICH Guidelines for Good Clinical Practice  
 (b) Biosafety in Microbiological and Biomedical Laboratories, 4th EDITION  
 (c) NIH Recommended Practices

**A. PURPOSE.**

The purpose of this SOP is to establish uniform Biosafety Level 3 survey procedures. Surveys will be conducted annually and will be performed as part of the orientation when new research endeavors are commenced.

**B. APPLICABILITY.**

This procedure applies to all participants who need access to the BSL-3 suite and will be performed by the Center of Environmental Health and Occupational Safety (EHS).

**C. RESPONSIBILITIES.**

1. The BSL-3 Oversight Committee identifies the environmental/safety training requirements of researchers working in the BSL-3 suite at USUHS.
2. EHS inspects all workspaces within the University in order to ensure a safe and healthful workplace. EHS will use, at a minimum, the following Safety Survey tool (or its equivalent) to perform periodic inspections of the BSL-3 suite.

**D. PROCEDURES - Biosafety Level 3 Suite Safety Survey.**

The following items, at a minimum, will be incorporated into any survey tool used in safety inspections of the Biosafety Level 3 suite and may be incorporated into the safe work practices orientation of new suite users, at the discretion of the PI:

Components	Yes	No	Comments
<u>Medical Surveillance</u>			
- An appropriate medical surveillance program is in place.	( )	( )	
- All personnel receive appropriate immunizations or tests for the agents handled or potentially present in the lab and periodic testing as recommended for agent handled.	( )	( )	
- If appropriate, a serum surveillance system is in place.	( )	( )	
- Persons at increased risk are not allowed in the suite without medical clearance from the occupational health physician.	( )	( )	
<u>Biosafety Manual</u>			
- This Instruction serves as the Bio-Safety Manual; procedures are adopted.	( )	( )	
- Personnel are advised of hazards.	( )	( )	
- Personnel are required to read the Instruction and follow guidelines on practices and procedures.	( )	( )	

## Enclosure 3

Components	Yes	No	Comments
<u>Training</u>			
- Site Specific training conducted and documented for each worker.	( )	( )	
- HAZCOM training current (annual).	( )	( )	
- EHS Laboratory Safety Training current (annual).	( )	( )	
- CDC Select Agent Training current.	( )	( )	
- Security Program for CDC Select Agents.	( )	( )	
- Personnel receive periodic updates or additional training as necessary for procedural changes.	( )	( )	
<u>Proficient Laboratory Practice</u>			
- The PI is responsible for ensuring that, before working with organisms at Bio-safety Level 3, all personnel demonstrate proficiency in standard microbiological practices and techniques, and in the practices and operations specific to the laboratory suite. (This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the PI or other component scientist proficient in safe microbiological practices and techniques.)	( )	( )	
- The PI has established policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g. immunization) and who comply with all entry and exit procedures, enter the laboratory or animal rooms.	( )	( )	
<u>Suite</u>			
- Lab is separated from unrestricted traffic flow in building.	( )	( )	
- Access to lab is through an anteroom with self-closing doors.	( )	( )	
- All penetrations in lab are sealed.	( )	( )	
- Exhaust air is single pass and exhausted away from occupied areas.	( )	( )	
- An insect and rodent program is in effect.	( )	( )	
- Laboratory doors are kept closed when experiments are in progress.	( )	( )	
<u>Access</u>			
- Chair of MIC in cooperation with BSL-3 Oversight committee controls access to BSL-3 suite.	( )	( )	
- No minors are allowed in BSL-3 suite.	( )	( )	
- Entry and exit logs are available for maintenance and housekeeping personnel.	( )	( )	
- Entry and exit information is available for routine workers.	( )	( )	
<u>Personal Protection</u>			
- Persons wash their hands after handling infectious materials, after removing gloves, and when they leave the laboratory.	( )	( )	
- Closed front gowns or coveralls are worn in laboratory.	( )	( )	
- Hand-washing sink is foot, elbow or automatically controlled.	( )	( )	
- Respiratory and face protection are used when in rooms containing infected animals.	( )	( )	

## Enclosure 3

Components	Yes	No	Comments
<u>Hand Protection</u>			
- Double gloves are worn when handling infectious material, potential contaminated equipment and work surfaces.	( )	( )	
<u>Respiratory Protection</u>			
- Respiratory protection is worn by all non-vaccinated personnel in the lab when aerosols are not safely contained in a bio-safety cabinet.	( )	( )	
<u>Hazard Warning Signage</u>			
- When infectious materials or infected animals are present in the laboratory or containment module, a hazard warning sign incorporating the universal biohazard symbol, is posted on all laboratory and animal room access doors.	( )	( )	
- The hazard warning sign identifies the agent, lists the name and telephone number of the PI or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for immunizations, respirators, or other personal protective equipment.	( )	( )	
<u>Practices</u>			
- Mucous membrane protection provided when working with infectious material outside of a bio-safety cabinet	( )	( )	
- Eating, drinking, smoking, handling contact lenses, applying cosmetics and storing food for human use are not permitted in the BSL-3 suite.	( )	( )	
- Animals and plants not related to the work being conducted are not permitted in BSL-3 suite.	( )	( )	
- Personnel are required to read and follow all instructions or practices and procedures.	( )	( )	
- All procedures are carefully performed to minimize the creation of aerosols or splatters.	( )	( )	
- Mouth pipetting is prohibited; mechanical pipetting devices used	( )	( )	
- All open manipulations involving infectious materials are conducted in biological safety cabinets or other physical containment devices within the containment module.	( )	( )	
- No work in open vessels is conducted on the open bench.	( )	( )	
- Clean up is facilitated by using plastic-backed paper toweling on the non-perforated work surfaces within biological safety cabinets.	( )	( )	
<u>Decontamination</u>			
- Laboratory equipment and work surfaces are decontaminated at least once a day with an effective disinfectant, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials.	( )	( )	
- Spills of infectious materials are contained, decontaminated, and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious material.	( )	( )	

