

# **Biological Safety**

## **BSL3 Laboratory**

### **Manual**





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## **Section 1 Responsibilities**

### **1.1 Department Chair**

Department Chair bears overall responsibility for implementation and maintenance of safe practices and procedures in their department. Department Chair, especially in the case of large departments, may share this responsibility with a departmental biological safety committee and/ or a unit director.

### **1.2 Principal Investigator**

The Principal Investigator (PI) has the responsibility, authority and support for assessing risks, establishing policies and procedures, training personnel and maintaining the facility and equipment.

The Principal Investigator performs appropriate risk assessment of research projects. The level of detail should be dependent on the hazard associated with the organism under study (e.g., an assessment of risk associated with research on BSL2 agents might reasonably be less detailed than a risk assessment of BSL3 or unknown agents). Each evaluation should be completed before work is undertaken and the project should be reassessed periodically as new data are obtained. The assessment should include an analysis of the risks posed by the organism under investigation and by any specific research methods that may affect that risk (e.g., procedures requiring highly concentrated amounts of virus or inoculation of laboratory animals). No human or animal pathogen should be studied without prior written approval of the Biological Safety Committee. The procedures for handling unclassified agents should be addressed by the Biological Safety Committee, the Office of Environmental Health and Safety (EHS), and the related departments. The agents must be registered and information about these agents must be provided to the Office of Environmental Health and Safety (EHS).

Principal Investigators are responsible for the application of appropriate safety practices and procedures within their laboratories and instructing students and staff of potential hazards.

The Principal Investigator also approves research personnel to work in the laboratory and assures that personnel are competent to conduct the work. Review and document once a year BSL3 responsibilities with research personnel. The review should include the following:

- Review signs and labels.
- Methods for recognizing tasks that may involve exposure.
- Review the use and limitations of engineering controls (e.g. review centrifugation protocols).
- Discuss autoclave function and proper procedure for the autoclaving of waste.
- Demonstrate work within a biological safety cabinet (BSC) with props to show safe work practices and how contaminated items are to be discarded.
- Review the use and limitations of safe work practices which all users of the facility are expected to follow.
- Review the use and limitations of PPE including types, location, removal, handling, decontamination and disposal. If applicable, include the use of purified air power respirator (PAPR) or disposal N95 respirators.
- Review and discuss spill procedures.
- Review exposure incident procedures.
- Walk-through of the facility showing the sign in/out procedure, donning and removing PPE, and location of all alarms as well as their meanings.
- Opportunity for questions.

Ensure that all employees working with BSL2+ and BSL3 agents receive specific training and demonstrate technical proficiency working with these agents in culture.

Develops policies governing the operation of the laboratory and implements protocols to ensure safe operation.

Complete and revise the Standard Operating Procedures for BSL3 Research Laboratories (see Section 10.4 and 10.5).

Maintain liaison with the maintenance staff and the EHS-Biosafety Office.

BSL3 research involving recombinant DNA must be registered with the Biological Safety Committee. The Principal Investigator must complete the "Registration of Experiments Involving Recombinant or Synthetic Nucleic Acid Molecules" application form. The application must have the details of the nature of the proposed experiment and an assessment of the levels of physical and biological containment required for them as established by the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids (NIH Guidelines). Note that NIH and Yale University requires registration and approval of any recombinant DNA research involving genetic material from BSL3 or BL4 agents (RG3/RG4).

### **1.3 Research Personnel**

All research personnel engaged in BSL2+ or BSL3 research must complete the requirements detailed in section 11 on BSL3 personnel training for approval to work independently in the laboratory. Research personnel must ensure that all work is conducted in compliance with NIH, CDC, OSHA and other applicable guidelines. Follow the Yale University Biological Safety Manual except where superseded by the BSL3 Manual. Follow all procedures outlined in the BSL3 Manual.

Learn the operating procedures for the laboratory, the potential hazards of the infectious agents in use and emergency procedures. Help maintain the facility in good working condition.

Report to the Principal Investigator any medical restrictions, reportable illnesses, and any event that may be an exposure or result in the creation of a potential hazard. Any irregular laboratory conditions or accidents must be reported immediately to the PI and EHS.

If inexperienced in handling human pathogens or tissue cultures, receive training and demonstrate proficiency in standard microbiological practices from the Principal Investigator.

Complete the medical surveillance requirements.

Perform assigned responsibilities. The operation of the facility is the responsibility of the users; therefore a number of tasks must be assigned. These tasks are as follows:

- Training new research personnel and visitors.
- Autoclaves and waste: Assume responsibility for autoclaving and decontaminating biological waste. Maintain autoclave log sheets to document destruction of each waste treatment cycle.
- Freezers: Ensure identity of materials. Remove damaged and unwanted materials from freezers. Maintain freezers in a clean and orderly fashion. Keep an inventory log of agent and materials, included the amounts in storage, owner and location within freezer.
- Cleaning: maintain the lab as a clean and uncluttered research environment.
- Vacuum trap and filter maintenance. Set up vacuum system protection filters and replace when needed. See section 3.5 for additional information on vacuum line traps and filter systems.



- Supplies: Maintenance of supplies, including personnel protective equipment
- Log Book: Maintenance of the BSL3 log book where incidents, shipping and receiving of infectious materials, repairs, etc., are recorded
- Entry/Exit Logs: Maintain authorized researcher and visitors entry/exit logs.
- Visitors: Ensure visitors are accompanied and complete the Biosafety Level 3 Visitor Clearance Form, see section 12.3.

## **1.4 Environmental Health and Safety (EHS)**

The Office of Environmental Health and Safety (EHS) provide consultation on operation of the BSL3 laboratory to ensure compliance with CDC/NIH, OSHA, USDA and state criteria. EHS provides information on regulations that apply to the laboratory.

EHS will perform BSL3 and all other applicable lab safety training for new personnel.

EHS will advise on safe methods for new procedures, and will provide assistance in the event of a biohazardous material spill.

EHS will inspect BSL3 labs semi-annually to ensure continued compliance with applicable safety regulations.

The Biological Safety Officer (BSO) is responsible for the implementation of policy guidelines recommended by the Institutional Biological Safety Committee (IBC). The BSO identifies potential problem areas and suggests to the IBC safety objectives to be achieved. In addition, the BSO is also the Institutional Biological Safety Officer for recombinant DNA research. Some of the specific biological safety services provided by the EHS include:

- Evaluation and inspection of laboratory facilities for work with infectious agents.
- Investigation of laboratory accidents and if needed, develop and communicate written preventive measures.
- Consultation to members of the Yale community in matters related to biological safety.
- Periodically survey Yale University's research labs to identify potential work with biohazards.
- Dissemination of information pertaining to safety in biological research through a periodic newsletter, lab inspections, demonstration or special training course as may be necessary.

## **1.5 Biological Safety Committee**

The Biological Safety Committee serves as the Yale Institutional Biosafety Committee (IBC) in compliance with the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids (NIH Guidelines).

The committee's BSL3 responsibilities include:

- Registration of laboratories working with BSL3 agents (see section 13.6 for a list of agents.).
- Registers laboratories and approves containment measures and procedures to be used.
- Advises facility users on policies related to BSL3 containment.
- In conjunction with EHS, develop BSL3 emergency response procedures.
- Periodically update laboratory registrations.
- In conjunction with Employee Health, determine the necessity for special medical monitoring.

- Advises Yale administration on the suspension of access privileges for staff found to be in violation of policies and procedures governing facility use.

It is the responsibility of the Biological Safety Committee to recommend to the President and Provost pertinent safety guidelines relating to procedures and facilities used in biological research.

## **Section 2 Medical Surveillance Program**

### **2.1 Medical Surveillance**

A medical surveillance program of University personnel engaged in biological research is conducted by Employee Health at the Yale Health Center located at 55 Lock Street. The purpose of the program is to conduct periodic health assessments of employees with attention devoted to factors or conditions associated with a particular biological agent a given individual might handle. For a particular employee, the medical surveillance program might well call for any of a number of precautionary measures, including immunizations, a periodic physical examination, and collection of a serum sample.

The purpose of the medical surveillance program is to:

- ensure individual employees are physically fit for the nature and extent of the work to be undertaken.
- recommend appropriate medical precautions.
- perform periodic reassessment of employees to determine if medical conditions associated with employment are prevalent and, if so, to undertake definitive measures to alleviate them.

The extent of medical surveillance for a given employee will vary greatly and be dependent upon:

- nature of the research project in which involved
- biological agents to which directly or potentially exposed
- factors relating to the current or previous health status of the individual.

The Principal Investigator is to provide Employee Health with guidelines and descriptions of conditions that may have significance for personnel assigned to the laboratory. It is the ultimate responsibility of the Principal Investigator or lab supervisor to inform Employee Health about employees new or currently working in BSL2 or BSL3 areas. The survey frequency of an employee working with BSL2 or BSL3 agents is determined by the type of research performed and the recommendation of the employee's supervisor or Principal Investigator.

Medical surveillance is provided without charge for any employee of Yale University working in biological research laboratories. For more information about this program contact the Employee Health at (432-0071). All BSL-3 Researchers must enroll in the Yale Employee Health medical surveillance program. To initiate your registration, call Employee Health at 432-7978 to schedule an appointment with the Employee Health Physician. Bring to Employee Health the “Yale University Biosafety Level 3 (BSL3) Worker Medical Clearance Form”. Complete the top portion of the form and have your Principal Investigator sign off on the form before you meet with Employee Health. Medical surveillance meeting with Employee Health is required prior to entering the BSL3 laboratory.

### **2.2 Employee Serum Storage**

Many infections do not result in an overt disease condition. Such infections are detected by development of antibodies to the agent in question. Therefore, Employee Health has established a program for persons engaged in certain types of biological research. This includes collection of pre-assignment serum as well as routine

periodic specimens. If an illness occurs, which may be related to the agent the person is working with, additional serum samples will be collected.

### **2.2.1 Enrolling in the Yale Serum Storage Program**

Researchers engaged in BSL3 research must enroll in the Yale Serum Storage Program. Before you can initiate BSL-3 research, you must provide a baseline serum sample which will either be banked by Employee Health or tested for antibodies to your research pathogen with your consent. The banked sample is a private confidential specimen that can't be tested without your consent. It provides a baseline sample that can be evaluated in the event of an exposure incident with Risk Group agents (or agents that require BSL3 work practices or containment).

Employee Health Office (432-7978) will coordinate your private meeting with the Employee Health Physician or representative and they will arrange for your baseline serum sample at the laboratory. The Employee Health Office is located on the 2<sup>nd</sup> floor of the Yale Health Center at 55 Locke Street, New Haven, CT. The lab is located on the lower level of the Yale Health Center at the same address. Must complete the medical surveillance meeting with Employee Health and provide a baseline serum sample (or test) before entering the BSL3 laboratory. After your visit with Employee Health, they will arrange for your baseline serum sample at the blood drawing laboratory for serum storage.

Yale Employee Health Office located at Yale Health Center, 55 Lock Street, 2<sup>nd</sup> floor.

## **2.3 Immunizations**

In certain situations personnel engaged in particular research activities will be immunized with appropriate vaccines, such as rabies, rubella and measles. Vaccines not commonly available will be obtained, whenever possible, for those engaged in specific research with potential exposure to the agent in question.

When indicated, appropriate follow-up serum samples will be collected at periodic intervals to measure vaccine-induced antibody production.

### **2.3.1 Yellow Fever Vaccine**

Recommended for all persons actively working with or entering areas where yellow fever virus is used.

### **2.3.2 Rabies Vaccine**

Recommended for all persons entering laboratories or animal facilities with rabies vaccination entrance requirements.

### **2.3.3 Venezuelan Equine Encephalitis Vaccine**

Recommended for all persons entering laboratories or animal facilities with VEE vaccination entrance requirements. Custodial and maintenance personnel shall not enter the designated areas until at least one day following the termination of experiments with the virus.

### **2.3.4 Rift Valley Fever Vaccine**

Recommended for selected persons traveling to Africa or planning to work with Rift Valley Fever at Plum Island, CDC, USAMRIID.

### **2.3.5 Japanese Encephalitis Vaccine**

Recommended for persons actively working with Japanese Encephalitis virus.

### **2.3.6 Eastern and Western Equine Encephalitis Vaccines**

Recommended for persons actively working with either or both viruses.

### **2.3.7 Hepatitis B Vaccine**

Recommended for persons working with human blood, body fluids or tissues.

### **2.3.8 Vaccinia Vaccine**

Employees are required to receive a medical evaluation and counseling regarding vaccinia immunization from Employee Health before beginning work with vaccinia. In cases where infected animals are not housed in filter-top cages or other primary containment devices vaccination shall be required for room entry.

## **2.4 Medical Restrictions**

### **2.4.1 Pregnancy**

It is recognized that exposure to certain infectious agents may adversely affect a fetus during pregnancy if the mother is infected with the agent. Therefore, if pregnancy is possible while you are working in an infectious disease laboratory or laboratory engaged in work with infectious agents you should consult your principle investigator or supervisor. The Employee Health is also available for questions regarding the potential harm from the biological agents present within your laboratory.

Women that are pregnant or become pregnant are encouraged to inform their supervisors or principal investigators and Employee Health. Employees are urged to discuss exposure issues with their supervisors or principal investigators regarding associated risks of research being conducted and pregnancy. Employee Health will give advice about precautions that may be necessary.

Employee Health is a resource for pregnant women to discuss questions or concerns they may have about risks in their work environment. Employee Health may also act as a liaison between pregnant employees and their respective supervisors or principal investigators.

### **2.4.2 Other Restrictions**

Restrictions or recommendations will be made on an individual basis after discussion with the Employee Health Physician and the employee's personal physician. Examples of conditions that may warrant special precautions are HIV infection, immunosuppressive conditions or drug therapy that suppresses the immune system. Therefore, if you have any of the above conditions, you must inform your physician and Employee Health about the situation.

## **2.5 Emergency Procedures for Exposure Incidents**

An "exposure incident" is specific contact (eye, mouth, other mucous membrane, non-intact skin, percutaneous or aerosol exposure) to potentially infectious materials that results from the performance of an employee's duties.

An employee who sustains a known or potential "exposure incident" must wash the area immediately with soap and water or immediately leave the laboratory in the event of an aerosol exposure. The employee must report the incident to his/her supervisor and the Biosafety Office.

The supervisor must complete an Employee First Report of Injury form and a Health Service Report form, documenting the route of exposure and the circumstances under which the incident occurred. Both forms are available at <http://www.yale.edu/hronline/workers-comp/>.

Employees must go to Employee Health within 1 to 2 hours following the exposure incident to consult with the Employee Health Physician. Call Employee Health at 432-0071. Employee Health hours are Monday to Friday 8:30am to 5:00pm. After hours go to Acute Care.

### **2.5.1 Percutaneous Injury Response**

Treat affected area:

- Wash well with disinfectant or antiseptic soap and water for 15 minutes.

### **2.5.2 Splash to Face Response**

- Flush affected area in eye wash for 15 minutes. If an eyewash is not immediately available, use another source of clean portable water and follow up with a full 15-minute eyewash as soon as feasible.

NOTE: If the accident generates an aerosol (i.e. spill outside the biosafety cabinet) leave the area promptly and follow the response procedures at a safer location. Identify a backup location prior to initiation of work.

### **2.5.3 Aerosol Exposure Response**

- Hold breath and immediately leave the room. Quickly remove outer gloves before leaving and drop gloves on floor before entering the ante room.
- Remove PPE carefully: turn exposed areas inward and place in a biohazard bag.
- Wash hands well with soap and water. Also, wash any exposed skin with disinfectant wipes or soap and water. Remove contaminated personal clothing and place in a separate biohazard bag.
- Post biohazard spill sign and record time of spill on sign. Lab should be evacuated for at least 30 minutes. PI and EHS must clear lab for reentry.

All BSL3 labs, especially those without a sink in the ante room, should have an emergency clothing kit that contains the following: two sets of scrubs (pants with tops in relevant sizes), a box of disinfectant hand wipes, two portable eyewash bottles, two biohazard bags, and booties or two pairs of shoes.

## **2.6 Post-Exposure Evaluation and Follow-Up**

Employee Health will provide the post-exposure evaluation and follow-up at no cost to employees following an "exposure incidents". The post-exposure monitoring periods are dependent on the type of exposure; which is related to the various incubation periods of the infectious agents.

Employees can obtain copies of their medical records by contacting University Health Services. These records are kept by the Health Information Services, 55 Lock Street, 432-0062.

All employees who have an "exposure incident" will be offered a confidential post-exposure medical evaluation and follow-up through the Employee Health. The post-exposure medical evaluation and follow-up includes the following:

- A review/evaluation of the route of exposure and the circumstances under which the incident occurred.
- For human blood and body fluid exposure:
  - An attempt to identify the source individual, if possible, and his/her HIV and HBV infection status.
  - The employee will be offered the option of having blood drawn for baseline serum collection (storage) or for HIV and HBV serological status testing depending on the incident.
- The employee will be offered post exposure prophylaxis when medically indicated.
- The employee will be given appropriate treatment and counseling concerning precautions to take during the period after the exposure incident. The employee will also be given information on what potential illnesses to be alert for and to report any unusual signs or symptoms to appropriate personnel.
- OSHA Bloodborne Pathogens Regulation requires, for blood and body fluid exposure, the University to provide the employee with a copy of the evaluating health care professional's written opinion within 15 working days of the completion of the original evaluation. The health care professional's written opinion will indicate: (1) that the employee has been informed of the results of the evaluation; and (2) that the employee has been told about any medical conditions resulting from exposure to blood or other potentially infectious materials requiring further evaluation or treatment.
- All other findings or diagnoses will remain confidential and will be recorded in the employee's medical record. All laboratory tests are conducted at no cost to the employee.

Contact Employee Health at (203)432-0071 if you require post-exposure evaluation or have follow-up questions.

## **Section 3 Laboratory Procedures**

### **3.1 Entry Procedures**

Entry into the facility is restricted to authorized individuals. Only individuals required for program or support needs are authorized to enter the facility while research is in progress. They must be advised of the potential biohazards and informed of laboratory procedures.

The Principal Investigator, lab supervisor, or an authorized BSL3 person must accompany maintenance and repair personnel. Visitors are not permitted in the BSL3 facility without prior consent from the PI. All visitors must complete and sign the Biosafety Level 3 Visitor Clearance Form (see section 12.3) prior to and following entry of the BSL3 facility. Visitors must be accompanied by an authorized BSL3 researcher or EHS representative for the duration of their visit to the facility.

- Before entering the anteroom to the laboratory, check the reading of the magnehelic pressure gauge. If your facility is equipped with a digital room pressure monitor that has both an audible and visual alarm, verify that the "green" enter light is on. There will not be an audible alarm during "green" or "clean to enter" conditions. Laboratory personnel must verify that the direction of the airflow is going into the BSL3 laboratory. If the BSL3 laboratory is positive to the corridor do not enter the laboratory and notify the PI, EHS and Yale BS&O (203-432-6888). Read and follow all entry procedures. Biohazard door signs and entry requirements and procedures must be posted.

- Upon entering the anteroom, sign the BSL3 Entry/Exit Log. Before walking into the laboratory, put on a clean gown and then proceed into the laboratory. Once in the laboratory, don gloves (2 pairs), face shield or respiratory protection (if applicable) and any other required personal protective equipment (PPE) for your project.

### **3.2 Exit Procedures**

- Before leaving the laboratory, remove outer gloves, gown, inner gloves and face protection and wash hands.
- Verify the airflow direction before entering the anteroom.
- Enter the anteroom and make sure door is closed.
- Sign the BSL3 Entry/Exit Log, exit anteroom, check all doors are closed and locked.

### **3.3 Personnel Practices**

Personnel practices or work practice controls reduce the likelihood of employee exposure to infectious agents by altering the way a task is performed. The protection provided by work practice controls is based upon employee behavior and attitude.

Proper work practice controls protect others from exposure to infectious agents in the work area or facility, reduce possible cross contamination and improve the quality of the work performed. Routine use of safe work practices also provides a margin of safety for unrecognized hazards.

Remember that safety is a shared responsibility. Your attitude and work practices are critical for your own health and safety, and for the welfare of those around you.

Organize and plan work procedures with safety in mind and keep an uncluttered work space. Always make sure all necessary safety materials and exposure control equipment are available and in good working order. Keep tuberculocidal disinfectant or 10% household chlorine bleach and paper towels nearby in case of a spill.

Always remove personal protective equipment (such as gowns, gloves, boot covers) and wash hands when leaving the facility.

Always wear appropriate personal protective equipment (PPE).

Beards or mustaches may be undesirable in work places with potential airborne contamination. Facial hair retains particulate contamination more persistently than clean shaven skin. Clean shaven faces enhance the fit of facial masks and are required when face fitting respirators are used.

Keep long hair tied back or covered.

Eating, drinking, smoking, applying cosmetics and lip balm, and handling contact lenses are prohibited in the laboratory.

Mouth pipetting is not permitted. Mechanical pipetting devices must be used.

### **3.4 General Laboratory Procedures**

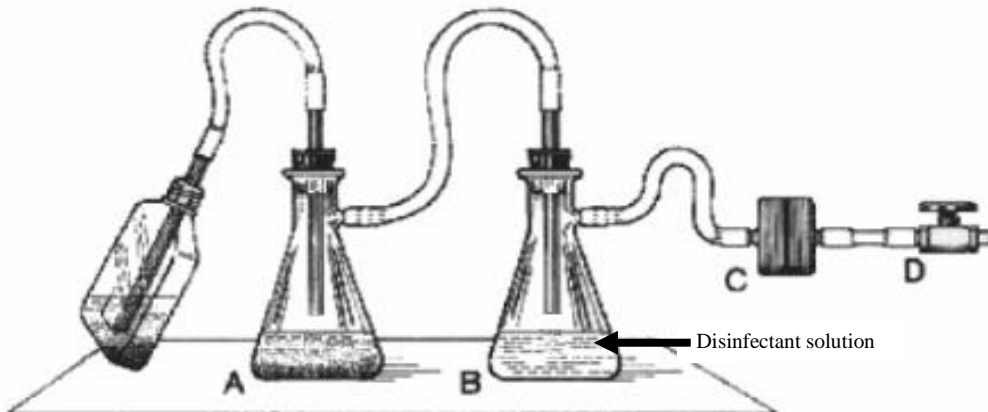
A significant concern in the BSL3 facility is the exposure to infectious aerosols. Various laboratory activities lead to the production of aerosols. The production of aerosols may result from pouring liquid cultures or supernatants, mixing a liquid culture using high speed devices such as a vortex, dropping a tube or flask of liquid culture, or the breaking of a tube during centrifugation. Any procedure that imparts energy to a cultured microorganism has the potential to generate aerosols. It is important to minimize or avoid production of aerosols and conduct all aerosol generating procedures in a biological safety cabinet. Confine aerosols as close as possible to their point of generation.