## Enclosure 3

Components	Yes	No	Comments
- Spill procedures are developed and posted.	( )	( )	
- Contaminated equipment is decontaminated before removal from the suite for repair or maintenance or packaging for transport, in accordance with applicable local, state, or federal regulations.	( )	( )	
- Cultures, tissues, specimens of body fluids, or wastes are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.	( )	( )	
- All potentially contaminated waste materials (e.g. gloves, lab coats, etc.) from laboratories are decontaminated before disposal or reuse.	( )	( )	
- Spills and accidents that result in overt or potential exposures to infectious materials are immediately reported to the PI and EHS.	( )	( )	
- Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained for all accidents and potential exposures.	( )	( )	
- Cages are washed in a cage washer. The mechanical cage washer has a final rinse temperature of at least 180 deg F.	( )	( )	
- An autoclave is available which is convenient to where the biohazard is contained. The autoclave is utilized to decontaminate infectious waste before moving it to other areas of the suite.	( )	( )	

### Waste Disposal

- A method for decontaminating all laboratory wastes is available in the suite and utilized, preferably within the laboratory (i.e. autoclave, chemical disinfection, incineration or other approved decontamination method).	( )	( )	
- All cultures, stocks, a potentially contaminate waste materials (i.e. gloves, labcoats etc...) and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving.	( )	( )	
- Infectious waste from BSL-3 laboratories is decontaminated before removal for off-site disposal.	( )	( )	
- Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak-proof container and closed for transport from the laboratory.	( )	( )	
- If waste is transported out of the laboratory, it is properly sealed and not transported in public corridors.	( )	( )	

### Sharps

- Policies for the safe handling of sharps are instituted.	( )	( )	
- Needles and syringes or other sharp instruments are restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.	( )	( )	
- Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials.	( )	( )	

## Enclosure 3

Components	Yes	No	Comments
- Used disposable needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they are carefully placed in conveniently located puncture-resistant containers used for sharps disposal.	( )	( )	
- Non-disposable sharps are placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.	( )	( )	
- Syringes which re-sheath the needle, needle-less systems, and other safe devices are used when appropriate.	( )	( )	
- Broken glassware is not handled directly by hand, but is removed by mechanical means such as a brush and dustpan, tongs or forceps.	( )	( )	
- Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, and disposed of according to local, state, or federal regulations.	( )	( )	

### Safety Equipment (Primary barriers)

- Protective laboratory clothing such as solid-front or wrap-around gowns, scrub suits, or coveralls are worn by workers when in the laboratory.	( )	( )	
- Protective clothing is worn only in the laboratory.	( )	( )	
- Reusable clothing is decontaminated before being laundered.	( )	( )	
- Clothing is changed when overtly contaminated.	( )	( )	
- Gloves are worn when handling infectious materials, infected animals, and when handling contaminated equipment.	( )	( )	
- Frequent changing of gloves accompanied by hand washing is established policy	( )	( )	
- Disposable gloves are not reused.	( )	( )	
- All manipulations of infectious materials, necropsy of infected animals, harvesting of tissues or fluids from infected animals or embryonated eggs, etc. are conducted in a Class III biological safety cabinet.	( )	( )	
- When a procedure or process cannot be conducted within a biological safety cabinet, then appropriate combinations of personal protective equipment (e.g. respirators, face shields) and physical containment devices (e.g. centrifuge safety cups or sealed rotors) are used.	( )	( )	

### Laboratory Facilities

- A working eyewash station is readily available inside BSL-3 suite	( )	( )	
- The laboratory is separated from areas that are open to unrestricted traffic flow within the building, and access to the laboratory is restricted.	( )	( )	
- Passage through a series of two self-closing doors is the basic requirement for entry into the laboratory from access corridors.	( )	( )	
- Electronic key card access system is functional.	( )	( )	
- A clothes-changing room is included in the passageway.	( )	( )	
- The BSL-3 suite contains a sink for hand washing.	( )	( )	
- The sink is hands-free or automatically operated and is located near the room exit door.	( )	( )	
- The sink drain is filled with disinfectant after each use.	( )	( )	

## Enclosure 3

Components	Yes	No	Comments
- The interior surfaces of walls, floors, and ceilings of areas where BSL-3 agents are handled are constructed for easy cleaning and decontamination.	( )	( )	
- Seams, if present, are sealed.	( )	( )	
- Penetrations in floors, walls, and ceiling surfaces are sealed.	( )	( )	
- Walls, ceilings, and floors are smooth, impermeable to liquids and resistant to the chemicals and disinfectants normally used in the laboratory.	( )	( )	
- Floors are monolithic and slip-resistant.	( )	( )	
- Openings such as around ducts and the spaces between doors and frames are sealed to facilitate decontamination.	( )	( )	
- Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and those chemicals used to decontaminate the work surfaces and equipment.	( )	( )	
- Laboratory furniture is capable of supporting anticipated loading and uses.	( )	( )	
- Spaces between benches, cabinets, and equipment are accessible for cleaning.	( )	( )	
- Chairs and other furniture used in laboratory work are covered with a non-fabric material that can be easily decontaminated.	( )	( )	

### Ventilation System

- A ducted exhaust air ventilation system is provided. This system creates directional airflow which draws air into the laboratory from "clean" areas and toward "contaminated" areas. The exhaust air is not re-circulated to any other area of the building.	( )	( )	
- The outside exhaust is dispersed away from occupied areas and air intakes, or the exhaust is HEPA-filtered.	( )	( )	
- Laboratory personnel verify that the direction of the airflow (into the laboratory) is proper.	( )	( )	
- A visual monitoring device that indicates and confirms directional inward airflow at the laboratory entry.	( )	( )	
- An HVAC control system is provided to prevent sustained positive pressurization of the laboratory.	( )	( )	
- Audible alarms notifying personnel of HVAC system failure are operational and tested.	( )	( )	
- Class III biological safety cabinets are used and directly connected to the supply system, in a manner that prevents positive pressurization of the cabinets.	( )	( )	
- Continuous flow centrifuges or other equipment that may produce aerosols are contained in devices that exhaust air through HEPA filters before discharge into the laboratory.	( )	( )	
- These HEPA systems are tested at least annually.	( )	( )	
- If vacuum service (i.e. central or local) is provided, each service connection should be fitted with liquid disinfectant traps and an in-line HEPA filter, placed as near as practicable to each use point or service cock.	( )	( )	
- Filters are installed to permit in-place decontamination and replacement.	( )	( )	
- Illumination is adequate from all activities, avoiding reflections and glare that could impede vision.	( )	( )	

### Enclosure 3

Components	Yes	No	Comments
- The completed Bio-safety suite design and operational procedures are documented. The suite was tested for verification that the design and operational parameters were met prior to operation.	( )	( )	
- Facilities are re-verified at least annually against these procedures as modified by operational experience.	( )	( )	



**BSL-3 SUITE SPECIFIC TRAINING CHECKLIST**

BSL-3 Suite Specific Training will include:

- Personal emergency procedures: personnel assistance alarms, communication equipment, fire alarms, emergency gas shutoff valves, fire extinguishers, and procedures during and after normal duty hours.
- Operations in Class III biological safety cabinets (BSC), i.e. cabinet function and procedures within the cabinets (for example, pipetting, disposal of infectious material, and clean-up).
- Use of syringes with infectious materials; the disposal of syringes, transport of syringes from cabinets to animals cages; how to expel air from loaded syringes or, when necessary, how to safely remove a needle from the syringe; and procedures for inoculating animals with infectious materials.
- Centrifugation procedures: use of sealed rotors and tubes, safety cups, evacuation of certain centrifuge chambers, and rotor-lifting devices.
- Use of disinfectant traps and HEPA filters on vacuum lines (central hose vacuum system, centrifuges, and portable vacuum pumps).
- Management of accidents (animal bites, self-inoculation with needles, infectious spills inside and outside biological safety cabinets, centrifuge accidents, etc.).
- Emergency response, training on the CDC related supplement to Instruction 3000, *Occupant Emergency Plan for the Uniformed Services University of the Health Sciences*.
- Autoclave operation and preventive maintenance.
- Use and operation of laminar flow animal housing systems.
- Handling, storage, and labeling of compressed gases.
- UV light pass-box procedures.



**BSL-3 USE APPLICATION FORM**

Revised 15 September 2003

INVESTIGATOR: \_\_\_\_\_ DEPT: \_\_\_\_\_

DATE: \_\_\_/\_\_\_/\_\_\_ PHONE: \_\_\_\_\_

PROTOCOL TITLE: \_\_\_\_\_

\_\_\_\_\_

PROTOCOL NO. \_\_\_\_\_ NEW RENEWAL CONVEYANCE

**LIST MEMBERS PERFORMING RESEARCH TO SUPPORT PROTOCOL:**

\_\_\_\_\_

\_\_\_\_\_

**SAFETY ASSURANCE:** A safety assurance will be provided by the EHS before a protocol is approved. This assurance will be primarily based upon safety training of the PI and a review of the PI's safety record. The PI is responsible for ensuring the training (to include site-specific training) of all working protocol members.

1. **WILL SCHEDULE 1 CONTROLLED SUBSTANCES BE USED?**  YES  NO  
If YES please indicate amounts \_\_\_\_\_
2. **WILL CLASS 4 (EXTREMELY HAZARDOUS) CHEMICALS BE USED?**  YES  NO  
If YES please indicate \_\_\_\_\_
3. **WILL CDC SELECT AGENTS BE USED?**  YES  NO  
If YES please indicate \_\_\_\_\_

**I WILL NEED TO USE THE BSL-3 SUITE FOR THIS RESEARCH AND UNDERSTAND THAT MY PROPOSAL WILL BE ROUTED TO THE BSL-3 OVERSIGHT COMMITTEE FOR REVIEW AND APPROVAL. USUHS INSTRUCTION 6403, "BIOHAZARD SUITE MANAGEMENT," PROVIDES THE REQUIREMENTS FOR THIS APPROVAL. NOTE: "BIOSAFETY IN MICROBIOLOGICAL & BIOMEDICAL LABORATORIES," 4<sup>TH</sup> EDITION, BY CDC, PROVIDES ADDITIONAL REQUIREMENTS.**

**4. STATE THE REASON WHY YOU ARE SEEKING ACCESS TO THE BSL-3 SUITE:**

BSL-3 AGENT USED IN PROTOCOL \_\_\_\_\_

OTHER \_\_\_\_\_

5. TRAINING

I AM FAMILIAR WITH THE USUHS INSTRUCTION 6403, "BIOHAZARD SUITE MANAGEMENT," AND TAKE RESPONSIBILITY FOR REVIEWING THESE INSTRUCTIONS, IN DETAIL, WITH ALL PROTOCOL MEMBERS.

I AM FAMILIAR WITH "BIOSAFETY IN MICROBIOLOGICAL & BIOMEDICAL LABORATORIES," 4<sup>TH</sup> EDITION BY CDC; I HAVE READ THE SECTIONS RELEVANT TO THE ANIMAL BIOSAFETY LEVEL 3 (ABSL-3) AND THE BIOHAZARDOUS AGENT USED IN MY PROTOCOL.

I HAVE COMPLETED/WILL COMPLETE ALL OF THE SITE-SPECIFIC TRAINING REQUIRED BY THE INSTRUCTION AND WILL ENSURE THAT ALL PROTOCOL MEMBERS RECEIVE TRAINING.

I HAVE COMPLETED/WILL COMPLETE ALL OF THE CDC SELECT AGENT TRAINING REQUIRED BY THE INSTRUCTION AND WILL ENSURE THAT ALL PROTOCOL MEMBERS RECEIVE TRAINING.

I HAVE CONTACTED/WILL CONTACT EHS, AND I WILL ENSURE THAT EHS SPONSORED BASIC BIOLOGICAL AND CHEMICAL SAFETY TRAINING IS COMPLETED, AND THAT ALL PROTOCOL MEMBERS RECEIVE TRAINING.