- Keep doors locked when the laboratory is unoccupied.
- Post the biohazard door sign on the access door to the facility. Ensure that any specific entry requirements (PPE and vaccination), the name of the agent (for compliance with federal and Yale University Regulations, the name of the pathogen family may be substituted for pathogen name as a security initiative), the Biosafety Level, and the name of an emergency contact person is posted on either the sign or the Laboratory Information Card.
- Label all equipment (e.g., incubators, freezers) used to store or ship infectious materials with biohazard warning labels and agent(s) name.
- Place the BSL3 wall notice (not a door sign) inside your laboratory to remind researchers of the core safety practices.
- Infectious materials may be transported to and from the BSL3 facility in sealed leakproof containment carriers inside labeled secondary containers, provided the transport is to another BSL3 facility.
- Opening containers of infectious materials or handling infectious materials must be done only in biological safety cabinets.
- All items must be decontaminated before leaving the BSL3 facility.
- Decontaminate all contaminated laboratory wastes and equipment before disposal or washing. Store contaminated materials that can be autoclaved in closed, leak-proof containers containing suitable disinfectant solution. Allow an appropriate length of contact time before removal from the laboratory.
- Decontaminate work surfaces when experiments conclude or after any spill.
- Always wear gloves when handling potentially infectious materials or surfaces.
- A fresh solution of 10% chlorine bleach should be made weekly and be available for decontamination.
- Avoid the use of needles and other sharps whenever possible. Needles shall never be recapped, removed, sheared, bent or broken. Needles and syringes must be discarded promptly in a needle sharps container after use. For BSL3 experiments, this requires placement of the sharps containers within the biosafety cabinet.
- Avoid the use of glass items and use plastic alternative items. Contact EHS for assistance on alternatives to glass items (e.g., tubes, pipettes, flasks).
- If sharps cannot be eliminated and must be used, work with EHS to identify a safe sharp alternative for evaluation by your laboratory.
- Conduct all procedures carefully to minimize the creation of aerosols.



### 3.5 Vacuum Line Traps and Filter Systems

HEPA filters or HEPA like filters must be installed between the collection flasks and the vacuum source. Note this system set up is required for both house vacuum systems and portable vacuum pumps. Infectious aerosols can contaminate the house system or be dispersed within the facility if filters are not in place. Vacuum line chemical traps and filters prevent suction of materials into the vacuum lines. The trap system also prevents vacuum lines from clogging with material. The trap system protects your work from materials in the vacuum line from backing-up into your facility. In-line HEPA filters are manufactured by Pall Trincor Corporation. The local distributor is Pond Technical Sales (203) 284-1500. Another manufacturer is Gelman Sciences. Their product is called Vacushield (HEPA like filter) and may be ordered through VWR Scientific or Baxter.



A. Collection Flask, B. Overflow flask, C. Filter, D. Vacuum Line

### 3.6 BSL3 Laboratory Practices

- Keep laboratory doors closed, and locked when unoccupied.
- Post biohazard sign on lab door, include agent, entry requirements, and an emergency contact phone number.
- Verify proper air direction before each entry into BSL3 areas.
- Label all equipment and storing infectious organisms with a biohazard sign.
- Only allow researchers authorized by EHS to perform BSL3 experiments.
- All researchers must have demonstrated proficiency in BSL3 microbiological practices.
- Contact Employee Health Office for enrollment in the BSL3 medical surveillance program (432-0071).
- Develop and maintain a lab BSL3 safety manual.
- Do not permit visitors until lab cleared by P.I. and EHS (785-3550).
- Keep animals and plants not used in BSL3 experiments out of the laboratory.
- Don't eat, smoke, drink, or store food in BSL3 areas.
- Wear solid-front or wrap-around gowns, gloves that can fit over gown cuffs, and safety glasses inside but not outside the laboratory.
- Do not mouth pipette. Use mechanical pipetting devices.
- **Perform all work with BSL3 agents inside a biological safety cabinet. Collect all BSL3 waste inside the biological safety cabinet.**
- Use physical containment devices, such as sealed rotors or safety buckets for centrifugation.

- Load and unload sealed centrifuge rotors and safety buckets inside a biological safety cabinet.
- Use a biological safety cabinet to contain aerosol-producing equipment.
- Wear respiratory protection devices when aerosols cannot be contained.
- Protect the vacuum system from contamination by installing a HEPA or HEPA-like filter in between the vacuum system and the collection flask.
- Avoid using hypodermic needles and other sharps. Use plastic supplies in place of glass.
- Use 2 leak proof, labeled unbreakable containers for the transport of BSL3 materials between laboratories. Consult EHS for interstate shipment requirements.
- Wash hands after completing experimental procedures and before leaving the laboratory.
- Disinfect work surfaces daily and immediately after a spill.
- Decontaminate all laboratory wastes and contaminated materials before disposal, washing, or reuse.
- For transport to the autoclave, package contaminated materials in closed, durable, leak-proof containers.
- Verify autoclave procedures periodically.
- Post incident and spill response procedures in the laboratory. Report spills and potential exposures to the P.I. and EHS. An eyewash should be readily available
- Maintain a biological spill kit in a location outside the laboratory to facilitate spill response and decontamination.
- Report any facility or equipment problems to EHS and Physical Plant.
- Control insect and rodent infestation.

### **3.7 Personal Protective Equipment (PPE)**

- Dedicate PPE for the experiment. PPE worn for BSL3 work should not be worn in other areas. Remove before leaving the laboratory.
- Wear a lab coat or solid-front gown, preferably with a knit or grip cuff.
- Double glove for all work within the biological safety cabinet (BSC). Remove the outer pair before exiting the BSC, and don a new pair each time you reenter the BSC.
- Ensure that your gloves extend over the sleeve of your lab coat. An opening at the wrist will allow aerosols generated within the BSC to contaminate your wrist and forearm, extend handwashing to your elbow.
- Sleeve covers can be worn to ensure coverage of the wrist and will also minimize contamination of the sleeves of your lab coat.
- Face Protection (mask and eyewear) can also be worn and will protect mucous membranes from exposure in the event a spill outside the BSC during transfer of material to and from the incubator. It will also help to prevent you from touching your eyes, nose and mouth when working within the BSC.
- Remove PPE before leaving the laboratory. Placing a hook within the anteroom will facilitate this requirement. Remove your outer gloves first, then your lab coat or gown, followed by the inner gloves. Take your face protection off last. Don't touch your face with gloved hands. Remove gloves and other clothing aseptically, from the inside out, and avoid touching the contaminated outer side of the glove.
- Decontaminate reusable PPE as soon as feasible after it has been contaminated. Small areas can be spot treated with a suitable disinfectant, such as 10% household bleach. Lab coats can also be autoclaved, or

sent to a laundry facility equipped to handle biohazardous PPE. Disposable PPE can be placed within a biohazard bag, treated and discarded as biomedical waste.

- Wash your hands with soap and water after removing PPE and before leaving the laboratory.
- Note: if a respirator is required for your research, you must complete a confidential medical health questionnaire for Employee Health and enroll in the Yale Respiratory Protection Program. Training is required before initial use and annually thereafter. Call 785-3550 if you have any questions.

### **3.8 Handwashing**

- Wash hands whenever PPE is removed and before leaving the laboratory.
- Wash with soap and warm water for at least 30 seconds. Since the contact time of most soaps is quite extensive for actual decontamination, mechanical friction from scrubbing and water dilution are essential for complete cleaning.
- No glove is 100% leak proof and you must change gloves often and as soon as feasible when visibly contaminated.
- Never wet or wash your gloves with water or disinfectant, as this will encourage wicking and increase permeability of the protective barrier.
- If you wish to wash gloves with disinfectant, check with EHS to see if your gloves can be washed. Break through times for that disinfectant and your glove material must be evaluated before initiation this of procedure.

### **3.9 Procedures for centrifugation**

As secondary containment is required, all centrifugation shall be done using centrifuge safety buckets or sealed centrifuge tubes in sealed rotors. Please notify EHS if you have a centrifuge that does not have adequate secondary containment for further evaluation.

The following procedures for centrifugation are recommended:

- Examine tubes and bottles for cracks or stress marks before using them.
- Fill and decant all centrifuge tubes and bottles within the biological safety cabinet. Never overfill centrifuge tubes as leakage may occur when tubes are filled to capacity. The maximum for centrifuge tubes is 3/4 full.
- Wipe outside of tubes with disinfectant before placing in safety cups or rotors.
- Place all sealed tubes in safety buckets or sealed rotors. Inspect the "O" ring seal of the safety bucket, the inside of safety buckets or rotors and correct rough walls caused by erosion or adhering of matter and remove debris from the rubber cushions. Seal rotor or bucket and wipe down with disinfectant, remove outer gloves, and transport to the centrifuge.
- Wait 2-5 minutes after the run to allow aerosols to settle in the event of a spill. Transport sealed rotor or safety bucket to biological safety cabinet to complete your experiment. Don new pair of outer gloves
- Open safety buckets or rotors in a biological safety cabinet. Determine prior to initiating BSL3 work if the rotor does not fit in the biological safety cabinet. You may need to use a smaller rotor or activate the sliding mechanism for the view screen of the biosafety cabinet. If the view screen is fixed and the rotor does not fit, BSL3 work may not be initiated. Contact EHS for immediate assistance.

- Decontaminate the rotor or safety bucket by spraying with an appropriate disinfectant and allowing to air dry. Wipe the throw line within the centrifuge with disinfectant. In the event of a spill during centrifugation, follow the spill response procedures outlined in the Biosafety Spill Response Guide.
- Avoid the use of a microfuge, which is difficult to contain. If you cannot avoid using a microfuge, use a model that has built in secondary containment (a sealed rotor) along with microfuge tubes equipped with an O-ring seal. Please contact EHS for assistance in identifying containment features for purchasing new microfuge or to evaluate upgrading your existing model.
- For high risk biohazard experiments, the following measure may be employed: a HEPA-filtered respirator must be worn when opening the centrifuge to remove the rotor. If construction of the centrifuge permits, the centrifuge chamber is to be connected to a vacuum pump with a HEPA filter installed between the centrifuge and the vacuum pump.
- A Class I biosafety cabinet may be installed over the ultra-centrifuge as an additional containment measure.

### **3.10 Biological Safety Cabinet (BSC) Protocol**

A properly balanced and used Class II Biological Safety Cabinet (BSC) will do an excellent job of controlling airborne contaminants only if appropriate contamination control procedures and aseptic techniques are also employed. Additional information on BSC policies and procedures can be found in the Clean Air Device Program Guide.

- All BSCs shall be professionally certified at the time of installation and annually thereafter. If a BSC is to be moved, it shall be professionally decontaminated before moving and recertified before work commences. Contact EHS at 737-2125 for assistance with certification, decontamination and other services.
- Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC may affect proper airflow and the level of protection provided. Also, avoid blocking the front or rear intake grills.
- Keep the front and rear grilles clear when working within the BSC. Avoid blocking the rear grille. Don't store items on top of the BSC. Remind fellow researchers to minimize traffic and work behind the operator, as this may interfere with cabinet airflow. Depending on the location of the BSC within the room, opening and closing the room door can significantly interfere with BSC airflow.
- Some BSCs are equipped with ultraviolet (UV) lights. If good procedures are followed, UV lights are not needed. All UV lights shall be turned off whenever the laboratory is occupied.
- Avoid using toxic, explosive, flammable, or radioactive substances in the BSC unless a safety professional has approved the procedure.
- Perform all work within a BSC. This includes discarding waste within the BSC. Moving your hands in and out of the BSC will disrupt the protective air curtain at the front access opening.
- To begin the BSC operation, turn on the lights, confirm the air intake and exhaust grills are clear, and turn on the blower. If a drain valve is present, make certain it is closed.
- Wash hands and arms with germicidal soap before and after work in the BSC. Operators shall wear long sleeved gowns with tight fitting cuffs and surgical gloves. This measure protects the operator's hands and arms from contamination and minimizes the shedding of skin flora into the work area.
- Disinfect interior surfaces of the work area using freshly prepared appropriate disinfectant.
- Everything needed for the complete procedure shall be placed in the cabinet before starting work. Wipe items down with disinfectant prior to placement within the BSC. Nothing shall pass in or out through the air barrier until the procedure is completed. Avoid overloading the work area, and thereby compromising the efficacy of the BSC.

- Work supplies are best arranged to segregate clean from dirty materials. Segregate clean areas from contaminated areas within the BSC by at least 12-14”.
- Wait five minutes after all materials have been placed in the BSC before beginning work. This will enable the BSC to purge airborne contaminants from the work area.
- Work as far to the back of the BSC workspace as possible.
- Always use mechanical pipetting aids.
- Avoid using open flames inside BSCs. If a flame must be present, use a burner with a pilot light and place it to the rear of the workspace. Flames disrupt the unidirectional airflow and contribute to the heat load inside the BSC. Flames have shortened the lifetime of HEPA filters, burned holes through HEPA filters and have caused explosions in BSCs. Never leave an open flame (burner or pilot light) unattended in your BSC.
- Consider using an electric micro-incinerator if a heat source is required.
- Do not work in a BSC while a warning light or alarm is signaling.
- Absorbent paper on the work surface of the BSC will safely absorb splatter.
- Collect all waste within the BSC. Waste containers should be placed inside the BSC to avoid breaking the air barrier and bringing contaminated items out into the room. Smaller biohazard waste bags may be utilized along with beakers or shallow trays containing disinfectant for the collection and disinfecting of pipettes and other contaminated items. Waste can also be collected within the BSC in the following manner.

Horizontal collection: Horizontal trays containing disinfectant allow total immersion of pipettes.

Vertical collection: Beakers containing disinfectant can be used if disinfectant is drawn up inside the pipette and allowed to run down the interior wall upon disposal into the beaker.

Bags: Bags have the potential for creating aerosols when moved. Seal autoclave bags within the cabinet and place within a second bag. Carefully add water to the primary bag before sealing (25 ml for smaller bags, 200 ml for larger bags). The addition of water will help to generate steam within the bag during the autoclave cycle.

- Store tissue culture flasks in the incubator within small secondary trays to help minimize contamination. Trays will also facilitate transfer to and from the BSC.
- Keep your hands away from your face (face protection helps to minimize the potential for this route of exposure).
- Avoid the use of glass Pasteur pipettes or needles and syringes. Substitute plastic for glass whenever feasible. Alternatives to glass Pasteur pipettes include: plastic pipettes, plastic transfer pipettes, plastic gel loading pipette tips and pipette tip extenders, aspirators, and flexible plastic aspiration pipettes. Campus stock rooms carry a 2ml plastic aspirating pipette without a cotton plug.
- If the use of sharps cannot be avoided, maintain a sharps container in the immediate vicinity of use. Discard intact needles and syringes immediately after use. Use a one-handed disposal technique method (keep a hand behind your back or by your side, don't place on or near the opening of the sharps container). Never recap, bend, break or otherwise manipulate sharps by hand. If you must remove the needle from the syringe, use the small opening on the top of the needle box for this purpose. Forceps, tweezers, or small pliers may also be utilized.
- Protect the house vacuum system or pump from contamination by installing a trap and filter system. Use a primary collection flask containing disinfectant, followed by an overflow flask, which leads through a HEPA or hydrophobic filter.
- After completion of work, enclose or cover all equipment and materials. Wipe items down with an appropriate disinfectant prior to removal from the BSC. Allow the BSC to run for five minutes to purge airborne contaminants from the work area.

- Decontaminate interior surfaces (work surface, grilles, sides, back and inside front view screen) with an appropriate disinfectant after removal of all materials, cultures and apparatus.
- If using 10% bleach solution on work surfaces, allow it to air dry then follow up with 70% ethanol wipe to prevent rusting of stainless steel surface.
- Periodically decontaminate under work grilles and work surfaces if these parts are removable.
- Decontaminate liquid waste with household bleach diluted 10% against the volume of the waste. Allow at least a 30-minute contact time for full decontamination.
- Transport waste to autoclave in a leakproof container.

### **3.11 Chemical Hood - Guidelines for Operation**

Use a chemical hood for containment of hazardous chemicals which are not appropriate for use in a biosafety cabinet. It is important to keep the sash open within its proper operation position.

- Verify that the chemical hood is exhausting.
- Work with the sash lowered to the 100±10 ft/min level. The sash must be below chin level.
- Work at least 6 inches inside the hood.
- Do not block the face of the hood (e.g., with shielding or large pieces of equipment).
- Do not block rear exhaust slot. Place bulky items to the rear and sides on a supporting mesh elevated at least two inches from the work surfaces to allow passage of air to the rear slot.

### **3.12 Waste Handling and Disposal Procedures**

All waste from a BSL3 facility must be considered infectious and should be decontaminated daily. Additional information on the disposal of medical waste is found on the EHS website at <http://ehs.yale.edu/>

Waste from BSL3 facilities are generally autoclaved in the lab, and then packed as “Anatomical/Pathological waste” to ensure it is incinerated as a final method of disposal.

Liquids are inactivated by autoclaving or by inactivating with household bleach. Final concentration of 10% bleach against the volume of waste to be inactivated. A minimum contact time of 30 minutes is required. The liquid is then disposed of down the sink using large amounts of water followed by a disinfectant.

Solid wastes are inactivated by autoclaving. Autoclaves are operated at 250 °F and 15 pounds per square inch pressure for 60 minutes. The biohazard autoclave bags should not be taped closed. After autoclaving, all waste leaving the facility must be doubled bagged before placing in a box-bag unit.

### **3.13 Equipment Repairs/Service**

Potentially contaminated and contaminated equipment sent out for repair/service or to be discarded must be decontaminated as thoroughly as possible. Notify EHS if all contaminated portions of the equipment could not be sufficiently decontaminated. Note: insufficiently decontaminated equipment cannot be discarded. Contact EHS to identify an adequate decontamination procedure.

- Affix a Biosafety Notice equipment tag to the equipment indicating when the equipment was decontaminated, what disinfectant was used, and the name of the person who performed the

decontamination. Tags are available through the Office of Environmental Health and Safety at 785-3550. Sample of the equipment decontamination tag is in section 10.6.

- Thorough decontamination of highly technical or sensitive equipment or equipment with limited access to contaminated areas may not be possible. Decontaminate the equipment to the degree possible (flushing lines or wiping down the exterior) and affix a label to the equipment before sending it out for repair. The label must indicate what portions of the equipment remain contaminated and include the biohazard symbol as well as the term "biohazard". The label must convey this information to all affected workers (service representatives, manufacturer, etc.). Notify EHS who will work with the repair or service technician to develop an appropriate decontamination procedure and identify required PPE for working on the equipment.

### **3.13.1 Service or repairs of the Biological Safety Cabinets (BSC) and the exhaust system's HEPA filters and pre-filters.**

EHS will approve a certified vendor for certifying and repairing BSC and servicing the exhaust system's HEPA filters and pre-filters. The approved certifier will perform formaldehyde decontamination on the exhaust system for changing filters. The certifier will be accompanied by a representative from EHS whenever entering the facility. See Section 7.0 for additional information on maintenance and visitor procedures.

## **3.14 Procedure for Laundering Laboratory Protective Clothing**

Disposable protective clothing, which is placed in biomedical waste after use, is preferred. Reusable laboratory protective clothing must be decontaminated by autoclaving before disposal or laundering.

## **3.15 Transport of Human Pathogens or Other Potentially Infectious Materials on Campus (between labs or buildings)**

Package the material within the BSC. Use two leakproof containers:

- sealed primary container
- sealed secondary container
- absorbent (paper towels) between the primary and secondary containers suitable for volume transported
- a biohazard sticker on outside of the secondary container with agent name, lab address and phone number

Utilize plastic containers whenever feasible, avoid glass.

Sealed plastic (not glass) primary vials can be transported within sealed-labeled plastic bags.

Place glass primary containers within a sealed rigid plastic container with absorbent and padding to cushion the vial during transport.

Wipe the exterior of both primary and secondary containers with disinfectant prior to transport.

Personal protective equipment (gloves, lab coat, and face protection) is not required for transporting human pathogens or other potentially infectious materials if properly packaged and decontamination has been performed. Do not wear gloves in hallways (don't touch elevator buttons, doorknobs, telephones, or other clean items or surfaces with gloved hands).

Remember: PPE should be removed before exiting the laboratory. Placing a hook in your lab may facilitate compliance with this requirement.

Handle your packages carefully (maintain in an upright position during transport).

## **Section 4 Decontamination of Spills**

### **4.1 Spills in BSC**

In the event of a spill, all surfaces and items shall be surface decontaminated before being removed from the BSC.

If the spill results in puddles:

- Flood the area with an appropriate disinfectant, such as 10% chlorine bleach and let it react for 15 to 30 minutes. If a drain system is involved, consult the BSC manufacturer's specific instructions regarding decontamination.
- After the bleach treatment, wipe the area clean with water followed by 70% ethanol.
- Discard any item that may have become contaminated.

After a spill is decontaminated, the area shall be thoroughly cleaned and dried. Residual materials can support the growth and multiplication of microorganisms, and can jeopardize the product protection normally provided by BSCs.

### **4.2 Spills in the Laboratory**

Use the guidelines below for response to spills of BSL3 material outside of the biosafety cabinet or any other incident that may have generated an aerosol in the containment laboratory, such as failure of physical containment devices during centrifugation.

#### **4.2.1 Immediate Action:**

- Hold breath and leave room immediately; notify others in the room to evacuate immediately.
- Remove outer gloves and drop on lab floor prior to entering the ante room
- Remove personal protective equipment (PPE) in the airlock or access zone; turn potentially contaminated clothing inward; remove gloves last, and wash any exposed skin areas with antiseptic soap and warm water.
- In the event of an exposure incident:
  - Needle sticks/puncture wounds: wash the affected area with disinfectant, antiseptic soaps and warm water for 15 minutes. Squeeze around the area to encourage the flow of blood out of wound.
  - Mucous membrane exposure: use eyewash for 15 minutes to flush the affected area.
- Post a BIOHAZARD SPILL SIGN at entry to BSL3 lab door with the recorded date and time of spill.
- Notify your Principal Investigator and EHS



- Do not reenter laboratory until it has been cleared for reentry by the P.I. or EHS. In general a period of at least 30 minutes should be allowed before clean up is attempted, but the time is contingent upon the supply and exhaust features of the lab.

### 4.3 Composition of BSL3 Spill Kit

Undiluted household bleach

Forceps: for handling sharps or collecting small objects

Paper towels or other suitable absorbent

Biohazard bags: for the collection of contaminated spill clean-up items

Sharps container: if necessary, for collection of needles or other sharps

Personal Protective Equipment: Gloves (household utility gloves afford additional protection), face protection such as masks & eyewear, back-fastening gowns, Tyvek jump suits (will not drag into spill area when cleaning), plastic booties, and a powered-air-purifying-respirator\* (PAPR) with HEPA filters.

\*researchers using respiratory protection equipment must be enrolled in the Yale University Respiratory Protection Program and have training in the use and fit of their respirators prior to use.

**Store the BSL3 spill kit and respirator in a secure location outside of the BSL3 laboratory.** This will prevent contaminating the kit or the need to reenter a BSL3 spill situation to obtain your response kit.

**Maintain the spill kit.** Replace spill kit components as they are used to prepare for the next incident

#### 4.3.1 Spills in Incubators

Since a spill in an incubator is a breach of containment, close the incubator and immediately leave the BSL3 facility. Follow the BSL3 spill response procedures outlined in section 4.2.1 to 4.2.2.