I HAVE CONTACTED/WILL CONTACT EHS AND WILL ENSURE THAT EHS SPONSORED MEDICAL SURVEILLANCE AND IMMUNIZATION IS COMPLETED FOR ALL PROTOCOL MEMBERS.

I HAVE REVIEWED THE USUHS BIOHAZARD SUITE USERS' GUIDELINES (ENCLOSURE 6 OF INSTRUCTION 3000); I WILL ENSURE THAT ALL PROTOCOL MEMBERS DO LIKEWISE, AND THAT ALL QUESTIONS ARE ADDRESSED.

I WILL ENSURE THAT PROTOCOL MEMBERS RECEIVE AGENT-SPECIFIC BIOLOGICAL/ CHEMICAL HAZARD TRAINING PRIOR TO WORKING IN THE BIOHAZARD SUITE.

I AM FAMILIAR WITH USUHS INSTRUCTION 6408, "CDC SELECT BIOLOGICAL AGENTS MANAGEMENT," AND TAKE RESPONSIBILITY FOR REVIEWING THIS INSTRUCTION, IN DETAIL, WITH ALL PROTOCOL MEMBERS.

I AM FAMILIAR WITH USUHS INSTRUCTION 3000, "OCCUPANT EMERGENCY PLAN FOR THE UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES," AND THE SUPPLEMENT FOR ADDRESSING CDC SELECT AGENTS, AND TAKE RESPONSIBILITY FOR REVIEWING THESE INSTRUCTIONS, IN DETAIL, WITH ALL PROTOCOL MEMBERS.

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Principal Investigator Signature & Date

## BIOHAZARD SUITE USER'S GUIDE

### 1. Approval and Responsibility.

a. Use of the BSL-3 suite must be reviewed in advance by the BSL-3 Oversight Committee following written application by the principal investigator (PI) (see Enclosure 5). Following approval, the PI and listed researchers are considered authorized users, contingent on completion of applicable training. Only work to be accomplished under an approved protocol may be performed in the BSL-3 suite. Project prioritization will be at the discretion of the Biohazard Oversight Committee with consideration to the following: (1) current research commitments of the suite; (2) faculty status; (3) funding; and, (4) nature of research including biological agents used, protocol requirements, training needs, equipment needs, etc., based on the biohazards involved.

b. The principal investigator (PI) is responsible for all procedures performed in the biohazard suite. He/she must ensure appropriate training of all personnel and be responsible for the supervision of all work performed under the protocol.

c. Improper use of the suite may result in loss of permission to use the suite.

### 2. Entry and Exit Procedures.

a. Only personnel with approved access (issued access cards) will be allowed to enter the suite unescorted. Escorted personnel will sign-in and sign-out of the suite indicating their escort on the log.

b. Directional airflow and relative pressure for each area must be observed and recorded prior to entry. If any discrepancies are observed, entry must not be attempted until EHS is notified and provides clearance.

c. Enter and exit via the door marked "Personnel Access Only." The other door (marked with appropriate signage) is to be used only for moving equipment into or out of the

suite (under EHS supervision only, or as an emergency exit).

d. Disposable wrap-around gowns, surgical masks, gloves and hair covers must be worn while working in the suite and must be supplied by each investigator. When leaving the suite, place these disposable garments in a biohazard bag in the air lock. This bag will then be autoclaved and discarded by the next person who uses the suite during his/her regular decontamination after work. Additional personal protective equipment such as eye protection or respirators will be worn if required by the particular protocol.

e. Each investigator must provide an appropriate disinfectant for the agents he/she is using.

f. Exit showers are not required, but hands will be washed with soap and water prior to leaving the suite.

g. A spare set of clothing (i.e., surgical greens) will be kept available in the biohazard suite in case of accidental contamination of personal clothing.

h. Should such a contamination occur, follow the posted spill and decontamination procedures, and consult the PI and EHS.

i. Essential notebooks are allowed in the common area and cubicles. Unnecessary papers and books are NOT to be brought into the biohazard suite because these are difficult to decontaminate. (Instead, workers may be advised to e-mail the notes to the main lab outside the BSL-3 using a computer provided in the BSL-3 suite.) They may be decontaminated with the ethylene oxide sterilizer.

### 3. Design of Biohazard Suite, Safety Alarms and Emergency Procedures.

a. The major barrier protecting the hallway from hazards from the suite is a negative air pressure within the suite maintained by exhaust fans on the roof. Air

flows from the corridors through the air lock, through the common area and out through each of the three cubicles through a HEPA filter. Each biohazard hood has a separate alarm should the air exhaust be inadequate to provide safe use.

b. Alarms: There are two alarm boxes in the common area. These include alarms for the exhaust fan of each of the biohazard hoods. In case the alarm is activated, the ringing can be stopped by pressing the button. However, all work will be terminated immediately and all persons in the suite will leave the potentially contaminated area immediately. Call EHS from the common area and begin emergency procedures if applicable.

c. Emergency Procedures:

(1) Detailed emergency procedures are available in the Memorandum SUBJECT: Instruction 3000: *Supplemental Addition to the Occupant Emergency Plan for the Uniformed Services University of the Health Sciences (USUHS)*, for operations involving CDC Select Agents.

(2) If a biohazard spill occurs, follow the procedures listed in Enclosure 8 of the above memorandum. Detailed procedures should be posted in lab. For additional assistance contact EHS from the common area of the BSL-3 suite.

(3) If a chemical spill occurs, safety materials will be available within each laboratory.

(4) If a power failure occurs, the biohazard suite is on emergency power and will function normally during power blackouts.

(5) If a steam failure occurs, the biohazard suite cannot be used because the autoclave will be inoperative. (Historically there have been many brief steam outages. It is necessary to close the suite only if it is reasonably expected that the steam will be out for an extended period of time (e.g. 24 hours).

(6) If a water failure occurs, (i.e. shutdown of city water) the suite cannot be used since eyewash stations and the sink will

not be available during a possible spill or exposure.