- Add chlorine bleach to the tray of water at the bottom of the incubator.
- Remove the materials in the incubator wiping them with 70% ethanol or suitable disinfectant and placing them into another incubator.
- Collect non-sharp contaminated materials in a biohazard waste bag and place all sharp contaminated materials in a sharps container.
- Wipe incubator surfaces with 10% chlorine bleach solution, followed by water and then wipe surfaces with 70% ethanol.

#### 4.3.2 Spills in Centrifuge

A high-risk aerosol breach outside of a primary containment requires immediate evacuation of the laboratory. Follow the BSL3 spill response procedures outlined in section 4.2.1 to 4.2.2.

- Stop work immediately. Presume the aerosolized material is contaminated. The incident should be treated as a potential exposure.
- Inform all others in the area that an aerosol may have been generated. All persons shall evacuate the room immediately for at least 30 minutes. Notify the supervisor or Principal Investigator and EHS at 785-3555.
- Label the area off-limits for at least 30 minutes.
- **As with any release of BSL3 materials outside of primary containment, EHS and the PI must clear the laboratory for reentry.**

- Put on appropriate personal protective equipment (gown, gloves, powered air purifying respirator) before entering the laboratory.
- Use absorbent materials to cover spill areas before the addition of a disinfectant. Absorbent materials reduce the potential of generating an additional aerosol due to the decontamination procedure itself.
- Decontaminate all exposed environmental surfaces before releasing the room for normal use.
- Remove rotor and place in a BSC. To decontaminate rotor, soak it in 10% bleach or other suitable disinfectant, followed by mild detergent, then water rinse. If using 10% bleach solution on work surfaces, allow it to air dry then follow up with 70% ethanol wipe to prevent rusting of stainless steel surface.

#### 4.3.3 Cleaning the BSL3 Spill:

Note: if the spill involves radioactive materials, also contact the Radiation Safety Office emergency line (5-3555).

Once clearance has been given to reenter the spill area by the P.I. in consultation with EHS, don the appropriate personal protective equipment and enter the lab.

- While wearing PPE, cover the spill area with paper towels or disinfectant soaked paper towels.
- **Slowly pour** concentrated disinfectant around the edge of the spill, working toward the center (this will avoid enlarging the contaminated area). **Avoid splashing or the creation of aerosols during this step.**
- Allow a 15 - 20-minute contact time for the disinfectant.

While waiting, decontaminate surrounding floor and work surface areas where splashes or larger aerosols may have settled around the spill. Use disinfectant soaked towels to wipe these areas (1 - 10% household bleach is suitable for this purpose).

- After the 15 - 20-minute contact time, place soiled paper towels inside the biohazard bag.
- Repeat the decontamination procedure.

After Performing the Decontamination:

- Decontaminate any reusable items such as forceps by wiping with, then soaking in a disinfectant solution. A soak in 1 - 10% household bleach for 15 - 20 minutes is sufficient. Since bleach is corrosive, follow the decontamination with a water wipe down to remove any corrosive residues.
- Remove PPE, turn any exposed areas inward and place in the biohazard bag. Generally, gloves are removed last. However, to avoid touching your face with gloved hands, remove gloves just before removing masks or eyewear.
- Wipe down the exterior portions of any reusable PPE such as the PAPR and utility gloves with the bleach solution. Perform the disinfectant wipe down twice.
- Wash your hands well with soap and water, at least 15 - 30 seconds.
- Autoclave all waste generated from the spill cleanup. Use fresh gloves for transport to the autoclave, and wash hands after removing gloves.

## Section 5 Work with Hazardous Biological Toxins

Researchers are required to list the biological toxins involved in their experiments on their initial FORM 01 registration and on all annual FORM 01 updates available at <https://ehsis.yale.edu/EHSIntegrator/Registration>. Work with high hazard biological toxins, those with an LD50 of <500 mg/kg, should be reviewed by the Office of Environmental Health & Safety to verify that appropriate safety practices and protective equipment are in

place to minimize exposure. At a minimum, the requirements established in the Yale Chemical Hygiene Plan and the biosafety practices outlined in the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories for the toxin or parent microorganism must be followed. Additional information on working with and handling toxins is in the Yale Biological Safety Manual.

## 5.1 Recombinant DNA Experiments Involving Toxins

Recombinant DNA Experiments involving the cloning of genes that code for toxic molecules that have an LD50 of less than 100 ug/kg body weight, must be approved before initiation. Toxins with very low LD50 values (< 1ug/kg body weight) require additional registration and approval. Table 1 details NIH recombinant DNA registration requirements for certain experiments involving toxins. The list is not inclusive.

Table 1. NIH recombinant DNA registration requirements for certain experiments involving toxins

LD50 Range	< 100 ng/kg	1 ug/kg $\geq$ x > 100 ng/kg	100 ug/kg > x > 1 ug/kg
Approval(s)	NIH-ORDA Approval IBC Approval	NIH-ORDA Registration IBC Approval	IBC Approval
Toxin(s)	Botulinum Tetanus Diphtheria Shigella dysenteriae	Abrin  Clostridium- perfringens	Staph aureus beta toxin Ricin Aflatoxin B1 Bordatella pertussis toxin Saxitoxin Tetrodotoxin Staphylococcal enterotoxin (B,F)

Please contact Biosafety at 785-3550 to update your FORM 01, register your recombinant DNA experiments, or for assistance in establishing safe working practices for your work with hazardous biological toxins.

## 5.2 Select Agent Toxins

The following toxins are not regulated if the amount under the control of a Principal Investigator, treating physician or veterinarian, or commercial manufacturer or distributor does not exceed, at any time, the amounts indicated in the table below.

<i>HHS Toxins [§73.3(d)(3)]</i>	<i>Amount</i>
Abrin	1,000 mg
Botulinum neurotoxins	1.0 mg
Short, paralytic alpha conotoxins	100 mg
Diacetoxyscirpenol (DAS)	10,000 mg
Ricin	1,000 mg
Saxitoxin	500 mg
Staphylococcal Enterotoxins (Subtypes A, B, C, D, and E)	100 mg
T-2 toxin	10,000 mg
Tetrodotoxin	500 mg

Note: Toxin quantities shown are maximum quantities of toxins, in aggregate per PI, allowed by USDA/CDC. Note: Yale's internal watch list limits PIs to only 50% of the quantities shown here.

## **5.3 Toxin Work Practices**

Safe working practices to minimize exposure via ingestion, inhalation, mucous membrane contact, and absorption or penetration through the skin.

### **5.3.1 Work Practices**

- An inventory control system should be in place.
- Toxins should store in locked storage rooms, cabinets, or freezers when not in use.
- Label toxin work areas within lab.
- Cover work surfaces with plastic backed absorbent paper.
- Avoid generating aerosols; handle the powdered form carefully.
- Use a chemical fume hood or biosafety cabinet when feasible.
- Avoid the use of needles or Pasteur pipettes.
- Substitute plastic for glass wherever possible.
- Decontaminate work surfaces with 5-10% household bleach or 0.1N sodium hydroxide.
- Treat liquid waste with 50% household bleach (soak overnight). For T-2 mycotoxin use a combination of 50% household bleach and 0.25N sodium hydroxide.
- Collect and autoclave waste at the end of the day.
- Autoclave or chemically disinfect contaminated protective clothing before reuse.

### **5.3.2 Personal Hygiene**

- Keep your hands away from your face.
- Do not eat, drink, or smoke in the lab.
- Do not mouth pipette.
- Always wash hand after removing protective clothing and before leaving the lab.

### **5.3.3 Labels and Transport**

- Post biohazard sign at lab entry.
- Restrict access to the lab.
- Label equipment biohazard label used with or storing toxins.
- For transport, use sealed, unbreakable, leakproof containers with a biohazard label, full toxin name, and lab phone number.

### **5.3.4 Protective Clothing Requirements**

- Lab coat buttoned to the top with knit or grip cuffs, use gloves that are long enough to cover the sleeves; a back-fastening gown is suitable; sleeve covers offer additional protection.
- Gloves (consider double gloving).
- Face protection such as a face shield or safety glasses and a mask to cover the eyes, nose and mouth.
- Dedicate protective clothing for work with toxins and do not wear outside the lab.
- Avoid contact when removing gloves.

### **5.3.5 Work with Powdered Form of Toxin**

- Carefully weigh and convert to aqueous form as soon as possible.

- Store powdered form in an unbreakable secondary container labeled with the biohazard label and complete toxin name to identify the hazard.
- Change gloves after handling powdered toxin being sure to avoid skin contact with the toxin while removing gloves; wash hands prior to donning new gloves.

### **5.3.6 Emergency Response**

- Flush skin or eyes with running water for 15 minutes, notify PI immediately, seek medical assistance.
- Follow BSL2 spill procedures: leave lab for 30 minutes, upon return, decontaminate spill with 25% household bleach solution for 30 minutes, collect and autoclave waste.

## Section 6 Select Agents

Select agents are materials that have been identified by the U.S. Government as agents with potential for use in biological terrorism or warfare. The Department of Health and Human Services (DHHS), through the U.S. Centers for Disease Control and Prevention (CDC) and the Animal Plant Health Inspection Service (APHIS), through the United States Department of Agriculture (USDA), regulate select agents in the United States and its territories. Each agency has developed and maintains a list of select agents, including human, animal, and plant pathogens, high-risk toxins of biological origin, and prions. The current list of select agents is below, and can also be accessed from CDC and USDA web sites:

- <https://www.cdc.gov/phpr/dsat.htm>
- <https://www.usda.gov/>
- [https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-and-animal-product-import-information/sa\\_ag\\_select\\_agent](https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-and-animal-product-import-information/sa_ag_select_agent)).

The Select Agent Registry home page may be accessed at <http://www.selectagents.gov/>.

The federal select agent regulations were updated and changes have taken effect as of March 21, 2017. The most significant changes are an update of the select agents and toxins list (as reflected in Appendix A of this plan) and the change in the permissible amounts of toxins allowed per laboratory for exemption from registration. The list of Tier 1 select agents and toxins has also been updated and are also included in Appendix A. Institutions that possess or work with Tier 1 agents will have to implement additional personnel screening and ongoing personal screening requirements beyond the current federal background checks. Yale University is not currently registered for Tier 1 agents. A personnel screening program will be developed prior to the receipt of Tier 1 agents. In addition to the enhanced screening requirements for personnel with access to Tier 1 select agents, additional physical security measures have been added for locations where Tier 1 select agents are either stored or used. Yale University's current select agent laboratory is in conformity with the additional physical security measures that are required for research with Tier 1 select agents and toxins.

National concerns over select agents have led to an expansion in security requirements for these materials. As a result, entities and researchers in possession of these materials have additional obligations and responsibilities for their safe storage, use, transfer, and disposal.

### 6.1 Possession, Use, or Transfer of Select Agents

In order to possess, use, send or receive Select Agents, an institution and each individual who will have access to the Select Agent(s) must first satisfy the following requirements. Each requirement must be approved prior to possession, use or transfer.

- Register with the applicable U.S. Governing bodies (CDC, APHIS, and/or USDA) through the Yale Office of Environmental Health & Safety (EHS).
- Register with the State of Connecticut Department of Public Health through Yale EHS. This is required for those Select Agents that are human pathogens.
- Official authorization granted for each individual requesting access to Select Agents provided by the U.S. Federal Bureau of Investigation, the applicable U.S. Governing body, and Yale University.

Please note that violations of Select Agent rules and regulations can lead to severe criminal or civil penalties. Imprisonment and fines up to \$250,000.00 may be levied against individuals who are found in violation of these laws.

## 6.2 List of Select Agents and Regulated Toxins

The current list of Select Agents below is current as of March 2017. The Select Agent List is available online at: <https://www.selectagents.gov/selectagentsandtoxinslist.html>.

Select Agent Regulations may be found at: <https://www.selectagents.gov/regulations.html>

The list of excluded agents and toxins can be found at:  
<https://www.selectagents.gov/SelectAgentsandToxinsExclusions.html>

### HHS Select Agents and Toxins

Abrin	SARS-associated coronavirus (SARS-CoV)
Botulinum neurotoxins*	Saxitoxin
Botulinum neurotoxin producing species of Clostridium*	South American Haemorrhagic Fever viruses
Short, paralytic alpha Conotoxins	<ul style="list-style-type: none"><li>• Chapare</li><li>• Guanarito</li><li>• Junin</li><li>• Machupo</li><li>• Sabia</li></ul>
Coxiella burnetii	Staphylococcal enterotoxins
Crimean-Congo haemorrhagic fever virus	T-2 toxin
Diacetoxyscirpenol	Tetrodotoxin
Eastern Equine Encephalitis virus	Tick-borne encephalitis complex (flavi) viruses
Ebola virus*	<ul style="list-style-type: none"><li>• Far Eastern Tick-borne encephalitis</li><li>• Siberian subtype</li><li>• Kyasanur Forest disease</li><li>• Omsk Hemorrhagic Fever</li></ul>
Francisella tularensis*	Variola major virus (Smallpox virus)*
Lassa fever virus	Variola minor virus (Alastrim)*
Lujo virus	Yersinia pestis*
Marburg virus*	
Monkeypox virus	
Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)	
Ricin	
Rickettsia prowazekii	

### Overlap Select Agents and Toxins

Bacillus anthracis*	Burkholderia mallei*
Bacillus anthracis Pasteur strain	Burkholderia pseudomallei*
Bacillus cereus Biovar anthracis*	Hendra virus
Brucella abortus	Nipah virus
Brucella melitensis	Rift Valley fever virus
Brucella suis	Venezuelan Equine Encephalitis virus

### USDA Veterinary Services (VS) Select Agents and Toxins

African horse sickness virus	Mycoplasma capricolum
African swine fever virus	Mycoplasma mycoides
Avian influenza virus (highly pathogenic)	Newcastle disease virus (virulent)
Bluetongue virus (exotic)	Peste des petits ruminants virus
Classical swine fever virus	Rinderpest virus*
Foot-and-mouth disease virus*	Sheep pox virus
Goat pox virus	Swine vesicular disease virus
Lumpy skin disease virus	

## USDA Plant Protection and Quarantine (PPQ) Select Agents and Toxins

Peronosclerospora philippinensis (Peronosclerospora sacchari)	Rathayibacter toxicus Sclerophthora rayssiae
Phoma glycinicola (formerly Pyrenochaeta glycines)	Synchytrium endobioticum Xanthomonas oryzae
Ralstonia solanacearum	

\* Denotes Tier 1 Agent

### 6.3 Tier 1 Select Agents

A subset of select agents and toxins have been designated as Tier 1 because these biological agents and toxins present the greatest risk of deliberate misuse with significant potential for mass casualties or devastating effect to the economy, critical infrastructure, or public confidence, and pose a severe threat to public health and safety:

Tier 1 Select Agents and Toxins		
HHS Agents and Toxins	Overlap Agents	USDA Agents
Botulinum neurotoxins Botulinum neurotoxin producing species of <i>Clostridium</i> Ebola virus <i>Francisella tularensis</i> Marburg virus Variola major virus (Smallpox virus) Variola minor virus (Alastrim) <i>Yersinia pestis</i>	<i>Bacillus anthracis</i> <i>Bacillus cereus var anthracis</i> <i>Burkholderia mallei</i> <i>Burkholderia pseudomallei</i>	Foot-And-Mouth Disease virus Rinderpest virus

Entities that possess, use, or transfer Tier 1 select agents and toxins must adhere to the additional personnel screening and ongoing personal screening requirements. Additional physical requirements are also required for Tier 1 Select Agents.

The Yale Select Agent laboratory has been designed to meet the additional physical security requirements. However, the finalized program for personnel screening has not been set forth as of this version of the manual as Yale University is not currently registered for experiments involving Select Agents.

### 6.4 Permissible Toxin Amounts

The following toxins are not regulated if the amount under the control of a Principal Investigator, treating physician or veterinarian, or commercial manufacturer or distributor does not exceed, at any time, the amounts indicated in the table below.



<i>HHS Toxins [§73.3(d)(3)]</i>	<i>Amount</i>
Abrin	1,000 mg
Botulinum neurotoxins	1.0 mg
Short, paralytic alpha conotoxins	100 mg
Diacetoxyscirpenol (DAS)	10,000 mg
Ricin	1,000 mg
Saxitoxin	500 mg
Staphylococcal Enterotoxins (Subtypes A, B, C, D, and E)	100 mg
T-2 toxin	10,000 mg
Tetrodotoxin	500 mg

Note: Toxin quantities shown are maximum quantities of toxins, in aggregate per PI, allowed by USDA/CDC. Note: Yale's internal watch list limits PIs to only 50% of the quantities shown here.

#### **6.4.1 Toxin due Diligence Requirements**

All Yale Principal Investigators in possession of ANY QUANTITY of the 9 Select Agent Toxins listed above must retain a record of ALL transfers of ANY QUANTITY of these toxins outside their laboratory. The required documentation must include the following information:

- **Name of the recipient**
- **Toxin and quantity transferred**
- **Purpose of use (knowledge of recipient's legitimate need for the toxins)**

#### **6.4.2 Reporting Suspected Violations or Suspicious Activity**

If a Yale Principal Investigator in possession of ANY QUANTITY of the 9 Select Agent Toxins listed above detects suspicious activity associated with a request for toxin or suspicious activity associated with a shipped toxin, s/he must immediately notify the Yale University EHS Contact or the Alternate EHS Contact and the Federal Select Agent program at the contact information below:

Yale's Contact: Mr. Kevin Charbonneau: 203-737-2139 (office) 203-410-8527 (cell)

Yale's Alternate Contact: Mr. Ben Fontes: 203-737-5009 (office) 203-410-6223 (cell)

Email CDC: [LRSAT@cdc.gov](mailto:LRSAT@cdc.gov)

CDC Select Agent Office: 404-718-2000

Anonymous OIG Hotline: 1-800-447-8477 (voice) or 1-800-223-8164 (FAX)

#### **6.5 Registration of Possession, Use or Transfer of Select Agents.**

All activity involving Select Agents must be registered with the Yale Office of Environmental Health & Safety prior to initiation. Please contact the Biosafety Office with Yale EHS or contact your EHS Safety Advisor at 785-3550 to initiate a registration for your proposed Select Agent activity. The following bullets summarize the Select Agent registration and compliance pathway at Yale University.

- Notify Yale EHS of your intent to possess, use or transfer Select Agents.

- Complete an update of your Yale EHS FORM 01 Registration available at <https://ehsis.yale.edu/EHSIntegrator/Registration> with the new agents. (If the Select Agent is a human pathogen, Yale will help you also register with the State of CT Dept. of Public Health).
- Complete FBI Form FD961 and file with Yale EHS for registration with applicable federal entity
- Complete 2 sets of FBI fingerprint cards for initiation of background investigation check
- Complete all Yale EHS applicable training programs (Biosafety, Bloodborne Pathogens, Laboratory Safety, Biosafety Level 3, Select Agent Biosecurity, and Shipping/Transport of Hazardous Biological Agents).
- Complete a Yale EHS Request to Use Infectious Agents Form(s) available at <http://ehs.yale.edu/sites/default/files/files/request-infectious-agents.pdf> and Researcher Experience Form.
- Complete the Yale EHS researcher experience requirements (for BL2+ or BL3 agents)
- Satisfactory completion of EHS laboratory inspection of proposed work practices, safety equipment, and facility for Yale, CDC/NIH, and Select Agent regulatory requirements. (Safety and Security)

Receive final approval and authorization from Yale EHS, FBI, and the applicable governing body that you and each individual requesting access to Select Agents, the proposed storage location for Select Agents, and the Select Agent research areas have been cleared. (This is provided in the form of an approval letter from the Yale Biological Safety Committee). **This is only a general description of the process and it could take 6 to 9 months (or longer) from the time of the initial application to the time a federal inspection is scheduled for the proposed select agent research.**

Your laboratory will be subject to Yale and federal inspection or audit prior to initiation of work and at any time during your possession of Select Agents.

## 6.6 Discovery of Select Agents or Unknown Samples

Please notify EHS immediately if:

- You identify any Select Agent pathogen or toxin listed on the current federal list that was not previously registered by your lab
- You discover a toxin not previously reported by your laboratory in excess of either the Yale Watch List or the federal maximum allowable quantities listed above.
- You discover any unknown materials in your laboratory for assistance with identification.

These discoveries must be reported to the applicable governmental institution.

## 6.7 Intrafacility Transfer of Select Agents

Select agent pathogens and toxins may not be transferred outside of, to, or within Yale University unless EHS and federal approval has been granted. An intrafacility transfer is defined as the transfer of a Select Agent from one EHS and federally registered Select Agent lab to a similarly registered laboratory. Select Agents may not be transferred to a laboratory that is not registered with EHS and the applicable governmental institution. Once approved, intrafacility transfers will be overseen by EHS. Please contact the EHS Biosafety Office for additional information.

## 6.8 Destruction of Select Agents or Unknown Samples

Select Agent pathogens or toxins may not be destroyed until Yale EHS and the applicable government institution has provided approval for the destruction. Once approval has been granted for the destruction of

Select Agents, Yale EHS will officially assume possession of the material and record its destruction. The governing institution will alert Yale if witnesses are required.

If you have any questions regarding the Yale of Federal Select Agent process, please don't hesitate to contact the EHS Biosafety Office or your EHS Safety Advisor at 785-3550.

## **6.9 Additional Information**

Information on the Select Agent Program may be found at the following web sites:

Yale EHS: <http://ehs.yale.edu>

Centers for Disease Control and Prevention: <http://www.selectagents.gov/>

Notice of Exclusion for attenuated strains SAs: <http://www.cdc.gov/od/sap/exclusion.htm>

United States Department of Agriculture: <http://www.usda.gov/>

## **Section 7 Physical Plant Personnel, Maintenance and Visitor Procedures**

### **7.1 Vaccination requirements**

Currently, there are no vaccination requirements for Physical Plant or other maintenance personnel. Hepatitis B virus vaccination is offered free of charge to all occupationally exposed employees as part of the Yale Bloodborne Pathogen Program.

### **7.2 Procedures for entering BSL3 facility**

Please notify EHS for registration and training of visitors prior to their arrival. Each BSL3 facility shall keep a logbook to record when visitors enter and leave the facility. Visitors are all those who normally do not work in the BSL3 facility. For example: Physical Plant employees, EHS Emergency Responders, outside consultants or visitors, etc.

Each BSL3 facility shall post entry requirement procedures. All visitors shall follow the facility entry requirements. All visitors must complete a Biosafety Level 3 Visitor Clearance Form (see section 12.3).

All visitors shall be escorted in the BSL3 facility by a responsible researcher and/or a representative from EHS.

Some BSL3 facilities may need to decontaminate the environmental surfaces prior to entry by Physical Plant personnel or outside consultants. The facility may need up to a three week notice prior to the start of the work project. The laboratory workers shall shut down the laboratory activities and decontaminate environmental surfaces prior to entry by Physical Plant personnel or outside consultants.

All visitors shall follow exit procedures including removing PPE and hand washing.

### **7.3 Personal Protective Equipment (PPE)**

Each BSL3 facility shall post the PPE entrance requirements for entering clean and dirty areas of the BSL3 facility.

Read the entrance sign on the outer and inner facility doors. Obey all posted entrance requirements and all other signs.