(7) If a fire occurs, there is a fire extinguisher in the suite. Call the Fire Department by telephone if the fire can't be extinguished by using a fire extinguisher.

d. Emergency procedures for the Security Department, and others, when users are not present in the suite:

(1) EHS will be notified at any time a malfunction is noticed in the biohazard suite. Initial entry to the suite shall be by a responsible researcher or a member of EHS. Other personnel shall not be permitted in the biohazard suite until clearance is obtained from EHS. Security maintains a contact roster for EHS personnel. Responsible researchers for each experiment will also be available after normal duty hours; and permission for others to enter the suite may require consultation and concurrence among them and the EHS representative(s).

(2) In the event that the responsible researcher cannot be contacted, the Chair, Microbiology and Immunology Department will be consulted.

(3) If the malfunction has a potential impact on any animals housed in the suite, the veterinarian on call should be contacted. The security desk maintains a list of telephone and pager numbers for the on-call veterinarian and animal technician.

4. Laboratory Procedures in the Suite.

a. Safety procedures. Each user is responsible for leaving the suite in a safe condition for the next user. Cleaning crews and workmen will be able to work safely in the area after completion of each laboratory procedure. Therefore, each user must decontaminate the inside of the biohazard hood, and any areas that may have been contaminated, with a disinfectant appropriate for his/her hazardous agent. All contaminated material must be autoclaved before leaving the biohazard suite.

All decontaminated material must be properly processed and logged. The user is responsible for removing this material from the autoclave and discarding it promptly. Each user will include the biohazard bag containing contaminated clothing from previous users, which will be found in the entryway.

b. Supplies and storage. There is little or no storage space in the biohazard suite, so investigators must minimize storage in the suite and bring with them the supplies which they expect to use. This includes disposable safety clothing, plastic ware, pipettes, slides, etc.

c. Transport and storage of infectious organisms.

(1) Glassware containing infectious liquids must be stored and transported in leak-proof (e.g. polyethylene) containers large enough to contain the fluid in case of breakage or leakage and with sufficient absorbent material surrounding the glass container.

(2) Infectious materials from the incubator or refrigerator must be marked with the user's name, date, and the hazard. In the refrigerator and incubator, all infectious materials must be in containers or trays capable of containing the fluid in case of breakage or spilling. All samples of potentially viable VEE virus will be stored in screw cap tubes.

(3) Infectious agents may be removed from the suite (when permitted by protocol) following surface decontamination of the vessel with disinfectant. Hazardous agents which must be stored at -70 degrees will be stored in containers in ultra-cold freezers in the users department with appropriate labeling. There is also an ultra-cold freezer in the suite. 20-degree compartments for the refrigerators are available in the suite.

d. General laboratory procedures.

(1) All work with infectious substances will be done in the biohazard hoods. No work will be conducted in open vessels on the bench top. (At times, work must be done in closed

vessels on the bench top, e.g., microscopic visualization.)

(2) Disposable gloves must be worn when handling infected materials. A suitable disinfectant, clearly labeled, must be available for surface decontamination of gloves and hands prior to removal from the safety cabinet.

(3) Acceptable microbiological techniques must be used at all times.

(4) Care will be taken to avoid or contain procedures that generate small particle aerosols. These include:

(a) High-speed blending, mixing and grinding.

(b) Agitation.

(c) Centrifugation with non-sealed table top centrifuges.

(d) Sonication.

(e) Opening vials of lyophilized cultures.

All of these above stated procedures will be done in the biohazard hood.

(5) Hands must be kept from the mouth, nose, eyes and face to minimize self-inoculation.

(6) Doors must be closed at all times when procedures are being done.

(7) Disposable items will be used whenever possible. Sharply pointed instruments such as needles, scissors, and glassware, which can be broken, will be replaced wherever possible by blunted needles, scissors or unbreakable equipment.

(8) Before opening lyophilized culture vials, wrap them in disinfectant wetted gauze.

(9) Decontaminate all work surfaces with the appropriate disinfectant following your laboratory work after any spill or potential release of infectious material.

e. Pipetting.

(1) No mouth pipetting is permitted at USUHS. Use pipette aids. If these become contaminated, it is the responsibility of the individual who caused the contaminant to decontaminate them.

(2) Use automatic pipetters. Do not use blowout pipettes or mix infectious materials by pipetting. (This creates aerosols.) Use of this type of pipetting must be restricted to inside hoods.

(3) All pipettes used for pipetting infectious or toxic materials must be plugged.

f. Syringes.

(1) Needle-locking hypodermic syringes and blunt-ended needles will be used with infectious materials whenever possible.

(2) Do not use syringes for making dilutions if other methods such as pipettes or bio-pipettes can be used for making dilutions, tissue culture inoculations, etc.

(3) When removing a needle from a rubber-stoppered bottle, wrap the needle and stopper in an alcohol-soaked pledgette to prevent aerosol formation.

(4) Self-retracting syringe/needle combination units will be used whenever possible for injections into animals.

g. Centrifuges.

(1) Tabletop centrifuges must be used in the biohazard hood. This has been tested, and has been found to be a safe procedure.

(2) Before centrifuging, check tubes for cracks and inspect the side of the trunnion cup for rough walls or bits of glass. To balance tubes, place a germicidal solution between the tube and the trunnion cup. Discard this balancing solution and rinse the trunnion cups at the end of the procedure.

(3) Avoid decanting centrifuge tubes. If absolutely necessary, wipe the outer rim with a disinfectant. Otherwise, when centrifuged, the drops of infectious material will spin off as an aerosol. Avoid filling the tubes to the point where the rim becomes wet with culture. Never overfill tubes intended for angle rotors to the point where the fluid will reach the rim of the tube under horizontal centrifuge force.

(4) All persons must be instructed in the operation, safety and maintenance of centrifuges prior to being authorized to use this

equipment. This is the direct responsibility of the supervising professional staff member.

(5) Wipe the inside of the centrifuge with disinfectant if there is any possibility of contamination.

(6) Ultra-centrifugation. Rotors for ultra-centrifugation may be brought from the departments into the suite and used with the precautions described above.

(7) Rotors must be decontaminated before leaving the suite. The ethylene oxide sterilizer is available for this purpose.

h. Radioisotopes.