### **7.4 Responsibilities of the BSL3 facility users**

Working with Physical Plant personnel, Consultants, visitors and EHS to develop and revise Biosafety procedures for Physical Plant personnel and Consultants.

Follow all established Biosafety Level 3 and laboratory specific protocols.

Follow and complete the checklist for maintenance work in BSL3 facilities for each maintenance request in a BSL3 lab (see section 12.4).

Provide laboratory specific information (including current information on all contact personnel) to Physical Plant, visitors and Office of Environmental Health and Safety.

Provide all necessary PPE for entering BSL3 facility to Physical Plant, visitors and EHS.

Post entry and exit requirements for BSL3 facility.

Supplement this document with specific entry requirement procedures for their specific BSL3 facility. A copy of the supplement shall be submitted to EHS.

## **7.5 Responsibilities of Physical Plant Personnel and Visitors**

Follow all posted entry/exit procedures.

Contact designated laboratory personnel and schedule times when Physical Plant personnel and visitors may enter the laboratory.

Avoid the manipulation of experimental materials and supplies on bench tops, in refrigerators, incubators, freezers and biological safety cabinets.

## **Section 8 Registering Your Work with the EHS-Biosafety Office**

The EHS-Biosafety Office must be contacted before:

- work with a new infectious agent is initiated
- changing the scope or location of existing work
- providing infectious agents to another investigator on or off campus
- arranging for visiting researchers to work in your laboratory.

Please note that Select Agents are organisms or toxins that have been identified as potential agents of biological or agricultural terrorism. Institutions and individuals who possess Select Agents must be registered and cleared by U.S. Government agencies including the Department of Health and Human Services (DHHS), Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), and the Federal Bureau of Investigation (FBI.) All transfers of Select Agents must be approved by the Government prior to transport.

You are required to register with EHS and CDC and/or USDA prior to working with or transferring select agents to or from Yale University. Registration is also required before use. For additional information, please consult the EHS web site at <http://ehs.yale.edu/select-agents-bsl-3-research> or contact EHS - Biosafety Office at 785-3550.

\*Work with Risk Group 4 agents (Biosafety Level 4) is not permitted at Yale University.

### **8.1 Registration of Biological Materials**

All Principal Investigators are required to complete and submit a "Form 01 Registration for the Use of Biological Materials" (Form 01) available at <https://ehs.yale.edu/EHSIntegrator/Registration>. The Office of Environmental

Health and Safety must maintain accurate information regarding the use of biological materials (e.g., microorganisms, cell lines, human materials, animals, and toxins) by University personnel. EHS policy requires all Principal Investigators to submit accurate information annually and when there are changes during the year regarding the addition or deletion of biological materials, addition or deletion of employees or changes in room locations.

Please note that the Form 01 is reviewed for compliance with the annual training requirements specified by the Occupational Safety and Health Administration (OSHA), the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids (NIH Guidelines)* and the Connecticut Department of Public Health. The Biosafety Office will assist in updating information during the annual biological and chemical safety lab inspection.

The Form 01 is available in online through the EHS Integrator at <https://ehsis.yale.edu/EHSIntegrator/Registration>.

## **8.2 Request to Use Infectious Agents**

Principal Investigators are required to complete a “Request to Use Infectious Agent Form” available at <http://ehs.yale.edu/sites/default/files/files/request-infectious-agents.pdf> and submit it to the Biosafety Office for the Biological Safety Committee approval to work with the infectious agent.

Registration with the State of Connecticut Department of Public Health (State) and the Biosafety Office is required before the initiation of research with a human etiologic agent. The Biosafety Office will assist with the State registration process and any required updates.

The Yale Animal Care and Use Committee, Yale Animal Resources Center, and Biosafety Office must approve all experiments involving the introduction of infectious agents or potentially hazardous biological materials into animals prior to initiation.

Principal Investigators and/or Lab Supervisors must contact the Biosafety Office (785-3550) before initiating work with Risk Group 2 or Risk Group 3 agents to ensure appropriate registration. The Classification of Etiologic Agents is available as part of the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids (NIH Guidelines)*. Copies of the NIH guidelines are available through the Biosafety Office or through the Internet at the following web address: [http://oba.od.nih.gov/rdna/nih\\_guidelines\\_oba.html](http://oba.od.nih.gov/rdna/nih_guidelines_oba.html). Call the EHS-Biosafety Office for assistance with agents that are not listed.

## **8.3 Recombinant DNA Registration**

Yale Biological Safety Committee approval is required prior to the initiation of most non-exempt recombinant DNA experiments. Principal Investigators are required to complete and submit a “Registration of Experiments Involving Recombinant or Synthetic Nucleic Acid Molecules” for each non-exempt project.

Principal Investigators and/or Lab Supervisors must contact the EHS-Biosafety Office to:

- register non-exempt recombinant DNA work
- update current registration if the scope of the work has changed
- ask any questions regarding recombinant DNA work.

The “Registration of Experiments Involving Recombinant or Synthetic Nucleic Acid Molecules” is available from the Biosafety Office or the EHS web site. The *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids (NIH Guidelines)* is available from the Biosafety Office and the EHS web site at <http://ehs.yale.edu/>.

Protocols for research involving recombinant DNA must be submitted to Biosafety at Yale Environmental Health and Safety. The Biosafety Officer will review and submit the application to the Biological Safety Committee.

## **Section 9 Autoclave Validation**

State regulation requires autoclaves used for final destruction of biomedical waste to be evaluated with respect to effectiveness of the sterilization process, temperature and pressure at least once during every forty hours of operation. A log recording temperature and pressure readings and results of biological tests is also required. EHS requires laboratory autoclaves to be tested quarterly if used for initial decontamination of biomedical waste before shipping off campus for incineration.

Steam sterilization is the most reliable means for complete destruction of all microbial life including bacterial spores. Autoclaves accomplish this by generating moist, high temperature, pressurized steam within a sealed chamber.

There are two types of steam sterilizers referred to as autoclaves:

- Gravity flow sterilizers must reach a temperature of 250°F (121°C) at 15 pounds per square inch of pressure for 60 minutes.
- Vacuum type sterilizers must reach a temperature of 270°F (132°C) at 27 pounds per square inch of pressure for 45 minutes.

Personal protection equipment (PPE) such as rubberized aprons, full face shields and heat and liquid resistant gloves must be worn when operating autoclaves.

Each appropriately packaged item in a load must be placed so that steam penetrates into and among all materials to be decontaminated. Tightly sealed or stoppered materials may not be effectively decontaminated and may become dangerously pressurized causing injury when removed from the autoclave.

Routine physical controls such as pressure gauges and temperature gauges are to be considered secondary methods of ensuring a sterilization cycle. Regular chemical monitoring of temperature and periodic biological monitoring must be performed and recorded.

### **9.1 Biological Indicator Tests**

Biological indicator tests must be conducted on steam sterilizers periodically to evaluate the effectiveness of the sterilization process. Tests are conducted using a spore strip or a vial test containing *Bacillus stearothermophilus*. Biological indicators placed at locations through the autoclave are the best indication of sterilization.

Note: Autoclave temperature sensitive tape is not a reliable means to determine if the time, temperature and pressure combination of the process was adequate to penetrate and kill microorganisms contained within the

load. Temperature sensitive tape only indicates that the desired temperature was reached, it does not indicate that the appropriate temperature and pressure were achieved.

- Tests must include the capacity of the sterilization process to kill *Bacillus stearothermophilus*.
- A log must be maintained to record:
  - dates of test
  - test results

## **9.2 Chemical Test**

Temperature sensitive tape must be affixed to the primary containers of medical waste to indicate the desired temperature was reached.

If the temperature sensitive tape does not indicate a temperature of at least 250°F (121°C) was reached during the sterilization process, the biomedical waste is not considered decontaminated.

## **Section 10 Forms**

Forms required to be submitted to EHS prior to working with agents requiring BSL3 containment are provided below.

### **10.1 Request to Use Infectious Agents Form**

Please complete the Request to Use Infectious Agents Form available at <http://ehs.yale.edu/sites/default/files/files/request-infectious-agents.pdf> and return your completed form to Yale Environmental Health and Safety.



## 10.2 Requirements for BSL3 Experimentation in Animals

The approval of your protocol is contingent upon the satisfactory completion of the following requirements.

### Registration

All animal protocols are first submitted to the Yale Animal Care and Use Committee (YACUC). If the animal work involves a hazardous biological, chemical, or radioactive agent, a “Request to use Hazardous Agents in Animals Form” must be completed and filed with YACUC. YACUC will process the form and forward it to the Yale Animal Resources Center (YARC). YARC will communicate this information to the Office of Environmental Health and Safety (EHS) for review. EHS will determine if additional registrations, such as rDNA and State of CT Dept. of Public Health, are necessary. EHS will submit registrations for the proposed work to the Yale Biological Safety Committee (BSC).

### Training

All researchers who plan experiments involving BSL3 agents must attend EHS Biosafety Level 3 laboratory safety training. Researchers should have prior experience with the microbiological techniques required for the safe handling of Level 3 agents. Worker experience forms can be used to document prior education and experience with these agents. Animal BSL3 researchers must also participate in a YARC Animal BSL3 Facility Orientation provided by YARC with EHS Biosafety personnel. The orientation is a scheduled walkthrough that covers:

- Entry & exit requirements;
- Personal protective equipment;
- \*Containment and \*\*Labeling;
- Transport of agents and animals;
- Cage handling and decontamination; and
- Disposal of carcasses, bedding and waste.

\*All rodents inoculated with BSL3 agents will be housed in Isolator cages. Caging and animals will be serviced in a Class II biological safety cabinet.

\*\*All agents in use in an animal room must be posted on the ABSL3 door sign.

The specific agent in use must be noted on the cage card.

Researchers complete the series by attending the YACUC training that is required for all animal users. An agent specific safety protocol will be generated by YARC, EHS Biosafety, and the P.I. for the proposed work.

### Medical Surveillance

ABSL3 researchers must enroll in the University medical surveillance program for an evaluation with the Employee Health Physician. A baseline serum sample will be collected and if necessary, appropriate immunization(s) will be administered. Researchers will not be cleared for ABSL3 work until the medical evaluation has been completed.

### Final YACUC Approval

Researchers will be allowed to initiate the experiments after YARC, EHS, and Medical Surveillance requirements have been satisfied. A written approval letter from the Yale BSC will also be required to initiate the BSL3 work. Yale BSC approval is contingent upon the preparation of a formal written risk assessment by the P.I. that outlines the standard operating procedures along with an equipment and supply inventory. The written risk assessment is discussed at a full BSC meeting (the P.I. is usually invited to present the proposal). Once the proposal is approved, a written letter will be submitted to the P.I., YARC, and YACUC outlining the conditions for the work.

YACUC will approve your project once all animal protocol issues have been resolved.

Security access for each individual will be authorized by YARC when the above requirements have been satisfactorily completed.

### 10.3 Registration for Biosafety Level 3 (BSL3) Research in Animals

YALE UNIVERSITY  
Office of Environmental Health & Safety  
Registration for Biosafety Level 3 (BSL3) Research in Animals

All researchers requesting access to BSL3 animal research facilities must complete the following registration form prior to initiation of research in animals.

NAME: \_\_\_\_\_ Net ID# \_\_\_\_\_

LAB ADDRESS: \_\_\_\_\_ PHONE: \_\_\_\_\_

YACUC PROTOCOL #: \_\_\_\_\_  
(if known)

Approvals Obtained

- Yale Animal Care and Use Committee (YACUC)
- Yale Animal Resources Center (YARC)  
Request to use Hazardous agents in animals
- Office of Environmental Health and Safety (EHS)  
Infectious agent, rDNA, State of CT.

Training

- YARC Orientation to BSL3 facilities (scheduled walkthrough)
- EHS BSL3 Laboratory Safety Training
- Worker Experience Forms (to document training in microbiological techniques)
- YACUC Orientation

Enrollment in Yale Medical Surveillance Program

- Consultation with Employee Health Physician, University Health Services

-Baseline serum, evaluation, and immunization(s) if necessary

Approval Letter from the Yale Biological Safety Committee (BSC)

- Formal Risk Assessment completed by Principal Investigator
- Standard Operating Procedures prepared
- Yale BSC approval letter received by the Principal Investigator, YARC, and YACUC

**For Administrative Use Only**

Approved by:

	Date	Comments
_____ EHS Biosafety	_____	_____
_____ Chief, Employee Health	_____	_____
_____ Yale Animal Resources Center	_____	_____
_____ Yale Biological Safety Committee	_____	_____
_____ Yale Animal Care & Use Committee	_____	_____



**10.5 Table of Physical Containment Devices & PPE to Be Used With Special Agents or Tasks within Research Protocol**

Principal Investigator: \_\_\_\_\_ Date: \_\_\_\_\_

Research Protocol: \_\_\_\_\_

Brief Description of Task (type manipulation and/or equipment used)	Physical Containment Devices Used (such as Biosafety Cabinet, Centrifuge Cups, etc.)	Personal Protective Equipment Worn



## 10.6 SAMPLE OF EQUIPMENT DECONTAMINATION TAG

### BIOSAFETY NOTICE

This equipment's exterior and interior surfaces were decontaminated, and are free of Biological Hazards. This notice does not apply to radiation or chemical hazards (if any).

This equipment is released for: (circle one)

Service/Repair

Relocation

Discard

Decontamination performed by: \_\_\_\_\_

Chemical or disinfectant used: \_\_\_\_\_

Date of decontamination: \_\_\_\_\_

Location of equipment: \_\_\_\_\_

Lab telephone number: \_\_\_\_\_

Note: The following areas \_\_\_\_\_ of this equipment remain contaminated and a biohazard warning label has been attached near the contaminated area.

Additional forms are available through the Office of Environmental Health and Safety.



Yale University

Office of Environmental Health and Safety 785-3550





**10.7 Annual BSL3 Responsibilities Review Form**

**Annual BSL3 Responsibilities Review**

This document records the review of BSL3 responsibilities with research personnel. The Review covered:

- Review signs and labels.
- Methods for recognizing tasks that may involve exposure.
- Review the use and limitations of engineering controls (e.g. review centrifugation protocols).
- Discuss autoclave function and proper procedure for the autoclaving of waste.
- Demonstrate work within a BSC with props to show safe work practices and how contaminated items are to be discarded.
- Review the use and limitations of safe work practices which all users of the facility are expected to follow.
- Review the use and limitations of PPE including types, location, removal, handling, decontamination and disposal - and using a PAPR - RACAL respirators.
- Review and discuss spill procedures.
- Review Exposure Incident procedures.
- Walk-through of the facility showing the sign in/out procedure, donning and removing PPE, show location of all alarms and their meanings.
- Opportunity for questions.

I certify that I have reviewed the above items with my research personnel and they understand the BSL3 practices for the safe handling and use of BSL3 agents.

\_\_\_\_\_  
(Signature of PI)

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

\_\_\_\_\_  
(Print Name of PI)

<b>Print Researchers Name</b>	<b>Signature of Researcher</b>

Return form to EHS-Biosafety, 135 College St or Fax 785-7588

## Section 11 BSL3 Personnel Training

### 11.1 BSL3 Personnel Training Record

#### TRAINING FOR BSL3 LABORATORY PERSONNEL

Name: \_\_\_\_\_

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

The following training is required for all those working directly with BSL3 agents. This document records that training has been received by the undersigned and the Principal Investigator. All new personnel must be registered with the Biosafety Committee prior to initiating work.

1. Read the most current version of the BSL3 Laboratory Safety Manual carefully. Date: \_\_\_\_\_
2. Review BSL3 Work Practices. Date: \_\_\_\_\_
3. Attend the EHS BSL3 Training (Biosafety). Date: \_\_\_\_\_
4. Successfully feed and maintain a suitable uninfected cell line (outside of the BSL3 laboratory). Date: \_\_\_\_\_
5. Gain two months experience working with viral pathogens classed at Biosafety Level 2 (BSL2). Date: \_\_\_\_\_
6. Demonstrate effective use of the biological safety cabinet and centrifuge for containment of aerosols generated during work with BSL3 agents. Date: \_\_\_\_\_
7. Watch procedures used for BSL3 agents. Date: \_\_\_\_\_
8. Work with the BSL3 agent under supervision for 15 hours. Date: \_\_\_\_\_

#### PREVIOUS LABORATORY EXPERIENCE:

\_\_\_\_\_  
(Signature of Research Participant) Date: \_\_\_\_\_

\*\*\*\*\*

I certify that I have examined \_\_\_\_\_ as described above and he/she has acceptable laboratory experience and knowledge of the required Biosafety Level 3 practices for the safe handling and use of BSL3 agents.

\_\_\_\_\_  
(Signature of an Approved BSL3 Researcher/PI) Date: \_\_\_\_\_

\_\_\_\_\_  
(Print Name of an Approved BSL3 Researcher/PI) Date: \_\_\_\_\_



## 11.2 Memorandum of Understanding and Agreement for PI

### MEMORANDUM OF UNDERSTANDING AND AGREEMENT for Principal Investigators

To: Yale Biological Safety Committee

I have read, understand and will comply with the Yale BSL3 Laboratory Safety Manual and the CDC/NIH BSL3 practices. As a Principal Investigator, I understand that it is my responsibility to inform individuals under my supervision of the risks associated with this research, to ensure their training in handling materials, and to ensure compliance with applicable safety regulations and emergency procedures.

Before transferring material from the BSL3 laboratory in which I work to other laboratories within the University, I will obtain the approval of the EHS Biological Safety Officer and conform to all regulations. Before transferring BSL3 material from the BSL3 laboratory in which I work to investigators at a laboratory outside the University, I will inform investigators of the nature of the material and the BSL3 practices required for safe handling, and I will contact the Biological Safety Officer to ensure the shipment is in compliance with applicable transport regulations.

I will notify the Yale Biological Safety Committee and the EHS Biological Safety Officer immediately concerning any research-related accident, exposure incident or release of BSL3 materials to the environment; any problems pertaining to the implementation of biological and physical containment procedures; or any violations of BSL3 requirements. Upon request I will promptly submit a written report about any of these matters.

\_\_\_\_\_  
Principal Investigator

\_\_\_\_\_  
Date

\_\_\_\_\_  
Department

\_\_\_\_\_  
Phone Number

\_\_\_\_\_  
Reviewed and Accepted by the  
Chairman, Yale Biological Safety Committee

\_\_\_\_\_  
Date



### 11.3 BSL3 Users Memorandum of Understanding and Agreement

#### Memorandum of Understanding and Agreement For Authorized Users

To: Yale Biological Safety Committee

I have read, understand, and will comply with the Yale BSL3 Laboratory Safety Manual and CDC/NIH BSL3 practices. I am familiar with the required biosafety practices, techniques and emergency procedures.

I have been informed of the risks associated with this research, and I am participating voluntarily. I have received instruction in the use of the BSL3 laboratory from my Principal Investigator and the Biological Safety Officer.

Before transferring material from the BSL3 laboratory in which I work to other Laboratories within the University, I will seek the approval of the EHS Biological Safety Officer and conform to all regulations. Before transferring BSL3 material from the BSL3 laboratory in which I work to investigators at a laboratory outside the University, I will inform investigators of the nature of the material and the BSL3 practices required for safe handling, and I will contact the Biological Safety Officer to ensure the shipment is in compliance with applicable transport regulations.

I will notify my supervisor, the Yale Biological Safety Committee and the EHS Biological Safety Officer immediately concerning any research-related accident, exposure incident or release of BSL3 materials to the environment; any problems pertaining to the implementation of biological or physical containment procedures; or any violations of BSL3 requirements. I will cooperate in any investigation of any of these matters.

\_\_\_\_\_  
Signature of Research Participant

\_\_\_\_\_  
Signature of Supervisor

\_\_\_\_\_  
Print Name

\_\_\_\_\_  
Print Name

\_\_\_\_\_  
Date

\_\_\_\_\_  
Date

\_\_\_\_\_  
Campus Address (Bldg./Rm.#)

\_\_\_\_\_  
Phone Number





## 11.4 Memorandum of Understanding and Agreement for PI Storing BSL3 Agents

### MEMORANDUM OF UNDERSTANDING AND AGREEMENT for Principal Investigators Storing BSL3 Agents

To: Yale Biological Safety Committee

I have read, understand and will comply with the Yale BSL3 Laboratory Safety Manual and the CDC/NIH BSL3 practices. As a Principal Investigator, I understand that it is my responsibility to inform individuals under my supervision of the risks associated with this research, to ensure their training in handling materials, and to ensure compliance with applicable safety regulations and emergency procedures.

Before transferring material from the BSL3 laboratory in which I work to other laboratories within the University, I will obtain the approval of the EHS Biological Safety Officer and conform to all regulations. Before transferring BSL3 material from the BSL3 laboratory in which I work to investigators at a laboratory outside the University, I will inform investigators of the nature of the material and the BSL3 practices required for safe handling, and I will contact the Biological Safety Officer to ensure the shipment is in compliance with applicable transport regulations.

I will notify the Yale Biological Safety Committee and the EHS Biological Safety Officer immediately concerning any research-related accident, exposure incident or release of BSL3 materials to the environment; any problems pertaining to the implementation of biological and physical containment procedures; or any violations of BSL3 requirements.

Upon request I will promptly submit a written report about any of these matters.

\_\_\_\_\_  
Principal Investigator

\_\_\_\_\_  
Date

\_\_\_\_\_  
Department

\_\_\_\_\_  
Phone Number

\_\_\_\_\_  
Reviewed and Accepted by the  
Chairman, Yale Biological Safety Committee

\_\_\_\_\_  
Date



## 11.5 Researcher Experience Form

### RESEARCHER EXPERIENCE FORM

Name: \_\_\_\_\_ Job Title: \_\_\_\_\_

Principal Investigator: \_\_\_\_\_ Date: \_\_\_\_\_

Net Id: \_\_\_\_\_

Education:

Date	Institution	Major Area	Degree

Laboratory experience related to work with microorganisms or cell culture:

Date	Institution	Description of work

12/2003



## 11.6 BSL3 Observation Checklist

Yale Biological Safety Committee  
BSL3 Subcommittee Observation Checklist

Date:

Time:

BSL3 Lab Location:

Type of Research:       In vitro       In vivo       Insectary       Other

Description if necessary:

Researcher(s) Observed:

Supporting Researcher(s):

BSL3 Subcommittee Observers:

Safety Requirement or Procedure	Observation Result (Pass/Fail/ N/A)	Comments:
Entry to BSL3 Lab		
Knowledge of airflow alarm operation		
PPE for BSL3 work (gown, double gloves, face protection, other)		
Preparation for BSL3 work & Set up of biosafety cabinet		
Vacuum system traps and filter		
Centrifugation Procedure (safety buckets/BSC load/unload)		
Sharps safety (absence of or safe use)		
Knowledge of effective operation of BSC (grilles clear, no flame, minimization of hand movement in and out of unit)		
BSL3 work practices (minimize aerosol formation, safe pipetting)		
Waste collection within BSC		
Safe entry/exit in BSC during work		
Labels (equipment, transport)		
Transport (labeled, leakproof)		
Storage (labeled, segregated)		
Collection and sealing waste inside BSC		
Disinfection/Decontamination of the BSC		
Awareness of Disinfectants effective against agent in use		
Removal of items from BSC		
Removal and disposal of PPE		
Continued on back of page		

<b>Safety Requirement or Procedure</b>	<b>Observation Result (Pass/Fail/ N/A)</b>	<b>Comments:</b>
Hand washing		
Exit from Laboratory		
Awareness of use of eyewash		
Knowledge of facility contingency plans (autoclave failure, failure of BSC)		
Awareness of BSL3 spill response procedures		
Awareness of Fire Alarm response		
Awareness of facility airflow alarm		
Awareness of post-exposure response procedures		
Awareness of signs/symptoms of infection of agent in use		
Awareness of Yale BSL3 Manual		
Awareness of site specific SOP		
Awareness of BSL3 Maintenance policies (general entry)		
Awareness of Select Agent Storage, Inventory, and Security Requirements		
Medical Surveillance Requirements completed		
Training Requirements completed		
Awareness of requirement to consult PI if skin non-intact		

Notes:

## Section 12 BSL3 Checklists

### 12.1 BSL3 Laboratory Checklist

#### LABORATORY BIOSAFETY CHECKLIST

Principal Investigator \_\_\_\_\_ Date \_\_\_\_\_

Bldg \_\_\_\_\_ Room # \_\_\_\_\_ Phone \_\_\_\_\_

Present Use:  cell culture  rDNA  infectious agents  animals  human material

Biosafety Level \_\_\_\_\_ Biosafety Officer \_\_\_\_\_

#### **BSL3: All work in a Biological Safety Cabinet or other physical containment device**

Agents in use:

Agents in storage:

Brief description of experiments:

List of authorized users:

Who is responsible for the BSL3 Laboratory when you are off-campus?  
(Are BSL3 experiments allowed when you are away, who directs the lab in your absence?)