Radioactive materials may be used in the suite following approval by the Radiation Safety Committee and establishment of safe disposal procedures for contaminated radioactive waste. Autoclaving contaminated radioactive material may be considered and accomplished after evaluation by, and approval of the Radiation Safety Officer.

i. Weighing.

When weighing out hazardous material in the biohazard hood, wear a mask and gloves. Wash the balance after completion of the weighing.

j. Incubator.

(1) The incubator in each cubicle is reserved for work done in that cubicle to prevent possible cross-contamination of agents and cultures.

(2) All plates or bottles in the incubator will be on trays containing paper, which will absorb spills if they occur. If spilling of contaminated material inside the incubator occurs, the investigator is responsible for decontamination in collaboration with EHS.

k. Refrigerator/Freezer.

(1) Refrigerator/freezer space in the suite is limited. Therefore, it is necessary that they be used only for short-term storage of materials with the exception of CDC Select Agents. Refrigerators or freezers containing CDC Select Agents will be secured (locked) with strict key control maintained. Any bio-hazardous material placed in the refrigerator or freezer

must be clearly labeled with the investigator's name, date, and agent, in a container, which could contain the material if it is spilled.

(2) Refrigerators and freezers will be cleared out periodically by participating researchers. Unlabeled material will be discarded.

**l. Sonication.**

(1) A sonicator is provided. This must be used in the biohazard hood. Ear protectors must be worn during sonication procedures.

(2) The sonicator can be surface-decontaminated with appropriate disinfectant and rinsed with water to prevent pitting.

(3) Sonication will be done in closed vessels whenever possible.

**m. Refractometer and spectrophotometer.**

(1) These instruments can be used in the biohazard suite. The cuvettes must be provided by each investigator. Cuvette holders which are potentially contaminated must be decontaminated by each investigator. All cuvettes containing hazardous material must be closed during operation.

(2) The refractometer can be used with small volumes (10 ml) of hazardous agent samples. Surfaces in contact with the contaminated agent must be swabbed with disinfectant and alcohol at the end of the procedure.

**n. Autoclave.**

(1) A steam autoclave has been provided and instructions for its use are posted. At the end of each laboratory procedure, (or at a minimum, the end of the workday) all contaminated material will be placed in trays and autoclaved. The following procedures are to be employed:

a. Syringes and needles must be placed in puncture-resistant cardboard boxes in biohazard bags.

b. Paper, plastic and disposable materials must be placed in biohazard containment bags.

c. Glassware must be submerged in

water containing disinfectants before it is autoclaved.

(2) Each investigator must autoclave his/her materials before leaving the suite. If the autoclave is already in use, all contaminated materials must be in closed containers labeled with the investigator's name while waiting to be autoclaved. The investigator must return promptly to autoclave the materials.

(3) Following decontamination, materials are to be removed promptly from the autoclave via the door in the glassware washing room adjacent to the biohazard suite. The investigator is responsible for making sure that the floor of the sterilizer is clean. No broken glassware, melted plastic or other materials will be left in the autoclave. Each load of processed material must be logged in.

(4) After autoclaving, materials can be disposed of by departmental personnel in the usual manner. This includes boxing in biohazard waste boxes at the present time.

(5) To prevent explosions, dry hypochlorides (bleach) or strong oxidizing materials must never be autoclaved with organic materials like paper, cloth, oil, etc.

(6) Cellulose nitrate ultracentrifuge tubes will be chemically decontaminated and are not to be autoclaved.

**o. Animals.**

(1) Should use of animals within the suite be required, this must be reviewed by the BSL-3 Suite Oversight Committee and approved by the Director, Department of Laboratory Animal Medicine (LAM).

(2) Rodents must be housed in microisolator cage units and cage units only opened in the biosafety cabinet.

(3) All animal manipulations will be performed in a biosafety cabinet.

**5. Accident, First Aid and Illness.**

a. It is the responsibility of each employee to notify his/her supervisor of any personal condition, which might prevent him/her from working safely in the suite. If

appropriate, refer to EHS for medical clearance to continue work in the suite. Responsibilities include:

- (1) Recent surgical procedures.
- (2) Cuts and abrasions interfering with the normal integrity of the skin.
- (3) Treatment with immuno-suppressive drugs.
- (4) Pregnancy.

b. Accidental exposure to infectious organisms.

(1) An employee who is accidentally exposed to an infectious agent will report immediately to his/her supervisor. Each PI should have an SOP that assists workers in determining when they can address spills, and when they should refer to EHS. If the agent enters the mouth, eyes, lungs, or penetrates or comes in contact with the skin, the supervisor will direct disinfecting procedures and see that the employee reports without delay to the Occupational Health Clinic. (Call 295-3305 or call EHS Emergency 295-3320.) This includes all animal bites and scratches.

(2) In case of a microbiological accident outside a safety cabinet such as a spill, follow the posted spill procedures. At a minimum:

- (a) If possible, contain the spill with containment equipment under the sink and disinfect.
- (b) Remove contaminated clothing, and place in the biosafety hood.
- (c) Don emergency clothing maintained in the laboratory.
- (d) Report the spill to the supervisor and EHS.
- (e) The individual(s) who clean(s) the room will wait 20 minutes after the spill to enter wearing protective clothing including surgical gowns, shoe protectors, face masks or respirators and rubber gloves.
- (f) Disinfectant will be applied liberally and allowed to soak for 20 minutes or more before other final clean-up measures are

implemented as directed by EHS.

c. First Aid.

(1) If an individual is injured in the suite he/she will:

- (a) Thoroughly clean the wound with soap and water.
- (b) Notify his/her supervisor of the injury.
- (c) Report the injury to EHS so that the proper treatment can be initiated quickly.

(d) If directed, leave the suite and get treatment at the Emergency Room of the hospital.

(2) Medical care will be provided. Emergent care shall take precedence over biohazard, decontamination, or administrative concerns.

(3) All potential exposures involving infectious materials will be promptly reported to the responsible supervisor and EHS. EHS will institute appropriate actions to obtain baseline and additional samples for laboratory diagnosis of possible resulting infection.

d. Animal bites.