Do above workers have education and experience with these agents?  Yes  No  
Have these workers been trained in the safe handling of these agents?  Yes  No  
Have workers received universal precautions training if applicable?  Yes  No  
Are all accidents and spills reported to the Principal Investigator?  Yes  No  
Are exposures immediately reported to Health Services for follow-up?  Yes  No

Are there written procedures for:  
-cleaning and decontaminating the laboratory and equipment?  Yes  No  
-spill, accident, and exposure incident response?  Yes  No  
-standard operating procedures?  Yes  No  
Is a biological spill kit available?  Yes  No

Are emergency response procedures posted for spills and accidents?  Yes  No  
Do lab workers know the proper response in event of a fire?  Yes  No  
Is fire extinguisher available and up to date?  Yes  No  
Is fire alarm audible from within BSL3 Lab?  Yes  No

Is there a written equipment and supply inventory for BSL3 experiments?  Yes  No  
Has plastic been substituted for glass wherever feasible?  Yes  No

#### MEDICAL SURVEILLANCE

- Is there a medical surveillance program for researchers? Yes No
- Is serum stored before, during, and at termination of BSL3 experiments? Yes No
- Do immunosuppressed researchers (i.e. pregnant, those on steroid or cytotoxic drug treatment) consult with PI prior to beginning work? Yes No
- Do workers with cuts, abrasions, dermatitis, eczema, or other form of compromised skin condition consult with PI before beginning work? Yes No
- Are workers restricted from working unless waterproof bandages can appropriately cover the affected area? Yes No

**LABORATORY FACILITIES:**

- Is lab separate from unrestricted traffic flow? Yes No
- Are there 2 sets of (self-closing) doors? Yes No
- Are doors kept closed? Yes No
- Do researchers follow appropriate entry procedures ( is outer door allowed to close before the inner door is opened)? Yes No
- Can lab personnel verify proper airflow direction? Yes No
- What is the magnehelic gauge reading? \_\_\_\_\_ “ WG

Sketch of BSL3 Facility and indication of airflow with smoke tube:

- Is access restricted? Yes No
- Is a door sign posted detailing name of agent, biosafety level, entry requirements, and name and location of PI or emergency contact? Yes No
- Is janitor access allowed? Yes No
- If yes, is access only allowed when work is not in progress, when all agents are properly stored away, and only to treat floors (wet mop only, no sweeping)? Yes No
- Is a foot, elbow, or automatically operated sink available near exit door? Yes No
- Is there a supply of soap and paper towels? Yes No
- Are interior surfaces water resistant? Yes No
- Are floor, walls, and ceiling impervious? Yes No
- Are joints and penetrations sealed or capable of sealing? Yes No
- Is lighting flush against ceiling? Yes No
- Are windows closed and sealed? Yes No
- Is an autoclave available for decontamination of biological waste? Yes No
- Location of autoclave \_\_\_\_\_
- Is waste transported to autoclave in a leakproof container? Yes No
- Is an eyewash available within the laboratory (or in adjacent room)? Yes No
- Is there a system of communication from the lab to the outside? Yes No
- Is emergency power available for the lab or equipment? Yes No
- Have back-up locations been identified for storage or use in the event of equipment failure? Yes No
- If so, how are materials transported in a non-breakable, labelled, leakproof, plastic container? Yes No



Is required equipment located within the lab? Yes No  
 centrifuge\_\_\_\_\_

shaker\_\_\_\_\_

sonicator\_\_\_\_\_

other\_\_\_\_\_

Are containment devices used (safety buckets, sealed rotors, or sealed tubes) or is the equipment placed inside the biological safety cabinet during use? Yes No

Type of vacuum system: central pump aspirator

Are vacuum traps and filters used to protect the vacuum system? Yes No

Are agents stored in a secure location with limited access? Yes No

Is the biohazard label placed on equipment used for work with these agents or storing them? Yes No

If liquid N<sub>2</sub> used, is storage limited to vapor phase only? Yes No

Is disinfectant available? Yes No  
 70% ethanol? Yes No  
 1-10% bleach? Yes No  
 1% wescodyne? Yes No  
 other\_\_\_\_\_ Yes No

Are work surfaces (biological safety cabinet) and lab equipment decontaminated after all experiments? Yes No

Is a pest control program in effect (are all insects and rodents reported)? Yes No

Are pipettors available (mouth pipetting is prohibited)? Yes No

Eating, drinking, smoking, and food storage not permitted in lab? Yes No

**WORK PRACTICES**

Are supplies of gloves, back-fastening gowns, tyvek suits, face, respiratory, or other protection available? Yes No

Are required PPE items worn for all work with infectious agents? Yes No

Do workers follow the required entry and exit requirements? Yes No

Do workers check airflow, bring in supplies, don PPE, and make sure door is closed behind them before entering the facility? Yes No

Do workers remove PPE, then step into clean area, wash hands thoroughly, and ensure that door is closed securely prior to leaving the facility? Yes No

Is there an appropriate storage and dressing area? Yes No

Are hands washed each time gloves are removed? Yes No

**BIOLOGICAL SAFETY CABINET**

Is there a biological safety cabinet available in the laboratory? Yes No

Are all manipulations performed inside a biological safety cabinet or other physical containment device? Yes No

Are all supplies placed inside the cabinet prior to initiation of work? Yes No

Is all waste collected inside the cabinet? Yes No

Is the waste bag sealed and wiped down with disinfectant prior to removal? Yes No

Are all items wiped down with disinfectant prior to removal from cabinet? Yes No

Is the interior of the cabinet wiped after experiments (grilles, work surface, sides, rear wall, and inside front view screen)? Yes No

Is the cabinet certified at least annually? Yes No

**TRANSPORT OF BSL3 MATERIALS**

- Are all materials packaged for transport inside the biological safety cabinet?  Yes  No
- Are BSL3 materials placed in leakproof container, then placed in a secondary leakproof, labelled, non-breakable plastic container for transport?  Yes  No
- Is outer surface of container wiped with disinfectant prior to removal from the cabinet?  Yes  No

**CENTRIFUGATION**

- Are centrifuge tubes placed into and removed from safety cups and sealed rotors inside the biological safety cabinet?  Yes  No
- Are tubes and bottles checked for deformities before each use?  Yes  No
- Are o-rings changed if cracked, worn or missing?  Yes  No
- Are primary containers limited to 3/4 full?  Yes  No
- Is the air evacuated through a vacuum trap and filter after each run or is respiratory protection worn when opening the centrifuge?  Yes  No
- Are safety cups and rotors disinfected after each use?  Yes  No
- Is the centrifuge interior decontaminated after each use?  Yes  No

Centrifuge (Model)	Tubes & Speed Adaptors (RPM)	Time (run time)	Time allowed before Opening centrifuge (minutes)
-----------------------	---------------------------------	--------------------	--

- 1.
- 2.
- 3.

**DECONTAMINATION AND DISPOSAL OF BIOLOGICAL WASTE**

- Are all containers of biological waste clearly labelled?  Yes  No
- Is all waste discarded within the biological safety cabinet?  Yes  No
- Is the outside of the bag wiped down with disinfectant before removal?  Yes  No
- Is waste transported to the autoclave in a durable, leakproof container?  Yes  No
- Is infectious waste autoclaved at the end of each work day?  Yes  No

Method for decontamination of laboratory waste?

Type of waste	Method	Contact time
plastics		
recyclable glassware		
liquids		
animals		
other		

- Are autoclaving procedures verified?  Yes  No
- Verification method?

LAB PERSONNEL PRESENT/NOTES:

## 12.2 BSL3 Facility Checklist

### BSL3 Facility Checklist

Principal Investigator \_\_\_\_\_ Bldg \_\_\_\_\_ Room# \_\_\_\_\_

Institution: \_\_\_\_\_ Phone # \_\_\_\_\_

Ventilation:

Description of air-handling system for suite: (Dedicated exhaust? Supply?)

Sketch of lab

1. Is all air discharged from the lab and not re-circulated back into the air supply or building, or other buildings?  Yes  No
2. Is there an airlock, vestibule, or anteroom?  
description: (Double door entry, restricted access)  Yes  No
3. Facility or room area (ft<sup>3</sup>) \_\_\_\_\_
5. Air supply data: \_\_\_\_\_ CFM
6. Air exhaust data: \_\_\_\_\_ CFM
7. # Air changes/hour
8. Is supply and exhaust for room interlocked?  Yes  No  
Will supply cut off if exhaust fails?  Yes  No
9. Is there an alarm for ventilation system failure?  Yes  No
10. Are system failure modes or alarms tested?  Yes  No
11. Is lab smoke tested to verify airflow direction?  
Does air travel from cleanest to dirtiest areas?  Yes  No  
 Yes  No
12. Can lab personnel verify proper air flow direction?  
Magnehelic gauge/Pressure Monitor reading \_\_\_\_\_ “ WG  Yes  No
13. Is Exhaust air HEPA-filtered?  Yes  No

Facility:

1. Is lab separate from unrestricted traffic flow?  Yes  No
2. Is entry through 2 sets of self-closing doors?  
Biohazard door sign posted?  Yes  No  
 Yes  No
3. Is a foot, elbow or automatically operated sink located near the exit door?  
Is the soap dispenser automatically operated also?  Yes  No  
Is there an adequate supply of soap and paper towels?  Yes  No

4. Are interior surfaces water resistant (impervious floor, walls, ceiling)? Yes No
5. Are joints and penetrations in lab sealed? Yes No  
Are windows closed and sealed? Yes No
6. Is lighting flush against ceiling? Yes No  
(solid ceiling, not dropped and easily cleanable)
7. Are alarms audible within the facility? Yes No
8. Is there a method of communication to outside of lab (phone, intercom, or sight lines)? (windows on entry doors) Yes No
9. Is there an emergency back-up system for problems with lab equipment? Yes No  
Have other locations or equipment been identified for back-up storage in case of equipment failure? Yes No
10. Is emergency power available? Yes No  
Has back-up power system been tested? Yes No
11. Is there an autoclave available within the facility? Yes No  
If no, autoclave location \_\_\_\_\_
12. Is an eyewash readily available in facility? Yes No  
If no, eyewash location? \_\_\_\_\_
13. Are vacuum lines protected with disinfectant traps and filters if used for aspiration of infectious materials? Yes No
14. Is a Biological Safety Cabinet available? Yes No  
Type of BSC: Class II Type A Type B1 B2 B3 Type A/B3  
Certification date \_\_\_\_\_  
Thimble connection Hard ducted Exhaust back to room  
If Thimble: 1" gap at top and 1.5" gap on all sides?  
Clearance behind and on sides of BSC? 2 – 12 " behind? 3" on sides?  
Supply air interference with BSC? Yes No

**Other pertinent BSL3 Items:**

- Adequate storage space for supplies of personal protective equipment?
- Exhaust HEPA filter certified?
- Magnehelic gauge in place across HEPA filter to track loading?
- Bag in/Bag out filters?
- Positive seal dampers in supply and exhaust ducts to facilitate decontamination?
- Port available upstream of the filter for leak testing?
- Moveable panel located on downstream side of filter for scanning filter face?
- Location of Exhaust HEPA filters? (at least 50 feet from supply air intake)
- Ducts within building under negative pressure?
- Exhaust stack exit velocity equal to 2500 fpm?
- 15% differential between supply and exhaust in lab?
- 12-15 air changes per hour?
- 50 CFM infiltration through entry door(s)?
- 0.05 " H<sub>2</sub>O pressure gauge at entry way(s)?
- Exhaust fan protected with a lock so it can't be shut down?
- Sink drains connected directly to a sanitary sewer?
- Sink available in anteroom?
- Coved floors continuous with walls in facility?
- Sight lines into laboratory from outside containment area?

- Interior surfaces (floors, walls, ceiling) impervious?
- Ventilation over the autoclave (size of area, temp. build up an issue?)
- Waste transport policy (from lab to autoclave) prepared?
- Animals and plants unrelated to BSL3 work prohibited from the lab?
- Glassware minimized or eliminated?
- Centrifuge containment available?
- BSL3 spill response kit available outside of the laboratory?
- Plan prepared for facility decontamination or shut down?
- Protocol for maintenance work in the BSL3 lab prepared?
- List of authorized BSL3 users on file with the biosafety committee?
- Local Fire and Police officials notified of facility emergency response plans and kept abreast of current work activities?

Animal Areas:

- |  |                              |                             |
|--|------------------------------|-----------------------------|
| 1. Are floor drains filled with disinfectant or water?               | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| 2. Is an inventory kept, and are methods in place to prevent escape? | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| 3. Is the floor free of seams or cracks?                             | <input type="checkbox"/> Yes | <input type="checkbox"/> No |

Continuous maintenance program is essential:

- ✓ Lab smoke tested periodically to ensure airflow is inward
- ✓ All facility problems reported to PI immediately
- ✓ BSC and Exhaust HEPA filters certified annually?
- ✓ Ongoing preventive maintenance program – fans inspected every 6 – 12 months (lab decontaminated over weekend and belts and other parts checked)
- ✓ Spare ventilation system parts kept on hand within the facility

Air balancing recommended annually (to update air changes per hour and pressure differential readings, compare the buildings pressure readings to the contractors)

**12.3 BSL3 Visitor Clearance Form**

**Yale University**  
and Safety

Office of Environmental Health

135 College Street, 1<sup>st</sup> Floor  
New Haven, Connecticut 06510-2411  
Telephone: 203-785-3550  
Fax: 203-785-7588

**Biosafety Level 3 Visitor Clearance Form**

This form must be completed by visitors to the BSL3 Laboratory

Name: \_\_\_\_\_

Affiliation: \_\_\_\_\_

Address: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

To be completed at start of visit:

Laboratory Location: \_\_\_\_\_

I have been instructed on the BSL3 facility entry & exit procedures, and have been provided personal protective equipment and instructed on its appropriate use. I will not be handling any infectious material, and I will not touch any laboratory equipment, work surfaces, or other items within the facility.

Signature: \_\_\_\_\_

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

To be completed, signed and dated at the end of the visit:

I have observed all the Biosafety requirements as stated above and those explained to me prior to entry into the facility. If an incident or breach in Biosafety precautions occurred, an incident report has been completed, and a medical evaluation has been arranged through the Yale University Employee Health Services.

Signature: \_\_\_\_\_

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

I, \_\_\_\_\_ (Safety Representative), have provided instructions on the BSL3 entry and exit procedures and information on the appropriate use of personal protective equipment for the visitor named above. The following personal protective equipment was provided and worn by the visitor:  Back fastening gown  gloves  N-95 respirator  mask  booties  eye protection  face shield.

Safety Representative Signature: \_\_\_\_\_ Date: \_\_\_\_\_



## 12.4 Checklist for Maintenance Work in BSL3 Facilities:

### CHECKLIST FOR MAINTENANCE WORK IN BSL3 FACILITIES:

The general framework for the performance of work by those who are not familiar with the hazards associated with the BSL3 laboratory will be to eliminate the hazard prior to entry into the facility. The following checklist should be completed for each maintenance request in a BSL3 lab.

- Notification of Entry: Request for service work submitted by lab personnel to Physical Plant and Occupational Health and Safety (785-3550).
- BSL3 work halted prior to entry:  
Last time and date of work with BSL3 agents: \_\_\_\_\_ / \_\_\_\_\_ / \_\_\_\_\_
- \*note all work with BSL3 agents must be conducted inside a biological safety cabinet or other physical containment device.
- All BSL3 agents have been securely stored away in incubators, freezers or other containment devices
- All BSL3 biological waste has been removed from the lab in sealed bags and taken to the autoclave for decontamination.
- Equipment that will be serviced by maintenance has been decontaminated with a suitable disinfectant. The attached Biological Safety Notice describes the decontamination procedure.
- Work surfaces around the work location within the BSL3 Lab have been decontaminated.

#### Laboratory Entry & Exit

- Entry and Exit procedures have been explained to all service personnel.  
(required PPE worn, log book signed, & workers have any required vaccinations).
- Work surface covers or plastic bags were provided to service personnel for placement of tools
- The workers were accompanied by an authorized BSL3 lab member at all times during the visit.**
- Workers followed the required exit procedures and washed hands before exiting.

I attest that the above information is accurate and complete:

Name(print) \_\_\_\_\_ Signed \_\_\_\_\_ Date: \_\_\_\_\_





## Section 13 Guidelines and Regulations

### 13.1 CDC/NIH Laboratory Biosafety Level Criteria – Biosafety Level 3

(The following information has been reprinted from the Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, Centers for Disease Control and Prevention, National Institutes of Health, U.S Department of Health and Human Services Public Health Service, December 2009.)

#### Biosafety Level 3 (BSL-3)

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices.

A BSL-3 laboratory has special engineering and design features.

The following standard and special safety practices, equipment, and facility requirements apply to BSL-3.

#### A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory. Laboratory Biosafety Level Criteria: BSL-3
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

- a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
- b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
- c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
- d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated

decontamination method). Depending on where the decontamination will be performed, the following methods should be used prior to transport:

- a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
- b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required. (See Appendix G.)
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

## B. Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
  - a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
  - b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor must be used.

### C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.
2. Workers in the laboratory where protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.
3. Eye and face protection (goggles, mask, face shield or other splash guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers:
  - a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
  - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
  - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. Eye, face, and respiratory protection must be used in rooms containing infected animals.

### D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors must be self-closing and have locks in accordance with the institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Laboratory access is restricted. Access to the laboratory is through two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.
2. Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.
3. The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.



## 13.2 CDC/NIH Vertebrate Animal Biosafety Level Criteria-Animal Biosafety Level 3

(The following information has been reprinted from the Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, Centers for Disease Control and Prevention, National Institutes of Health, U.S Department of Health and Human Services Public Health Service, December 2009.)

Animal Biosafety Level 3 involves practices suitable for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission, and agents causing serious or potentially lethal disease. ABSL-3 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-2.

The ABSL-3 laboratory has special engineering and design features.

ABSL-3 requires that: 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 potentially lethal 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, must be conducted in BSCs or by use of other physical containment equipment.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Employee occupational health programs must be implemented.

The following standard and special safety practices, safety equipment, and facility requirements apply to ABSL-3.

### A. Standard Microbiological Practices

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies.
2. Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review.
3. Prior to beginning a study, animal protocols must be reviewed and approved by the IACUC5 and the Institutional Biosafety Committee.
4. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals.
5. The safety manual must be available and accessible. Personnel are advised of potential and special hazards, and are required to read and follow instructions on practices and procedures.
6. Consideration must be given to specific biohazards unique to the animal species and protocol in use.
7. The supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.
8. An appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered.
9. Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with the research, animal husbandry duties, animal care, and manipulations.
10. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of childbearing age should be provided information regarding immune competence and conditions that

may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

11. Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.
12. A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/or animals are housed or are manipulated. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is used within an animal room.
13. Security-sensitive agent information and occupational health requirements should be posted in accordance with the institutional policy.
14. Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.<sup>1,3,4</sup>
15. Access to the animal room is limited to the fewest number of individuals possible. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or are manipulated.
16. All persons, including facility personnel, service workers, and visitors, are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.
17. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
18. Gloves are worn to prevent skin contact with contaminated, infectious/hazardous materials and when handling animals. Double-glove practices should be used when dictated by risk assessment.
19. Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.
20. Persons must wash their hands after removing gloves and before leaving the areas where infectious materials and/or animals are housed or are manipulated.
21. Eye, face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.
22. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
23. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
24. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
25. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.
26. When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions must always be taken with sharp items. These include:
  - a. Use of needles and syringes or other sharp instruments in the animal facility is limited to situations where there is no alternative such as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
  - b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used, disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.
  - c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.



- d. Broken glassware must not be handled directly; it should be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
  - e. Use of equipment with sharp edges and corners should be avoided.
27. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.
  28. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/ or animals are housed or are manipulated.
  29. An effective integrated pest management program is required. (See Appendix G.)
  30. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof containers for appropriate disposal in compliance with applicable institutional, local and state requirements.
  31. Decontaminate all potentially infectious materials before disposal using an effective method.

## B. Special Practices

1. Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms.
2. When appropriate, a base line serum sample should be stored.
3. All procedures involving the manipulation of infectious materials, handling of infected animals or the generation of aerosols must be conducted within BSCs or other physical containment devices when practical.
4. When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used.
5. Restraint devices and practices are used to reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications).
6. The risk of infectious aerosols from infected animals or their bedding also can be reduced if animals are housed in containment caging systems, such as solid wall and bottom cages covered with filter bonnets, open cages placed in inward flow ventilated enclosures, HEPA-filter isolators and caging systems, or other equivalent primary containment systems.
7. Actively ventilated caging systems must be designed to prevent the escape of microorganisms from the cage. Exhaust plenums for these systems should be sealed to prevent escape of microorganisms if the ventilation system becomes static, and the exhaust must be HEPA filtered. Safety mechanisms should be in place that prevent the cages and exhaust plenums from becoming positive to the surrounding area should the exhaust fan fail. The system should also be alarmed to indicate operational malfunctions.
8. A method for decontaminating all infectious materials must be available within the facility, preferably within the areas where infectious materials and/or animals are housed or are manipulated (e.g., autoclave, chemical disinfection, or other approved decontamination methods).
9. Consideration must be given to means for decontaminating routine husbandry equipment, sensitive electronic and medical equipment.
10. Decontaminate all potential infectious materials (including animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse) by an appropriate method before removal from the areas where infectious materials and/or animals are housed or manipulated.
11. It is recommended that animal bedding and waste be decontaminated prior to manipulation and before removal from the areas where infectious materials and/or animals are housed or are manipulated, preferably within the caging system.
12. Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements.



13. Equipment, cages, and racks should be handled in a manner that minimizes contamination of other areas.
14. Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated.
15. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
16. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

### C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. Properly maintained BSCs and other physical containment devices or equipment should be used for all manipulations for infectious materials and when possible, animals. These manipulations include necropsy, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals.
2. The risk of infectious aerosols from infected animals or bedding can be reduced by primary barrier systems. These systems may include solid wall and bottom cages covered with filter bonnets, ventilated cage rack systems, or for larger cages placed in inward flow ventilated enclosures or other equivalent systems or devices.
3. A risk assessment should determine the appropriate type of personal protective equipment to be utilized.
4. Personnel within the animal facility where protective clothing, such as uniforms or scrub suits. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home. Disposable personal protective equipment such as non-woven olefin cover-all suits, wrap-around or solid-front gowns should be worn over this clothing, before entering the areas where infectious materials and/or animals are housed or manipulated. Front-button laboratory coats are unsuitable.
5. Disposable personal protective equipment must be removed when leaving the areas where infectious materials and/or animals are housed or are manipulated. Scrub suits and uniforms are removed before leaving the animal facility.
6. Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.
7. All personnel entering areas where infectious materials and/or animals are housed or manipulated wear appropriate eye, face and respiratory protection. To prevent cross contamination, boots, shoe covers, or other protective footwear, are used where indicated.
8. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.
9. Gloves are worn to protect hands from exposure to hazardous materials.
10. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.
11. Procedures may require the use of wearing two pairs of gloves (double-glove).
12. Gloves are changed when contaminated, glove integrity is compromised, or when otherwise necessary.
13. Gloves must not be worn outside the animal rooms.
14. Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials.
15. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

16. Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

#### D. Laboratory Facilities (Secondary Barriers)

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.
2. Access to the animal facility is restricted.
3. Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open.
4. Entry into the containment area is via a double-door entry, which constitutes an anteroom/airlock and a change room. Showers may be considered based on risk assessment. An additional double-door access anteroom or double-doored autoclave may be provided for movement of supplies and wastes into and out of the facility.
5. A hand-washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility. The sink should be hands-free or automatically operated.
6. If the animal facility has multiple segregated areas where infectious materials and/or animals are housed or are manipulated, a sink must also be available for hand washing at the exit from each segregated area.
7. Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.
8. The animal facility is designed, constructed, and maintained to facilitate cleaning, decontamination and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant.
9. Penetrations in floors, walls and ceiling surfaces are sealed, including openings around ducts and doorframes, to facilitate pest control, proper cleaning and decontamination. Walls, floors and ceilings should form a sealed and sanitizable surface.
10. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Flooring is seamless, sealed resilient or poured floors, with integral cove bases.
11. Decontamination of an entire animal room should be considered when there has been gross contamination of the space, significant changes in usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the animal room must be based on the risk assessment.
12. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
13. Furniture should be minimized. Chairs used in animal areas must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Equipment and furnishings with sharp edges and corners should be avoided.
14. External windows are not recommended; if present, all windows must be sealed and must be resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.
15. Ventilation of the facility should be provided in accordance with the Guide for Care and Use of Laboratory Animals.<sup>1</sup> The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms. This system creates directional airflow, which draws air into the animal room from “clean” areas and toward “contaminated” areas.

16. Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process. HEPA filtration and other treatments of the exhaust air may not be required, but should be considered based on site requirements, specific agent manipulations and use conditions. The exhaust must be dispersed away from occupied areas and air intakes.
17. Personnel must verify that the direction of the airflow (into the animal areas) is proper. It is recommended that a visual monitoring device that indicates directional inward airflow be provided at the animal room entry. The ABSL-3 animal facility shall be designed such that under failure 84 Biosafety in Microbiological and Biomedical Laboratories conditions the airflow will not be reversed. Alarms should be considered to notify personnel of ventilation and HVAC system failure.
18. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas, to facilitate cleaning and minimize the accumulation of debris or fomites.
19. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
20. Cages are washed in a mechanical cage washer. The mechanical cage washer has a final rinse temperature of at least 180°F. Cages should be autoclaved or otherwise decontaminated prior to removal from ABSL-3 space. The cage wash facility should be designed and constructed to accommodate high-pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures during the cage cleaning process.
21. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
22. BSCs (Class II, Class III) must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. Class II BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
23. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or exhausted directly to the outside through a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance.
24. Class III BSCs must supply air in such a manner that prevents positive pressurization of the cabinet or the laboratory room.
25. All BSCs should be used according to manufacturers' specifications.
26. When applicable, equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the animal facility. These HEPA filters should be tested and/or replaced at least annually.
27. An autoclave is available which is convenient to the animal rooms where the biohazard is contained. The autoclave is utilized to decontaminate infectious materials and waste before moving it to the other areas of the facility. If not convenient to areas where infectious materials and/or animals are housed or are manipulated, special practices should be developed for transport of infectious materials to designated alternate location/s within the facility.
28. Emergency eyewash and shower are readily available; location is determined by risk assessment.
29. The ABSL-3 facility design and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to use. Facilities should be re-verified at least annually against these procedures as modified by operational experience.
30. Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services, and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment of the site conditions, or other applicable federal, state or local regulations.a. Floors must be slip resistant,

impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.

- a. b. Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
- b. c. Ceilings should be constructed, sealed, and finished in the same general manner as walls.

Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment.

1. 4. Laboratory furniture
2. re must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.
  - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
3. 5. All windows in the laboratory must be sealed.
4. 6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
5. 7. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
6. 8. An eyewash station must be readily available in the laboratory.
7. 9. A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.
  - a. Laboratory personnel must be able to verify directional airflow. A visual monitoring device, which confirms directional airflow, must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.
  - b. The laboratory exhaust air must not re-circulate to any other area of the building.
  - b. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.
8. HEPA filter housings should have gas-tight isolation dampers, decontamination ports, and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.
9. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer’s recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.
10. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, or other validated decontamination method).

11. Equipment that may produce infectious aerosols must be contained in primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.
12. Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.
13. Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following: an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices, such as biometrics.
14. The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.

### **13.3 CDC/NIH Guidelines for Work with Toxins of Biological Origin**

(The following information has been reprinted from the Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, Centers for Disease Control and Prevention, National Institutes of Health, U.S Department of Health and Human Services Public Health Service, May 1999.)

In recognition of the growing number of microbiological and biomedical laboratories working with toxins of biological origin, the following is provided as a guideline for working with these toxins.

The material below is adapted from the Biological Defense Safety Program, Technical Safety Requirements (DA Pamphlet 385-69) and Appendix A of the United States Department of Labor Occupational Safety and Health Association rule "Occupational Exposure to Hazardous Chemicals in Laboratories".

Laboratory managers and facility safety officials are encouraged to utilize the references listed below and to consult with subject matter experts before using any toxin, to ensure that appropriate facilities, containment equipment, policies and procedures, personnel training programs and medical surveillance protocols specific to the toxin and the laboratory are in place.

#### General

The laboratory facilities, equipment, and procedures appropriate for work with toxins of biological origin must reflect the intrinsic level of hazard posed by a particular toxin as well as the potential risks inherent in the operations performed. If both toxins and infectious agents are used, both must be considered when containment equipment is selected and policies and procedures are written. If animals are used, animal safety practices must also be considered.

#### Standard Practices

Standard practices listed under BSL 2 and BSL 3 should be reviewed and incorporated as appropriate into protocols for work with toxins.

#### Special Practices

Special practices listed under BSL 2 and BSL 3 should be reviewed and incorporated as appropriate into protocols for work with toxins.

1. Each laboratory should develop a chemical hygiene plan specific to the toxin(s) used in that laboratory. The chemical hygiene plan should 1) identify the hazards that will be encountered in normal use of the toxin, and those that could be encountered in case of a spill or other accident, and 2) specify the policies and practices to be used to minimize risks (e.g., containment and personal protective equipment, management of spills, management of accidental exposures, medical surveillance).

2. Training specific to the toxin(s) used should be required and documented for all laboratory personnel working with toxins, before starting work with the toxin and at intervals thereafter.

3. An inventory control system should be in place.

4. Toxins should be stored in locked storage rooms, cabinets, or freezers when not in use.

5. Access to areas containing toxins should be restricted to those whose work assignments require access.

6. Preparation of primary containers of toxin stock solutions and manipulations of primary containers of dry forms of toxins should be conducted in a chemical fume hood, a glove box, or a biological safety cabinet or equivalent containment system approved by the safety officer. HEPA and/or charcoal filtration of the exhaust air may be required, depending on the toxin.
7. The user should verify inward airflow of the hood or biological safety cabinet before initiating work.
8. All work should be done within the operationally effective zone of the hood or biological safety cabinet.
9. When toxins are in use, the room should be posted to indicate "Toxins in Use - Authorized Personnel Only." Any special entry requirements should be posted on the entrance(s) to the room. Only personnel whose presence is required should be permitted in the room while toxins are in use.
10. All high risk operations should be conducted with two knowledgeable individuals present. Each must be familiar with the applicable procedures, maintain visual contact with the other, and be ready to assist in the event of an accident.
11. Before containers are removed from the hood, cabinet, or glove box, the exterior of the closed primary container should be decontaminated and placed in a clean secondary container. Toxins should be transported only in leak/spill-proof secondary containers.
12. Contaminated and potentially contaminated protective clothing and equipment should be decontaminated using methods known to be effective against the toxin before removal from the laboratory for disposal, cleaning or repair. If decontamination is not possible/practical, materials (e.g., used gloves) should be disposed of as toxic waste. Materials contaminated with infectious agents as well as toxins should also be autoclaved or otherwise rendered non-infectious before leaving the laboratory.
13. The interior of the hood, glove box, or cabinet should be decontaminated periodically, for example, at the end of a series of related experiments. Until decontaminated, the hood, box, or cabinet should be posted to indicate that toxins are in use, and access to the equipment and apparatus restricted to necessary, authorized personnel.

#### Safety Equipment

The safety equipment guidelines listed under BSL 2 and BSL 3 (see Section III) should be reviewed and incorporated as appropriate into protocols for work with toxins.

1. When using an open-fronted fume hood or biological safety cabinet, protective clothing, including gloves and a disposable long-sleeved body covering (gown, laboratory coat, smock, coverall, or similar garment) should be worn so that hands and arms are completely covered.
2. Eye protection should be worn if an open-fronted containment system is used.
3. Other protective equipment may be required, depending on the characteristics of the toxin and the containment system. For example, use additional respiratory protection if aerosols may be generated and it is not possible to use containment equipment or other engineering controls.
4. When handling dry forms of toxins that are electrostatic:
  - a. Do not wear gloves (such as latex) that help to generate static electricity
  - b. Use glove bag within a hood or biological safety cabinet, a glove box, or a class III biological safety cabinet.
5. When handling toxins that are percutaneous hazards (irritants, necrotic to tissue, or extremely toxic from dermal exposure), select gloves that are known to be impervious to the toxin.

6. Consider both toxin and diluent when selecting gloves and other protective clothing.

7. If infectious agents and toxins are used together in an experimental system, consider both when selecting protective clothing and equipment.

#### Laboratory Facilities

Laboratory facility recommendations listed under BSL 2 and BSL 3 and OSHA standards should be reviewed and incorporated as appropriate into protocols for work with toxins.

1. Vacuum lines. When vacuum lines are used with systems containing toxins, they should be protected with a HEPA filter to prevent entry of toxins into the lines. Sink drains should be similarly protected when water aspirators are used.





## 13.4 OSHA Bloodborne Pathogen Standard

### The Standard

#### General Industry

Part 1910 of title 29 of the Code of Federal Regulations is amended as follows:

#### PART 1910-[AMENDED]

##### Subpart Z-Amended

1. The general authority citation for subpart Z of 29 CFR part 1910 continues to read as follows and a new citation for 1910.1030 is added:

Authority: Secs. 6 and 8, Occupational Safety and Health Act, 29 U.S.C.655, 657, Secretary of Labor's Orders Nos.12-71 (36 FR 8784), 8-76 (41 FR 25059), or 9-83 (48 FR 35736), as applicable; and 29 CFR part 1911.

.....  
Section 1910.1030 also issued under 29 U.S.C. 653.  
.....

2. Section 1910.1030 is added to read as follows:

##### 1910.1030(a)

**Scope and Application.** This section applies to all occupational exposure to blood or other potentially infectious materials as defined by paragraph (b) of this section.

##### 1910.1030(b)

**Definitions.** For purposes of this section, the following shall apply:

**Assistant Secretary** means the Assistant Secretary of Labor for Occupational Safety and Health, or designated representative.

**Blood** means human blood, human blood components, and products made from human blood.

**Bloodborne Pathogens** means pathogenic microorganisms that are present in human blood and can cause disease in humans. These pathogens include, but are not limited to, hepatitis B virus (HBV) and human immunodeficiency virus (HIV).

**Clinical Laboratory** means a workplace where diagnostic or other screening procedures are performed on blood or other potentially infectious materials.

**Contaminated** means the presence or the reasonably anticipated presence of blood or other potentially infectious materials on an item or surface.

**Contaminated Laundry** means laundry which has been soiled with blood or other potentially infectious materials or may contain sharps.

**Contaminated Sharps** means any contaminated object that can penetrate the skin including, but not limited to, needles, scalpels, broken glass, broken capillary tubes, and exposed ends of dental wires.

**Decontamination** means the use of physical or chemical means to remove, inactivate, or destroy bloodborne pathogens on a surface or item to the point where they are no longer capable of transmitting infectious particles and the surface or item is rendered safe for handling, use, or disposal.

**Director** means the Director of the National Institute for Occupational Safety and Health, U.S. Department of Health and Human Services, or designated representative.

**Engineering Controls** means controls (e.g., sharps disposal containers, self-sheathing needles, safer medical devices, such as sharps with engineered sharps injury protections and needleless systems) that isolate or remove the bloodborne pathogens hazard from the workplace.

**Exposure Incident** means a specific eye, mouth, other mucous membrane, non-intact skin, or parenteral contact with blood or other potentially infectious materials that results from the performance of an employee's duties.

**Handwashing Facilities** means a facility providing an adequate supply of running potable water, soap and single use towels or hot air drying machines.

**Licensed Healthcare Professional** is a person whose legally permitted scope of practice allows him or her to independently perform the activities required by paragraph (f) Hepatitis B Vaccination and Post-exposure Evaluation and Follow-up.

**HBV** means hepatitis B virus.

**HIV** means human immunodeficiency virus.

**Needleless systems** means a device that does not use needles for:

(1) The collection of bodily fluids or withdrawal of body fluids after initial venous or arterial access is established; (2) The administration of medication or fluids; or (3) Any other procedure involving the potential for occupational exposure to bloodborne pathogens due to percutaneous injuries from contaminated sharps.

**Occupational Exposure** means reasonably anticipated skin, eye, mucous membrane, or parenteral contact with blood or other potentially infectious materials that may result from the performance of an employee's duties.

**Other Potentially Infectious Materials** means (1) The following human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids; (2) Any unfixed tissue or organ (other than intact skin) from a human (living or dead); and (3) HIV-containing cell or tissue cultures, organ cultures, and HIV- or HBV-containing culture medium or other solutions; and blood, organs, or other tissues from experimental animals infected with HIV or HBV.

**Parenteral** means piercing mucous membranes or the skin barrier through such events as needlesticks, human bites, cuts, and abrasions.

**Personal Protective Equipment** is specialized clothing or equipment worn by an employee for protection against a hazard. General work clothes (e.g., uniforms, pants, shirts or blouses) not intended to function as protection against a hazard are not considered to be personal protective equipment.

**Production Facility** means a facility engaged in industrial-scale, large-volume or high concentration production of HIV or HBV.

**Regulated Waste** means liquid or semi-liquid blood or other potentially infectious materials; contaminated items that would release blood or other potentially infectious materials in a liquid or semi-liquid state if compressed; items that are caked with dried blood or other potentially infectious materials and are capable of releasing these materials during handling; contaminated sharps; and pathological and microbiological wastes containing blood or other potentially infectious materials.

**Research Laboratory** means a laboratory producing or using research-laboratory-scale amounts of HIV or HBV. Research laboratories may produce high concentrations of HIV or HBV but not in the volume found in production facilities.

**Sharps with engineered sharps injury protections** means a nonneedle sharp or a needle device used for withdrawing body fluids, accessing a vein or artery, or administering medications or other fluids, with a built-in safety feature or mechanism that effectively reduces the risk of an exposure incident.

**Source Individual** means any individual, living or dead, whose blood or other potentially infectious materials may be a source of occupational exposure to the employee. Examples include, but are not limited to, hospital and clinic patients; clients in institutions for the developmentally disabled; trauma victims; clients of drug and alcohol treatment facilities; residents of hospices and nursing homes; human remains; and individuals who donate or sell blood or blood components.

**Sterilize** means the use of a physical or chemical procedure to destroy all microbial life including highly resistant bacterial endospores.

**Universal Precautions** is an approach to infection control. According to the concept of Universal Precautions, all human blood and certain human body fluids are treated as if known to be infectious for HIV, HBV, and other bloodborne pathogens.

**Work Practice Controls** means controls that reduce the likelihood of exposure by altering the manner in which a task is performed (e.g., prohibiting recapping of needles by a two-handed technique).

#### **1910.1030(c) Exposure Control --**

##### **1910.1030(c)(1) Exposure Control Plan.**

**1910.1030(c)(1)(i)**  
Each employer having an employee(s) with occupational exposure as defined by paragraph (b) of this section shall

establish a written Exposure Control Plan designed to eliminate or minimize employee exposure.

##### **1910.1030(c)(1)(ii)**

The Exposure Control Plan shall contain at least the following elements:

##### **1910.1030(c)(1)(ii)(A)**

The exposure determination required by paragraph (c)(2),

##### **1910.1030(c)(1)(ii)(B)**

The schedule and method of implementation for paragraphs (d) Methods of Compliance, (e) HIV and HBV Research Laboratories and Production Facilities, (f) Hepatitis B Vaccination and Post-Exposure Evaluation and Follow-up, (g) Communication of Hazards to Employees, and (h) Recordkeeping, of this standard, and

##### **1910.1030(c)(1)(ii)(C)**

The procedure for the evaluation of circumstances surrounding exposure incidents as required by paragraph (f)(3)(i) of this standard.

##### **1910.1030(c)(1)(iii)**

Each employer shall ensure that a copy of the Exposure Control Plan is accessible to employees in accordance with 29 CFR 1910.1020(e).

##### **1910.1030(c)(1)(iv)**

The Exposure Control Plan shall be reviewed and updated at least annually and whenever necessary to reflect new or modified tasks and procedures which affect occupational exposure and to reflect new or revised employee positions with occupational exposure. The review and update of such plans shall also:

##### **1910.1030(c)(1)(iv)(A)**

Reflect changes in technology that eliminate or reduce exposure to bloodborne pathogens; and

##### **1910.1030(c)(1)(iv)(B)**

Document annually consideration and implementation of appropriate commercially available and effective safer medical devices designed to eliminate or minimize occupational exposure.

##### **1910.1030(c)(1)(v)**

An employer, who is required to establish an Exposure Control Plan shall solicit input from non-managerial employees responsible for direct patient care who are potentially exposed to injuries from contaminated sharps in the identification, evaluation, and selection of effective engineering and work practice controls and shall document the solicitation in the Exposure Control Plan.

##### **1910.1030(c)(1)(vi)**

The Exposure Control Plan shall be made available to the Assistant Secretary and the Director upon request for examination and copying.

##### **1910.1030(c)(2)**

##### **Exposure Determination.**

##### **1910.1030(c)(2)(i)**

Each employer who has an employee(s) with occupational exposure as defined by paragraph (b) of this section shall prepare an exposure determination. This exposure determination shall contain the following:

**1910.1030(c)(2)(i)(A)**

A list of all job classifications in which all employees in those job classifications have occupational exposure;

**1910.1030(c)(2)(i)(B)**

A list of job classifications in which some employees have occupational exposure, and

**1910.1030(c)(2)(i)(C)**

A list of all tasks and procedures or groups of closely related task and procedures in which occupational exposure occurs and that are performed by employees in job classifications listed in accordance with the provisions of paragraph (c)(2)(i)(B) of this standard.

**1910.1030(c)(2)(ii)**

This exposure determination shall be made without regard to the use of personal protective equipment.

**1910.1030(d)**

**Methods of Compliance --**

**1910.1030(d)(1)**

**General.** Universal precautions shall be observed to prevent contact with blood or other potentially infectious materials. Under circumstances in which differentiation between body fluid types is difficult or impossible, all body fluids shall be considered potentially infectious materials.

**1910.1030(d)(2)**

**Engineering and Work Practice Controls.**

**1910.1030(d)(2)(i)**

Engineering and work practice controls shall be used to eliminate or minimize employee exposure. Where occupational exposure remains after institution of these controls, personal protective equipment shall also be used.

**1910.1030(d)(2)(ii)**

Engineering controls shall be examined and maintained or replaced on a regular schedule to ensure their effectiveness.

**1910.1030(d)(2)(iii)**

Employers shall provide handwashing facilities which are readily accessible to employees.

**1910.1030(d)(2)(iv)**

When provision of handwashing facilities is not feasible, the employer shall provide either an appropriate antiseptic hand cleanser in conjunction with clean cloth/paper towels or antiseptic towelettes. When antiseptic hand cleansers or towelettes are used, hands shall be washed with soap and running water as soon as feasible.

**1910.1030(d)(2)(v)**

Employers shall ensure that employees wash their hands immediately or as soon as feasible after removal of gloves or other personal protective equipment.

**1910.1030(d)(2)(vi)**

Employers shall ensure that employees wash hands and any other skin with soap and water, or flush mucous membranes with water immediately or as soon as feasible following contact of such body areas with blood or other potentially infectious materials.

**1910.1030(d)(2)(vii)**

Contaminated needles and other contaminated sharps shall not be bent, recapped, or removed except as noted in

paragraphs (d)(2)(vii)(A) and (d)(2)(vii)(B) below. Shearing or breaking of contaminated needles is prohibited.

**1910.1030(d)(2)(vii)(A)**

Contaminated needles and other contaminated sharps shall not be bent, recapped or removed unless the employer can demonstrate that no alternative is feasible or that such action is required by a specific medical or dental procedure.

**1910.1030(d)(2)(vii)(B)**

Such bending, recapping or needle removal must be accomplished through the use of a mechanical device or a one-handed technique.

**1910.1030(d)(2)(viii)**

Immediately or as soon as possible after use, contaminated reusable sharps shall be placed in appropriate containers until properly reprocessed. These containers shall be:

**1910.1030(d)(2)(viii)(A)**

Puncture resistant;

**1910.1030(d)(2)(viii)(B)**

Labeled or color-coded in accordance with this standard;

**1910.1030(d)(2)(viii)(C)**

Leakproof on the sides and bottom; and

**1910.1030(d)(2)(viii)(D)**

In accordance with the requirements set forth in paragraph (d)(4)(ii)(E) for reusable sharps.

**1910.1030(d)(2)(ix)**

Eating, drinking, smoking, applying cosmetics or lip balm, and handling contact lenses are prohibited in work areas where there is a reasonable likelihood of occupational exposure.