(1) Use of animals in the suite requires advance approval by the BSL-3 Oversight Committee and the Director, LAM.

(2) All animal bites or scratches from animals infected with infectious materials will be reported promptly to the responsible supervisor and EHS.

(3) EHS will institute appropriate actions to obtain baseline and additional samples for laboratory diagnosis of possible resulting infection. If an individual is bitten by a contaminated animal, he/she will be sent to the Emergency Room at the hospital for examination and treatment, and an Animal Bite Report will be prepared.

(4) The Director of LAM will be notified so that an evaluation can be made if there are any additional zoonotic diseases of concern.

e. Suspected occupational illness.

(1) Civilian employees who become sick (e.g., unexplained elevated temperature) with a suspected occupational illness, on duty will report to their supervisor and EHS. If an occupational illness is suspected, CA Form 16 and CA Form 2 must be prepared.

(2) Civilian employees who become sick while off duty with a suspected occupational illness must notify their supervisor and EHS. They may then report to the Emergency Room of the hospital or to their own physician.

(3) Military employees who becomesick while on or off duty will report to their supervisor and EHS and go immediately to the Emergency Room.

(4) Illness following special immunizations for biohazards work will be reported to the supervisor and EHS if it occurs following occupational immunization. Form CA 1 will be required. Illnesses after the first 12 days are not considered part of the immunization reaction.



## **SPECIFIC SAFETY ITEMS RELATED TO VENEZUELAN EQUINE ENCEPHALITIS (VEE)**

### **I. Venezuelan Equine Encephalitis Virus.**

VEE is an alphavirus that produces an often sub-clinical viral “flu” syndrome with fever, chills, headache, nausea, vomiting, as well as pharyngeal and conjunctival injection. Infections are commonly mild and only rarely involve encephalitis.

### **II. Transmission:**

A. Bite from infected mosquito (in most cases worldwide).

B. Infections from laboratory aerosols are common. There have been approximately 150 reported laboratory infections to date with one reported fatality.

C. Infection from needle stick injury possible while injecting VEE into animals.

D. Although viral particles have been isolated from infected animal blood and exudates, no confirmed cases of animal-to-human transmission have been documented. Likewise, no human-to-human transmission has been demonstrated, although it is theoretically possible.

### **III. Medical**

A. Infectious Dose: 1 viral unit.

B. Incubation Period: usually 2-6 days.

C. Clinical Manifestations - Mild viral syndrome lasting 3-5 days post inoculation. Symptoms include biphasic fever, severe headache, chills, myalgia, retro-orbital pain, nausea, vomiting, conjunctival and pharyngeal injection. In some cases CNS involvement occurs. Encephalitis is an uncommon complication that can lead to disorientation, convulsions, paralysis, coma, and/or death.

D. Therapy.

1. Supportive.

2. Currently there is no available drug therapy for VEE.

E. Prophylaxis - TC-83 is a currently available live-attenuated virus vaccine. Personnel determined by the command to meet the requirements in Enclosure (8) will be eligible to receive TC-83 and will do so at the command's discretion.

### **IV. Susceptibility**

A. Drugs – no known susceptibility to drug therapy.

B. Disinfectants – susceptible to 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, formaldehyde.

C. Physical inactivation – moist and dry heat; desiccation.

D. Viability outside host:

1. Has been isolated from pharyngeal secretions and is stable in aerosols.
2. Stable in dried blood and exudates.

**V. Special Precautions.**

A. Containment.

1. BSL-3 standard and special precautions IAW BMBL 4<sup>th</sup> Ed.
2. Vaccination (TC-83) IAW Enclosure (8).
3. BSL-3 certified cabinets and facilities design to include HEPA filtration of exhaust air prior to discharge.

B. Personal Protective Equipment (PPE).

1. At a minimum:
  - a) Power Air-Purifying Respirator (PAPR).
  - b) Tyvek suit.
  - c) Shoe covers.
  - d) Protective gloves.
2. Recommended: TC-83 vaccination.

C. Handling.

1. For the handling of specimens during routine research please refer to Enclosure 6, Section 4.

2. Spills.

(a) Spills inside biological safety cabinet. The occurrence of a spill in a biological safety cabinet poses less of a problem than a spill in an open laboratory as long as the spilled materials is contained in the biological safety cabinet. A Biosafety Cabinet is designed to contain spills and associated aerosols that are released during work within the cabinet. Provided that the Biosafety Cabinet is operating properly and has been inspected and certified, aerosols produced by a spill should be contained. A spill of a bio-hazardous material should be attended to immediately. All workers using the Biosafety Cabinets should have a supply of absorbent materials and decontaminating agent within the cabinet. This avoids the need to withdraw your arms from within the cabinet should a spill occur and allows you to decontaminate yourself prior to leaving the cabinet. Decontamination of the work zone can usually be accomplished by direct application of concentrated liquid disinfectants along with a thorough wipe down procedure. Formaldehyde gas decontamination may be required to treat inaccessible sections of the cabinet interior following a spill. Contact EHS after a major spill to determine the need for decontamination with formaldehyde. In general one should:

- (1) Alert people in the immediate area of the spill.

(2) Chemical decontamination procedures should be initiated immediately while the biological safety cabinet continues to operate.

(3) Wear gloves during the decontamination procedure. The spill should be covered with paper towels or other absorbent materials and the area should be soaked with a proven decontamination agent (e.g., Lysol Disinfectant spray, 1:10 dilution of Clorox containing sodium hypochlorite [NaOCl] or 70% ethanol) for 30 minutes.

(4) Use paper towels to wipe up the spill, working from the edges into the center.

(5) Decontaminate equipment and utensils. Items that are not readily or easily surface decontaminated should be carefully placed into autoclave bags and removed for further treatment (e.g., decontamination by autoclaving or other approved methods).

(6) Contaminated gloves and clothes (your sleeves are most likely contaminated and, if disposable sleeves are used, they should be removed after decontamination is complete, or remove and decontaminate the lab coat or Tyvek suit by autoclaving or soaking in decontaminant).