**1910.1030(d)(2)(x)**

Food and drink shall not be kept in refrigerators, freezers, shelves, cabinets or on countertops or benchtops where blood or other potentially infectious materials are present.

**1910.1030(d)(2)(xi)**

All procedures involving blood or other potentially infectious materials shall be performed in such a manner as to minimize splashing, spraying, spattering, and generation of droplets of these substances.

**1910.1030(d)(2)(xii)**

Mouth pipetting/suctioning of blood or other potentially infectious materials is prohibited.

**1910.1030(d)(2)(xiii)**

Specimens of blood or other potentially infectious materials shall be placed in a container which prevents leakage during collection, handling, processing, storage, transport, or shipping.

**1910.1030(d)(2)(xiii)(A)**

The container for storage, transport, or shipping shall be labeled or color-coded according to paragraph (g)(1)(i) and closed prior to being stored, transported, or shipped. When a facility utilizes Universal Precautions in the handling of all specimens, the labeling/color-coding of specimens is not necessary provided containers are recognizable as containing specimens. This exemption only applies while such specimens/containers remain within the facility. Labeling or

color-coding in accordance with paragraph (g)(1)(i) is required when such specimens/containers leave the facility.

**1910.1030(d)(2)(xiii)(B)**

If outside contamination of the primary container occurs, the primary container shall be placed within a second container which prevents leakage during handling, processing, storage, transport, or shipping and is labeled or color-coded according to the requirements of this standard.

**1910.1030(d)(2)(xiii)(C)**

If the specimen could puncture the primary container, the primary container shall be placed within a secondary container which is puncture-resistant in addition to the above characteristics.

**1910.1030(d)(2)(xiv)**

Equipment which may become contaminated with blood or other potentially infectious materials shall be examined prior to servicing or shipping and shall be decontaminated as necessary, unless the employer can demonstrate that decontamination of such equipment or portions of such equipment is not feasible.

**1910.1030(d)(2)(xiv)(A)**

A readily observable label in accordance with paragraph (g)(1)(i)(H) shall be attached to the equipment stating which portions remain contaminated.

**1910.1030(d)(2)(xiv)(B)**

The employer shall ensure that this information is conveyed to all affected employees, the servicing representative, and/or the manufacturer, as appropriate, prior to handling, servicing, or shipping so that appropriate precautions will be taken.

**1910.1030(d)(3)**

**Personal Protective Equipment --**

**1910.1030(d)(3)(i)**

**Provision.** When there is occupational exposure, the employer shall provide, at no cost to the employee, appropriate personal protective equipment such as, but not limited to, gloves, gowns, laboratory coats, face shields or masks and eye protection, and mouthpieces, resuscitation bags, pocket masks, or other ventilation devices. Personal protective equipment will be considered "appropriate" only if it does not permit blood or other potentially infectious materials to pass through to or reach the employee's work clothes, street clothes, undergarments, skin, eyes, mouth, or other mucous membranes under normal conditions of use and for the duration of time which the protective equipment will be used.

**1910.1030(d)(3)(ii)**

**Use.** The employer shall ensure that the employee uses appropriate personal protective equipment unless the employer shows that the employee temporarily and briefly declined to use personal protective equipment when, under rare and extraordinary circumstances, it was the employee's professional judgment that in the specific instance its use would have prevented the delivery of health care or public safety services or would have posed an increased hazard to the safety of the worker or co-worker. When the employee makes this judgement, the circumstances shall be investigated and documented in order to determine whether changes can be instituted to prevent such occurrences in the future.

**1910.1030(d)(3)(iii)**

**Accessibility.** The employer shall ensure that appropriate personal protective equipment in the appropriate sizes is readily accessible at the worksite or is issued to employees. Hypoallergenic gloves, glove liners, powderless gloves, or other similar alternatives shall be readily accessible to those employees who are allergic to the gloves normally provided.

**1910.1030(d)(3)(iv)**

**Cleaning, Laundering, and Disposal.** The employer shall clean, launder, and dispose of personal protective equipment required by paragraphs (d) and (e) of this standard, at no cost to the employee.

**1910.1030(d)(3)(v)**

**Repair and Replacement.** The employer shall repair or replace personal protective equipment as needed to maintain its effectiveness, at no cost to the employee.

**1910.1030(d)(3)(vi)**

If a garment(s) is penetrated by blood or other potentially infectious materials, the garment(s) shall be removed immediately or as soon as feasible.

**1910.1030(d)(3)(vii)**

All personal protective equipment shall be removed prior to leaving the work area.

**1910.1030(d)(3)(viii)**

When personal protective equipment is removed it shall be placed in an appropriately designated area or container for storage, washing, decontamination or disposal.

**1910.1030(d)(3)(ix)**

**Gloves.** Gloves shall be worn when it can be reasonably anticipated that the employee may have hand contact with blood, other potentially infectious materials, mucous membranes, and non-intact skin; when performing vascular access procedures except as specified in paragraph (d)(3)(ix)(D); and when handling or touching contaminated items or surfaces.

**1910.1030(d)(3)(ix)(A)**

Disposable (single use) gloves such as surgical or examination gloves, shall be replaced as soon as practical when contaminated or as soon as feasible if they are torn, punctured, or when their ability to function as a barrier is compromised.

**1910.1030(d)(3)(ix)(B)**

Disposable (single use) gloves shall not be washed or decontaminated for re-use.

**1910.1030(d)(3)(ix)(C)**

Utility gloves may be decontaminated for re-use if the integrity of the glove is not compromised. However, they must be discarded if they are cracked, peeling, torn, punctured, or exhibit other signs of deterioration or when their ability to function as a barrier is compromised.

**1910.1030(d)(3)(ix)(D)**

If an employer in a volunteer blood donation center judges that routine gloving for all phlebotomies is not necessary then the employer shall:

**1910.1030(d)(3)(ix)(D)(1)**

Periodically reevaluate this policy;

**1910.1030(d)(3)(ix)(D)(2)**

Make gloves available to all employees who wish to use them for phlebotomy;

**1910.1030(d)(3)(ix)(D)(3)**

Not discourage the use of gloves for phlebotomy; and

**1910.1030(d)(3)(ix)(D)(4)**

Require that gloves be used for phlebotomy in the following circumstances:

**1910.1030(d)(3)(ix)(D)(4)(i)**

When the employee has cuts, scratches, or other breaks in his or her skin;

**1910.1030(d)(3)(ix)(D)(4)(ii)**

When the employee judges that hand contamination with blood may occur, for example, when performing phlebotomy on an uncooperative source individual; and

**1910.1030(d)(3)(ix)(D)(4)(iii)**

When the employee is receiving training in phlebotomy.

**1910.1030(d)(3)(x)**

**Masks, Eye Protection, and Face Shields.** Masks in combination with eye protection devices, such as goggles or glasses with solid side shields, or chin-length face shields, shall be worn whenever splashes, spray, spatter, or droplets of blood or other potentially infectious materials may be generated and eye, nose, or mouth contamination can be reasonably anticipated.

**1910.1030(d)(3)(xi)**

**Gowns, Aprons, and Other Protective Body Clothing.**

Appropriate protective clothing such as, but not limited to, gowns, aprons, lab coats, clinic jackets, or similar outer garments shall be worn in occupational exposure situations. The type and characteristics will depend upon the task and degree of exposure anticipated.

**1910.1030(d)(3)(xii)**

Surgical caps or hoods and/or shoe covers or boots shall be worn in instances when gross contamination can reasonably be anticipated (e.g., autopsies, orthopaedic surgery).

**1910.1030(d)(4)**

**Housekeeping --**

**1910.1030(d)(4)(i)**

**General.** Employers shall ensure that the worksite is maintained in a clean and sanitary condition. The employer shall determine and implement an appropriate written schedule for cleaning and method of decontamination based upon the location within the facility, type of surface to be cleaned, type of soil present, and tasks or procedures being performed in the area.

**1910.1030(d)(4)(ii)**

All equipment and environmental and working surfaces shall be cleaned and decontaminated after contact with blood or other potentially infectious materials.

**1910.1030(d)(4)(ii)(A)**

Contaminated work surfaces shall be decontaminated with an appropriate disinfectant after completion of procedures; immediately or as soon as feasible when surfaces are overtly contaminated or after any spill of blood or other potentially infectious materials; and at the end of the work shift if the

surface may have become contaminated since the last cleaning.

**1910.1030(d)(4)(ii)(B)**

Protective coverings, such as plastic wrap, aluminum foil, or imperviously-backed absorbent paper used to cover equipment and environmental surfaces, shall be removed and replaced as soon as feasible when they become overtly contaminated or at the end of the workshift if they may have become contaminated during the shift.

**1910.1030(d)(4)(ii)(C)**

All bins, pails, cans, and similar receptacles intended for reuse which have a reasonable likelihood for becoming contaminated with blood or other potentially infectious materials shall be inspected and decontaminated on a regularly scheduled basis and cleaned and decontaminated immediately or as soon as feasible upon visible contamination.

**1910.1030(d)(4)(ii)(D)**

Broken glassware which may be contaminated shall not be picked up directly with the hands. It shall be cleaned up using mechanical means, such as a brush and dust pan, tongs, or forceps.

**1910.1030(d)(4)(ii)(E)**

Reusable sharps that are contaminated with blood or other potentially infectious materials shall not be stored or processed in a manner that requires employees to reach by hand into the containers where these sharps have been placed.

**1910.1030(d)(4)(iii)**

**Regulated Waste --**

**1910.1030(d)(4)(iii)(A)**

**Contaminated Sharps Discarding and Containment.**

**1910.1030(d)(4)(iii)(A)(1)**

Contaminated sharps shall be discarded immediately or as soon as feasible in containers that are:

**1910.1030(d)(4)(iii)(A)(1)(i)**

Closable;

**1910.1030(d)(4)(iii)(A)(1)(ii)**

Puncture resistant;

**1910.1030(d)(4)(iii)(A)(1)(iii)**

Leakproof on sides and bottom; and

**1910.1030(d)(4)(iii)(A)(1)(iv)**

Labeled or color-coded in accordance with paragraph (g)(1)(i) of this standard.

**1910.1030(d)(4)(iii)(A)(2)**

During use, containers for contaminated sharps shall be:

**1910.1030(d)(4)(iii)(A)(2)(i)**

Easily accessible to personnel and located as close as is feasible to the immediate area where sharps are used or can be reasonably anticipated to be found (e.g., laundries);

**1910.1030(d)(4)(iii)(A)(2)(ii)**

Maintained upright throughout use; and

**1910.1030(d)(4)(iii)(A)(2)(iii)**

Replaced routinely and not be allowed to overfill.

**1910.1030(d)(4)(iii)(A)(3)**

When moving containers of contaminated sharps from the area of use, the containers shall be:

**1910.1030(d)(4)(iii)(A)(3)(i)**

Closed immediately prior to removal or replacement to prevent spillage or protrusion of contents during handling, storage, transport, or shipping;

**1910.1030(d)(4)(iii)(A)(3)(ii)**

Placed in a secondary container if leakage is possible. The second container shall be:

**1910.1030(d)(4)(iii)(A)(3)(ii)(A)**

Closable;

**1910.1030(d)(4)(iii)(A)(3)(ii)(B)**

Constructed to contain all contents and prevent leakage during handling, storage, transport, or shipping; and

**1910.1030(d)(4)(iii)(A)(3)(ii)(C)**

Labeled or color-coded according to paragraph (g)(1)(i) of this standard.

**1910.1030(d)(4)(iii)(A)(4)**

Reusable containers shall not be opened, emptied, or cleaned manually or in any other manner which would expose employees to the risk of percutaneous injury.

**1910.1030(d)(4)(iii)(B)**

**Other Regulated Waste Containment --**

**1910.1030(d)(4)(iii)(B)(1)**

Regulated waste shall be placed in containers which are:

**1910.1030(d)(4)(iii)(B)(1)(i)**

Closable;

**1910.1030(d)(4)(iii)(B)(1)(ii)**

Constructed to contain all contents and prevent leakage of fluids during handling, storage, transport or shipping;

**1910.1030(d)(4)(iii)(B)(1)(iii)**

Labeled or color-coded in accordance with paragraph (g)(1)(i) this standard; and

**1910.1030(d)(4)(iii)(B)(1)(iv)**

Closed prior to removal to prevent spillage or protrusion of contents during handling, storage, transport, or shipping.

**1910.1030(d)(4)(iii)(B)(2)**

If outside contamination of the regulated waste container occurs, it shall be placed in a second container. The second container shall be:

**1910.1030(d)(4)(iii)(B)(2)(i)**

Closable;

**1910.1030(d)(4)(iii)(B)(2)(ii)**

Constructed to contain all contents and prevent leakage of fluids during handling, storage, transport or shipping;

**1910.1030(d)(4)(iii)(B)(2)(iii)**

Labeled or color-coded in accordance with paragraph (g)(1)(i) of this standard; and

**1910.1030(d)(4)(iii)(B)(2)(iv)**

Closed prior to removal to prevent spillage or protrusion of contents during handling, storage, transport, or shipping.

**1910.1030(d)(4)(iii)(C)**

Disposal of all regulated waste shall be in accordance with applicable regulations of the United States, States and Territories, and political subdivisions of States and Territories.

**1910.1030(d)(4)(iv)**

**Laundry.**

**1910.1030(d)(4)(iv)(A)**

Contaminated laundry shall be handled as little as possible with a minimum of agitation.

**1910.1030(d)(4)(iv)(A)(1)**

Contaminated laundry shall be bagged or containerized at the location where it was used and shall not be sorted or rinsed in the location of use.

**1910.1030(d)(4)(iv)(A)(2)**

Contaminated laundry shall be placed and transported in bags or containers labeled or color-coded in accordance with paragraph (g)(1)(i) of this standard. When a facility utilizes Universal Precautions in the handling of all soiled laundry, alternative labeling or color-coding is sufficient if it permits all employees to recognize the containers as requiring compliance with Universal Precautions.

**1910.1030(d)(4)(iv)(A)(3)**

Whenever contaminated laundry is wet and presents a reasonable likelihood of soak-through of or leakage from the bag or container, the laundry shall be placed and transported in bags or containers which prevent soak-through and/or leakage of fluids to the exterior.

**1910.1030(d)(4)(iv)(B)**

The employer shall ensure that employees who have contact with contaminated laundry wear protective gloves and other appropriate personal protective equipment.

**1910.1030(d)(4)(iv)(C)**

When a facility ships contaminated laundry off-site to a second facility which does not utilize Universal Precautions in the handling of all laundry, the facility generating the contaminated laundry must place such laundry in bags or containers which are labeled or color-coded in accordance with paragraph (g)(1)(i).

**1910.1030(e)**

**HIV and HBV Research Laboratories and Production Facilities.**

**1910.1030(e)(1)**

This paragraph applies to research laboratories and production facilities engaged in the culture, production, concentration, experimentation, and manipulation of HIV and HBV. It does not apply to clinical or diagnostic laboratories engaged solely in the analysis of blood, tissues, or organs. These requirements apply in addition to the other requirements of the standard.

**1910.1030(e)(2)**

Research laboratories and production facilities shall meet the following criteria:

**1910.1030(e)(2)(i)**

**Standard Microbiological Practices.** All regulated waste shall either be incinerated or decontaminated by a method such as autoclaving known to effectively destroy bloodborne pathogens.

**1910.1030(e)(2)(ii)**  
**Special Practices.**

**1910.1030(e)(2)(ii)(A)**

Laboratory doors shall be kept closed when work involving HIV or HBV is in progress.

**1910.1030(e)(2)(ii)(B)**

Contaminated materials that are to be decontaminated at a site away from the work area shall be placed in a durable, leakproof, labeled or color-coded container that is closed before being removed from the work area.

**1910.1030(e)(2)(ii)(C)**

Access to the work area shall be limited to authorized persons. Written policies and procedures shall be established whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements, and who comply with all entry and exit procedures shall be allowed to enter the work areas and animal rooms.

**1910.1030(e)(2)(ii)(D)**

When other potentially infectious materials or infected animals are present in the work area or containment module, a hazard warning sign incorporating the universal biohazard symbol shall be posted on all access doors. The hazard warning sign shall comply with paragraph (g)(1)(ii) of this standard.

**1910.1030(e)(2)(ii)(E)**

All activities involving other potentially infectious materials shall be conducted in biological safety cabinets or other physical-containment devices within the containment module. No work with these other potentially infectious materials shall be conducted on the open bench.

**1910.1030(e)(2)(ii)(F)**

Laboratory coats, gowns, smocks, uniforms, or other appropriate protective clothing shall be used in the work area and animal rooms. Protective clothing shall not be worn outside of the work area and shall be decontaminated before being laundered.

**1910.1030(e)(2)(ii)(G)**

Special care shall be taken to avoid skin contact with other potentially infectious materials. Gloves shall be worn when handling infected animals and when making hand contact with other potentially infectious materials is unavoidable.

**1910.1030(e)(2)(ii)(H)**

Before disposal all waste from work areas and from animal rooms shall either be incinerated or decontaminated by a method such as autoclaving known to effectively destroy bloodborne pathogens.

**1910.1030(e)(2)(ii)(I)**

Vacuum lines shall be protected with liquid disinfectant traps and high-efficiency particulate air (HEPA) filters or filters of equivalent or superior efficiency and which are checked routinely and maintained or replaced as necessary.

**1910.1030(e)(2)(ii)(J)**

Hypodermic needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., the needle is integral to the syringe) shall be used for the injection or aspiration of other potentially infectious materials. Extreme caution shall

be used when handling needles and syringes. A needle shall not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe shall be promptly placed in a puncture-resistant container and autoclaved or decontaminated before reuse or disposal.

**1910.1030(e)(2)(ii)(K)**

All spills shall be immediately contained and cleaned up by appropriate professional staff or others properly trained and equipped to work with potentially concentrated infectious materials.

**1910.1030(e)(2)(ii)(L)**

A spill or accident that results in an exposure incident shall be immediately reported to the laboratory director or other responsible person.

**1910.1030(e)(2)(ii)(M)**

A biosafety manual shall be prepared or adopted and periodically reviewed and updated at least annually or more often if necessary. Personnel shall be advised of potential hazards, shall be required to read instructions on practices and procedures, and shall be required to follow them.

**1910.1030(e)(2)(iii)**

**Containment Equipment.**

**1910.1030(e)(2)(iii)(A)**

Certified biological safety cabinets (Class I, II, or III) or other appropriate combinations of personal protection or physical containment devices, such as special protective clothing, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals, shall be used for all activities with other potentially infectious materials that pose a threat of exposure to droplets, splashes, spills, or aerosols.

**1910.1030(e)(2)(iii)(B)**

Biological safety cabinets shall be certified when installed, whenever they are moved and at least annually.

**1910.1030(e)(3)**

HIV and HBV research laboratories shall meet the following criteria:

**1910.1030(e)(3)(i)**

Each laboratory shall contain a facility for hand washing and an eye wash facility which is readily available within the work area.

**1910.1030(e)(3)(ii)**

An autoclave for decontamination of regulated waste shall be available.

**1910.1030(e)(4)**

HIV and HBV production facilities shall meet the following criteria:

**1910.1030(e)(4)(i)**

The work areas shall be separated from areas that are open to unrestricted traffic flow within the building. Passage through two sets of doors shall be the basic requirement for entry into the work area from access corridors or other contiguous areas. Physical separation of the high-containment work area from access corridors or other areas or activities may also be provided by a double-doored clothes-change room (showers may be included), airlock, or other access facility that



requires passing through two sets of doors before entering the work area.

**1910.1030(e)(4)(ii)**

The surfaces of doors, walls, floors and ceilings in the work area shall be water resistant so that they can be easily cleaned. Penetrations in these surfaces shall be sealed or capable of being sealed to facilitate decontamination.

**1910.1030(e)(4)(iii)**

Each work area shall contain a sink for washing hands and a readily available eye wash facility. The sink shall be foot, elbow, or automatically operated and shall be located near the exit door of the work area.

**1910.1030(e)(4)(iv)**

Access doors to the work area or containment module shall be self-closing.

**1910.1030(e)(4)(v)**

An autoclave for decontamination of regulated waste shall be available within or as near as possible to the work area.

**1910.1030(e)(4)(vi)**

A ducted exhaust-air ventilation system shall be provided. This system shall create directional airflow that draws air into the work area through the entry area. The exhaust air shall not be recirculated to any other area of the building, shall be discharged to the outside, and shall be dispersed away from occupied areas and air intakes. The proper direction of the airflow shall be verified (i.e., into the work area).

**1910.1030(e)(5)**

**Training Requirements.** Additional training requirements for employees in HIV and HBV research laboratories and HIV and HBV production facilities are specified in paragraph (g)(2)(ix).

**1910.1030(f)**

**Hepatitis B Vaccination and Post-exposure Evaluation and Follow-up --**

**1910.1030(f)(1)**

**General.**

**1910.1030(f)(1)(i)**

The employer shall make available the hepatitis B vaccine and vaccination series to all employees who have occupational exposure, and post-exposure evaluation and follow-up to all employees who have had an exposure incident.

**1910.1030(f)(1)(ii)**

The employer shall ensure that all medical evaluations and procedures including the hepatitis B vaccine and vaccination series and post-exposure evaluation and follow-up, including prophylaxis, are:

**1910.1030(f)(1)(ii)(A)**

Made available at no cost to the employee;

**1910.1030(f)(1)(ii)(B)**

Made available to the employee at a reasonable time and place;

**1910.1030(f)(1)(ii)(C)**

Performed by or under the supervision of a licensed physician or by or under the supervision of another licensed healthcare professional; and

**1910.1030(f)(1)(ii)(D)**

Provided according to recommendations of the U.S. Public Health Service current at the time these evaluations and procedures take place, except as specified by this paragraph (f).

**1910.1030(f)(1)(iii)**

The employer shall ensure that all laboratory tests are conducted by an accredited laboratory at no cost to the employee.

**1910.1030(f)(2)**

**Hepatitis B Vaccination.**

**1910.1030(f)(2)(i)**

Hepatitis B vaccination shall be made available after the employee has received the training required in paragraph (g)(2)(vii)(I) and within 10 working days of initial assignment to all employees who have occupational exposure unless the employee has previously received the complete hepatitis B vaccination series, antibody testing has revealed that the employee is immune, or the vaccine is contraindicated for medical reasons.

**1910.1030(f)(2)(ii)**

The employer shall not make participation in a prescreening program a prerequisite for receiving hepatitis B vaccination.

**1910.1030(f)(2)(iii)**

If the employee initially declines hepatitis B vaccination but at a later date while still covered under the standard decides to accept the vaccination, the employer shall make available hepatitis B vaccination at that time.

**1910.1030(f)(2)(iv)**

The employer shall assure that employees who decline to accept hepatitis B vaccination offered by the employer sign the statement in Appendix A.

**1910.1030(f)(2)(v)**

If a routine booster dose(s) of hepatitis B vaccine is recommended by the U.S. Public Health Service at a future date, such booster dose(s) shall be made available in accordance with section (f)(1)(ii).