(7) Remove protective gear. Individuals involved in the spill and clean-up should remove protective clothing (either disposing as bio-hazardous waste or decontaminating), wash their hands and face with an appropriate decontamination soap, and report to the USU Occupational Health Nurse or Family Health Clinic for any required evaluation or follow-up.

(b) Spills outside biological safety cabinet. Biological spills outside biological safety cabinets will generate aerosols that can be dispersed in the air throughout the laboratory. Appropriate protective equipment is particularly important.

(1) Minor Spills (less than 10 ml and generating little aerosol) on equipment, laboratory benches, walls, or floors:

a) Close laboratory doors and post warning signs to prevent others from entering the laboratory.

b) Thoroughly wash hands and other apparently contaminated areas with soap and water. Put on clean disposable gloves.

c) Cover the spill area with paper towels soaked in appropriate decontamination solution such as 1% sodium hypochlorite or 70% ethanol solution.

d) Wipe up the spill with the soaked paper towels and place the used towels in an autoclave pan and autoclave.

e) Pour decontaminating solution around and on the area of the spill. Let stand for 30 minutes then wipe up with paper towels. Place gloves and paper towels in autoclave pan and autoclave.

f) Wash hands and other apparently contaminated areas again with soap and water.

g) Remove all PPE immediately upon leaving the work area and as soon as possible if overtly contaminated. Contaminated PPE will be DISPOSED of as bio-hazardous waste or decontaminated.

(2) Major Spills (more than 10 ml or with considerable aerosol):

a) Close laboratory doors and post warning signs to prevent others from entering the laboratory.

b) Leave the biological safety cabinet operating and cultures inside cabinet.

c) Wash hands and other apparently contaminated areas with soap and water.

d) Report the accident to the Principal Investigator and to the EHS at 295-9443.

e) If personal clothing is contaminated, remove all outer clothing and place it in the autoclave or container for autoclaving. Put on clean garments.

f) Leave the laboratory for 20 minutes to allow dissipation of aerosols created by the spill.

g) Upon returning to the laboratory to start decontamination, check to see if laboratory doors are closed and appropriate signs are displayed. Put on gloves and other protective equipment as previously mentioned above.

h) Pour a decontamination solution around the spill and allow this solution to flow into the spill. Paper towels soaked with decontamination solution may be used to cover the area. Do not pour decontamination solution directly onto the spill in order to avoid additional release of aerosols.

i) Let decontamination solution/microorganism mixture stand for 30 minutes or longer to allow adequate contact time.

j) Transfer all contaminated materials to autoclave pan, cover with suitable cover, and autoclave according to standard directions.

k) Remove gloves and other contaminated garments and place them in an autoclave container for autoclaving.

l) Thoroughly wash hands, face, and other apparently contaminated areas.

m) Special care in decontamination may be necessary. The Principal Investigator and/or the Biosafety Officer may require the collection of sample cultures to determine that the area has been effectively decontaminated.

3. Disposal of VEE waste will be done in accordance with Enclosure (6), Section 4.a. All infectious waste will be autoclaved according to the following schedule:

- a. Solid and Liquid Waste – 1 hour.
- b. Infected Animal Carcasses – 8 hour.

4. Surface Decontamination Procedure. After any procedure using VEE virus the following surfaces will be disinfected with 70% EtOH or an appropriate disinfectant spray:

- a. Biohazard hood.
- b. Sink area and bench top.
- c. Freezer handles.
- d. Microscope.
- e. Refrigerator handles.
- f. Incubator handles.
- g. Door knobs.
- h. Small equipment (pipetters, etc).
- i. Any other surface or object that might have been touched or handled.

5. Storage of VEE specimens will be done in accordance with Enclosure 6, sections 4 b-c.

**VEE (TC-83) VACCINATION SELECTION CRITERIA**

References: (a) USAMRIID memo dated 14 August 2003, RE: SIP at USAMRIID  
(b) BMBL (4<sup>th</sup> Ed.), May 1999, pp. 189-199

1. VEE is a zoonotic arbovirus, which has been known to cross-infect humans. There is no record of person-to-person transmission but there have been 150 documented occurrences in humans with one fatality. Many of those cases were laboratory-associated infections. Given the potential for morbidity and mortality associated with VEE laboratory work and based on the recommendations for vaccination in Reference (b), the following are the recommended criteria for vaccination of personnel who work with VEE at USUHS.

2. Personnel recommended for vaccination are those who do not demonstrated an antibody titer to VEE on testing and who will in the course of their regular duties:

- a. Be potentially exposed to aerosols that may contain VEE particulates.
- b. Manually manipulate open or closed vessels containing VEE viral solutions regardless of concentration.
- c. Come into physical contact with animal bedding related to VEE work.
- d. Manipulate VEE infected laboratory animals.
- e. Come into contact with potentially infected animal blood (dried or moist) and/or exudates.
- f. Manipulate syringes or other “sharps” used in VEE work.

3. Additionally, personnel receiving TC-83 vaccination must not:

- a. Be immune-compromised.
- b. Have a history or family history of diabetes or glucose intolerance.
- c. Have received any other viral vaccination within 30 days of being vaccinated with TC-83.

Maintenance and housecleaning personnel are not recommended for vaccination since PPE is sufficient protection for their work in the laboratory when no VEE bench work is occurring.



**USUHS  
WORKPLACE HAZARD ANALYSIS**

NAME OF PARTICIPANT: \_\_\_\_\_

<b>Description of Task to be performed by Participant</b>	<b>Potential Safety or Health Hazard(s) *</b>	<b>Recommended Precautions</b>	<b>Equipment to be Used (Respirator, Protective Gloves, Bio-Safety Cabs, Blue Suits)</b>

Analyzed and prepared by: \_\_\_\_\_  
Principal Investigator

Date: \_\_\_\_\_

Reviewed by: \_\_\_\_\_  
Chairman, BSL-3 Committee

Date: \_\_\_\_\_

Approved by: \_\_\_\_\_  
Responsible Official, Select Agents Program

Date: \_\_\_\_\_