**1910.1030(f)(3)**

**Post-exposure Evaluation and Follow-up.** Following a report of an exposure incident, the employer shall make immediately available to the exposed employee a confidential medical evaluation and follow-up, including at least the following elements:

**1910.1030(f)(3)(i)**

Documentation of the route(s) of exposure, and the circumstances under which the exposure incident occurred;

**1910.1030(f)(3)(ii)**

Identification and documentation of the source individual, unless the employer can establish that identification is infeasible or prohibited by state or local law;

**1910.1030(f)(3)(ii)(A)**

The source individual's blood shall be tested as soon as feasible and after consent is obtained in order to determine HBV and HIV infectivity. If consent is not obtained, the employer shall establish that legally required consent cannot be obtained. When the source individual's consent is not

required by law, the source individual's blood, if available, shall be tested and the results documented.

**1910.1030(f)(3)(ii)(B)**

When the source individual is already known to be infected with HBV or HIV, testing for the source individual's known HBV or HIV status need not be repeated.

**1910.1030(f)(3)(ii)(C)**

Results of the source individual's testing shall be made available to the exposed employee, and the employee shall be informed of applicable laws and regulations concerning disclosure of the identity and infectious status of the source individual.

**1910.1030(f)(3)(iii)**

Collection and testing of blood for HBV and HIV serological status;

**1910.1030(f)(3)(iii)(A)**

The exposed employee's blood shall be collected as soon as feasible and tested after consent is obtained.

**1910.1030(f)(3)(iii)(B)**

If the employee consents to baseline blood collection, but does not give consent at that time for HIV serologic testing, the sample shall be preserved for at least 90 days. If, within 90 days of the exposure incident, the employee elects to have the baseline sample tested, such testing shall be done as soon as feasible.

**1910.1030(f)(3)(iv)**

Post-exposure prophylaxis, when medically indicated, as recommended by the U.S. Public Health Service;

**1910.1030(f)(3)(v)**

Counseling; and

**1910.1030(f)(3)(vi)**

Evaluation of reported illnesses.

**1910.1030(f)(4)**

**Information Provided to the Healthcare Professional.**

**1910.1030(f)(4)(i)**

The employer shall ensure that the healthcare professional responsible for the employee's Hepatitis B vaccination is provided a copy of this regulation.

**1910.1030(f)(4)(ii)**

The employer shall ensure that the healthcare professional evaluating an employee after an exposure incident is provided the following information:

**1910.1030(f)(4)(ii)(A)**

A copy of this regulation;

**1910.1030(f)(4)(ii)(B)**

A description of the exposed employee's duties as they relate to the exposure incident;

**1910.1030(f)(4)(ii)(C)**

Documentation of the route(s) of exposure and circumstances under which exposure occurred;

**1910.1030(f)(4)(ii)(D)**

Results of the source individual's blood testing, if available; and

**1910.1030(f)(4)(ii)(E)**

All medical records relevant to the appropriate treatment of the employee including vaccination status which are the employer's responsibility to maintain.

**1910.1030(f)(5)**

**Healthcare Professional's Written Opinion.** The employer shall obtain and provide the employee with a copy of the evaluating healthcare professional's written opinion within 15 days of the completion of the evaluation.

**1910.1030(f)(5)(i)**

The healthcare professional's written opinion for Hepatitis B vaccination shall be limited to whether Hepatitis B vaccination is indicated for an employee, and if the employee has received such vaccination.

**1910.1030(f)(5)(ii)**

The healthcare professional's written opinion for post-exposure evaluation and follow-up shall be limited to the following information:

**1910.1030(f)(5)(ii)(A)**

That the employee has been informed of the results of the evaluation; and

**1910.1030(f)(5)(ii)(B)**

That the employee has been told about any medical conditions resulting from exposure to blood or other potentially infectious materials which require further evaluation or treatment.

**1910.1030(f)(5)(iii)**

All other findings or diagnoses shall remain confidential and shall not be included in the written report.

**1910.1030(f)(6)**

**Medical Recordkeeping.** Medical records required by this standard shall be maintained in accordance with paragraph (h)(1) of this section.

**1910.1030(g)**

**Communication of Hazards to Employees --**

**1910.1030(g)(1)**

**Labels and Signs --**

**1910.1030(g)(1)(i)**

**Labels.**

**1910.1030(g)(1)(i)(A)**

Warning labels shall be affixed to containers of regulated waste, refrigerators and freezers containing blood or other potentially infectious material; and other containers used to store, transport or ship blood or other potentially infectious materials, except as provided in paragraph (g)(1)(i)(E), (F) and (G).

**1910.1030(g)(1)(i)(B)**

Labels required by this section shall include the following legend:



**Biohazard**

**1910.1030(g)(1)(i)(C)**

These labels shall be fluorescent orange or orange-red or predominantly so, with lettering and symbols in a contrasting color.

**1910.1030(g)(1)(i)(D)**

Labels shall be affixed as close as feasible to the container by string, wire, adhesive, or other method that prevents their loss or unintentional removal.

**1910.1030(g)(1)(i)(E)**

Red bags or red containers may be substituted for labels.

**1910.1030(g)(1)(i)(F)**

Containers of blood, blood components, or blood products that are labeled as to their contents and have been released for transfusion or other clinical use are exempted from the labeling requirements of paragraph (g).

**1910.1030(g)(1)(i)(G)**

Individual containers of blood or other potentially infectious materials that are placed in a labeled container during storage, transport, shipment or disposal are exempted from the labeling requirement.

**1910.1030(g)(1)(i)(H)**

Labels required for contaminated equipment shall be in accordance with this paragraph and shall also state which portions of the equipment remain contaminated.

**1910.1030(g)(1)(i)(I)**

Regulated waste that has been decontaminated need not be labeled or color-coded.

**1910.1030(g)(1)(ii)**

**Signs.**

**1910.1030(g)(1)(ii)(A)**

The employer shall post signs at the entrance to work areas specified in paragraph (e), HIV and HBV Research Laboratory and Production Facilities, which shall bear the following legend:



**Biohazard**

(Name of the Infectious Agent)

(Special requirements for entering the area)

(Name, telephone number of the laboratory director or other responsible person.)

**1910.1030(g)(1)(ii)(B)**

These signs shall be fluorescent orange-red or predominantly so, with lettering and symbols in a contrasting color.

**1910.1030(g)(2)**

**Information and Training.**

**1910.1030(g)(2)(i)**

Employers shall ensure that all employees with occupational exposure participate in a training program which must be provided at no cost to the employee and during working hours.

**1910.1030(g)(2)(ii)**

Training shall be provided as follows:

**1910.1030(g)(2)(ii)(A)**

At the time of initial assignment to tasks where occupational exposure may take place;

**1910.1030(g)(2)(ii)(B)**

Within 90 days after the effective date of the standard; and

**1910.1030(g)(2)(ii)(C)**

At least annually thereafter.

**1910.1030(g)(2)(iii)**

For employees who have received training on bloodborne pathogens in the year preceding the effective date of the standard, only training with respect to the provisions of the standard which were not included need be provided.

**1910.1030(g)(2)(iv)**

Annual training for all employees shall be provided within one year of their previous training.

**1910.1030(g)(2)(v)**

Employers shall provide additional training when changes such as modification of tasks or procedures or institution of new tasks or procedures affect the employee's occupational exposure. The additional training may be limited to addressing the new exposures created.

**1910.1030(g)(2)(vi)**

Material appropriate in content and vocabulary to educational level, literacy, and language of employees shall be used.

**1910.1030(g)(2)(vii)**

The training program shall contain at a minimum the following elements:

**1910.1030(g)(2)(vii)(A)**

An accessible copy of the regulatory text of this standard and an explanation of its contents;

**1910.1030(g)(2)(vii)(B)**

A general explanation of the epidemiology and symptoms of bloodborne diseases;

**1910.1030(g)(2)(vii)(C)**

An explanation of the modes of transmission of bloodborne pathogens;

**1910.1030(g)(2)(vii)(D)**

An explanation of the employer's exposure control plan and the means by which the employee can obtain a copy of the written plan;

**1910.1030(g)(2)(vii)(E)**

An explanation of the appropriate methods for recognizing tasks and other activities that may involve exposure to blood and other potentially infectious materials;

**1910.1030(g)(2)(vii)(F)**

An explanation of the use and limitations of methods that will prevent or reduce exposure including appropriate engineering controls, work practices, and personal protective equipment;

**1910.1030(g)(2)(vii)(G)**

Information on the types, proper use, location, removal, handling, decontamination and disposal of personal protective equipment;

**1910.1030(g)(2)(vii)(H)**

An explanation of the basis for selection of personal protective equipment;

**1910.1030(g)(2)(vii)(I)**

Information on the hepatitis B vaccine, including information on its efficacy, safety, method of administration, the benefits of being vaccinated, and that the vaccine and vaccination will be offered free of charge;

**1910.1030(g)(2)(vii)(J)**

Information on the appropriate actions to take and persons to contact in an emergency involving blood or other potentially infectious materials;

**1910.1030(g)(2)(vii)(K)**

An explanation of the procedure to follow if an exposure incident occurs, including the method of reporting the incident and the medical follow-up that will be made available;

**1910.1030(g)(2)(vii)(L)**

Information on the post-exposure evaluation and follow-up that the employer is required to provide for the employee following an exposure incident;

**1910.1030(g)(2)(vii)(M)**

An explanation of the signs and labels and/or color coding required by paragraph (g)(1); and

**1910.1030(g)(2)(vii)(N)**

An opportunity for interactive questions and answers with the person conducting the training session.

**1910.1030(g)(2)(viii)**

The person conducting the training shall be knowledgeable in the subject matter covered by the elements contained in the training program as it relates to the workplace that the training will address.

**1910.1030(g)(2)(ix)**

Additional Initial Training for Employees in HIV and HBV Laboratories and Production Facilities. Employees in HIV or HBV research laboratories and HIV or HBV production facilities shall receive the following initial training in addition to the above training requirements.

**1910.1030(g)(2)(ix)(A)**

The employer shall assure that employees demonstrate proficiency in standard microbiological practices and techniques and in the practices and operations specific to the facility before being allowed to work with HIV or HBV.

**1910.1030(g)(2)(ix)(B)**

The employer shall assure that employees have prior experience in the handling of human pathogens or tissue cultures before working with HIV or HBV.

**1910.1030(g)(2)(ix)(C)**

The employer shall provide a training program to employees who have no prior experience in handling human pathogens. Initial work activities shall not include the handling of infectious agents. A progression of work activities shall be assigned as techniques are learned and proficiency is developed. The employer shall assure that employees participate in work activities involving infectious agents only after proficiency has been demonstrated.

**1910.1030(h)  
Recordkeeping --**

**1910.1030(h)(1)  
Medical Records.**

**1910.1030(h)(1)(i)**

The employer shall establish and maintain an accurate record for each employee with occupational exposure, in accordance with 29 CFR 1910.1020.

**1910.1030(h)(1)(ii)**

This record shall include:

**1910.1030(h)(1)(ii)(A)**

The name and social security number of the employee;

**1910.1030(h)(1)(ii)(B)**

A copy of the employee's hepatitis B vaccination status including the dates of all the hepatitis B vaccinations and any medical records relative to the employee's ability to receive vaccination as required by paragraph (f)(2);

**1910.1030(h)(1)(ii)(C)**

A copy of all results of examinations, medical testing, and follow-up procedures as required by paragraph (f)(3);

**1910.1030(h)(1)(ii)(D)**

The employer's copy of the healthcare professional's written opinion as required by paragraph (f)(5); and

**1910.1030(h)(1)(ii)(E)**

A copy of the information provided to the healthcare professional as required by paragraphs (f)(4)(ii)(B)(C) and (D).

**1910.1030(h)(1)(iii)**

Confidentiality. The employer shall ensure that employee medical records required by paragraph (h)(1) are:

**1910.1030(h)(1)(iii)(A)**

Kept confidential; and

**1910.1030(h)(1)(iii)(B)**

Not disclosed or reported without the employee's express written consent to any person within or outside the workplace except as required by this section or as may be required by law.

**1910.1030(h)(1)(iv)**

The employer shall maintain the records required by paragraph (h) for at least the duration of employment plus 30 years in accordance with 29 CFR 1910.1020.

**1910.1030(h)(2)  
Training Records.**

**1910.1030(h)(2)(i)**  
Training records shall include the following information:

**1910.1030(h)(2)(i)(A)**  
The dates of the training sessions;

**1910.1030(h)(2)(i)(B)**  
The contents or a summary of the training sessions;

**1910.1030(h)(2)(i)(C)**  
The names and qualifications of persons conducting the training; and

**1910.1030(h)(2)(i)(D)**  
The names and job titles of all persons attending the training sessions.

**1910.1030(h)(2)(ii)**  
Training records shall be maintained for 3 years from the date on which the training occurred.

**1910.1030(h)(3)  
Availability.**

**1910.1030(h)(3)(i)**  
The employer shall ensure that all records required to be maintained by this section shall be made available upon request to the Assistant Secretary and the Director for examination and copying.

**1910.1030(h)(3)(ii)**  
Employee training records required by this paragraph shall be provided upon request for examination and copying to employees, to employee representatives, to the Director, and to the Assistant Secretary.

**1910.1030(h)(3)(iii)**  
Employee medical records required by this paragraph shall be provided upon request for examination and copying to the subject employee, to anyone having written consent of the subject employee, to the Director, and to the Assistant Secretary in accordance with 29 CFR 1910.1020.

**1910.1030(h)(4)  
Transfer of Records.**

**1910.1030(h)(4)(i)**  
The employer shall comply with the requirements involving transfer of records set forth in 29 CFR 1910.1020(h).

**1910.1030(h)(4)(ii)**  
If the employer ceases to do business and there is no successor employer to receive and retain the records for the prescribed period, the employer shall notify the Director, at least three months prior to their disposal and transmit them to the Director, if required by the Director to do so, within that three month period.

**1910.1030(h)(5)  
Sharps injury log.**

**1910.1030(h)(5)(i)**

The employer shall establish and maintain a sharps injury log for the recording of percutaneous injuries from contaminated sharps. The information in the sharps injury log shall be recorded and maintained in such manner as to protect the confidentiality of the injured employee. The sharps injury log shall contain, at a minimum:

**1910.1030(h)(5)(i)(A)**  
The type and brand of device involved in the incident,

**1910.1030(h)(5)(i)(B)**  
The department or work area where the exposure incident occurred, and

**1910.1030(h)(5)(i)(C)**  
An explanation of how the incident occurred.

**1910.1030(h)(5)(ii)**  
The requirement to establish and maintain a sharps injury log shall apply to any employer who is required to maintain a log of occupational injuries and illnesses under 29 CFR 1904.

**1910.1030(h)(5)(iii)**  
The sharps injury log shall be maintained for the period required by 29 CFR 1904.6.

**1910.1030(i)  
Dates --**

**1910.1030(i)(1)  
Effective Date.** The standard shall become effective on March 6, 1992.

**1910.1030(i)(2)**  
The Exposure Control Plan required by paragraph (c) of this section shall be completed on or before May 5, 1992.

**1910.1030(i)(3)**  
Paragraph (g)(2) Information and Training and (h) Recordkeeping shall take effect on or before June 4, 1992.

**1910.1030(i)(4)**  
Paragraphs (d)(2) Engineering and Work Practice Controls, (d)(3) Personal Protective Equipment, (d)(4) Housekeeping, (e) HIV and HBV Research Laboratories and Production Facilities, (f) Hepatitis B Vaccination and Post-Exposure Evaluation and Follow-up, and (g)(1) Labels and Signs, shall take effect July 6, 1992.

[56 FR 64004, Dec. 06, 1991, as amended at 57 FR 12717, April 13, 1992; 57 FR 29206, July 1, 1992; 61 FR 5507, Feb. 13, 1996; 66 FR 5325 Jan., 18, 2001]

**Appendix A to Section 1910.1030 -Hepatitis B Vaccine Declination (Mandatory)**

I understand that due to my occupational exposure to blood or other potentially infectious materials I may be at risk of acquiring hepatitis B virus (HBV) infection. I have been given the opportunity to be vaccinated with hepatitis B vaccine, at no charge to myself. However, I decline hepatitis B vaccination at this time. I understand that by declining this vaccine, I continue to be at risk of acquiring hepatitis B, a serious disease. If in the future I continue to have occupational exposure to blood or other potentially infectious materials and I want to be vaccinated with hepatitis B

vaccine, I can receive the vaccination series at no charge to me.

[56 FR 64004, Dec. 06, 1991, as amended at 57 FR 12717, April 13, 1992; 57 FR 29206, July 1, 1992; 61 FR 5507, Feb. 13, 1996]

### **13.5 State of Connecticut BSL3 Law**

Substitute House Bill No. 5521

PUBLIC ACT NO. 96-149

AN ACT CONCERNING BIOLEVEL-THREE LABORATORIES.

Be it enacted by the Senate and House of Representatives in General Assembly convened:

(NEW) (a) For purposes of this section, (1) a "biolevel-three laboratory" or "laboratory" means a laboratory which is operated by an institution of higher education and is designed and equipped under guidelines issued by the National Institutes of Health and the National Centers for Disease Control as a biolevel-three laboratory, and (2) "biolevel-three agent" means an agent classified as a biolevel-three agent by the National Institutes of Health and the National Centers for Disease Control.

(b) If an institution which operates a biolevel-three laboratory establishes a biosafety committee pursuant to the National Institutes of Health or the National Centers for Disease Control guidelines, such committee shall (1) forward the minutes of its meetings to the Department of Public Health and (2) meet at least annually with a representative of the Department of Public Health to review safety procedures and discuss health issues relating to the operation of the laboratory.

(c) Each such institution shall report to the Department of Public Health any infection or injury relating to work at the laboratory with biolevel-three agents and any incidents relating to such work which result in a recommendation by the institution that employees or members of the public be tested or monitored for potential health problems because of the possibility of infection or injury or incidents which pose a threat to public health.

(d) Each such institution shall report to the Department of Public Health any sanctions imposed on the laboratory or on the institution for incidents occurring at the laboratory by the National Institutes of Health, the National Centers for Disease Control, the United States Department of Defense or any other government agency.

Approved May 31, 1996. Effective October 1, 1996.





## 13.6 CLASSIFICATION OF HUMAN ETIOLOGIC AGENTS

This appendix includes those biological agents known to infect humans as well as selected animal agents that may pose theoretical risks if inoculated into humans. Included are lists of representative genera and species known to be pathogenic; mutated, recombined, and non-pathogenic species and strains are not considered. Non-infectious life cycle stages of parasites are excluded.

This appendix reflects the current state of knowledge and should be considered a resource document. Included are the more commonly encountered agents and is not meant to be all-inclusive. Information on agent risk assessment may be found in the Agent Summary Statements of the CDC/NIH publication, Biosafety in Microbiological and Biomedical Laboratories (see [Sections V-C, V-D, V-E, and V-F](#), Footnotes and References of Sections I through IV. Further guidance on agents not listed may be obtained through:

[Centers for Disease Control and Prevention](#), Biosafety Branch, Atlanta, Georgia 30333, Phone: (404) 639-3883, Fax: (404) 639-2294; National Institutes of Health, Division of Safety, Bethesda, Maryland 20892, Phone: (301) 496-1357; National Animal Disease Center, U.S. Department of Agriculture, Ames, Iowa 50010, Phone: (515) 862-8258.

The following list is from the Guidelines for Research Involving Recombinant DNA Molecules (April 2002):

### 13.6.1 Basis for the Classification of Biohazardous Agents by Risk Group (RG)

Risk Group 1 (RG1) Agents that are not associated with disease in healthy adult humans.

Risk Group 2 (RG2) Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.

Risk Group 3 (RG3) Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).

Risk Group 4 (RG4) Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).

### 13.6.2 Risk Group 1 (RG1) Agents

RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include asporogenic *Bacillus subtilis* or *Bacillus licheniformis*; adeno-associated virus (AAV) types 1 through 4; and recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus. A strain of *Escherichia coli* is an RG1 agent if it (1) does not possess a complete lipopolysaccharide (i.e., lacks the O antigen); and (2) does not carry any active virulence factor (e.g., toxins) or colonization factors and does not carry any genes encoding these factors.

Those agents not listed in Risk Groups (RGs) 2, 3 and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

### 13.6.3 Risk Group 2 (RG2) Agents

RG2 agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.

Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia

--*Acinetobacter baumannii* (formerly *Acinetobacter calcoaceticus*)

--*Actinobacillus*

--*Actinomyces pyogenes* (formerly *Corynebacterium pyogenes*)

--*Aeromonas hydrophila*

- Amycolata autotrophica
- Archanobacterium haemolyticum (formerly Corynebacterium haemolyticum)

Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia (continue)

- Arizona hinshawii - all serotypes
- Bacillus anthracis
- Bartonella henselae, B. quintana, B. vinsonii
- Bordetella including B. pertussis
- Borrelia recurrentis, B. burgdorferi
- Burkholderia (formerly Pseudomonas species) except those listed in RG3
- Campylobacter coli, C. fetus, C. jejuni
- Chlamydia psittaci, C. trachomatis, C. pneumoniae
- Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani
- Corynebacterium diphtheriae, C. pseudotuberculosis, C. renale
- Dermatophilus congolensis
- Edwardsiella tarda
- Erysipelothrix rhusiopathiae
- Escherichia coli - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including E. coli O157:H7
- Haemophilus ducreyi, H. influenzae
- Helicobacter pylori
- Klebsiella - all species except K. oxytoca (RG1)
- Legionella including L. pneumophila
- Leptospira interrogans - all serotypes
- Listeria
- Moraxella
- Mycobacterium (except those listed in (RG3)) including M. avium complex, M. asiaticum, M. bovis BCG vaccine strain, M. chelonae, M. fortuitum, M. kansasii, M. leprae, M. malmoense, M. marinum, M. paratuberculosis, M. scrofulaceum, M. simiae, M. szulgai, M. ulcerans, M. xenopi
- Mycoplasma, except M. mycoides and M. agalactiae which are restricted animal pathogens
- Neisseria gonorrhoeae, N. meningitidis
- Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N. transvalensis
- Rhodococcus equi
- Salmonella including S. arizonae, S. cholerasuis, S. enteritidis, S. gallinarum-pullorum, S. meleagridis, S. paratyphi, A, B, C, S. typhi, S. typhimurium
- Shigella including S. boydii, S. dysenteriae, type 1, S. flexneri, S. sonnei
- Sphaerophorus necrophorus
- Staphylococcus aureus
- Streptobacillus moniliformis
- Streptococcus including S. pneumoniae, S. pyogenes
- Treponema pallidum, T. carateum
- Vibrio cholerae, V. parahemolyticus, V. vulnificus
- Yersinia enterocolitica

Risk Group 2 (RG2) - Fungal Agents

- Blastomyces dermatitidis
- Cladosporium bantianum, C. (Xylohypha) trichoides
- Cryptococcus neoformans
- Dactylaria galopava (Ochroconis gallopavum)
- Epidermophyton

- Exophiala (Wangiella) dermatitidis
- Fonsecaea pedrosoi
- Microsporum
- Paracoccidioides braziliensis
- Penicillium marneffeii
- Sporothrix schenckii
- Trichophyton

Risk Group 2 (RG2) - Parasitic Agents

- Ancylostoma human hookworms including *A. duodenale*, *A. ceylanicum*
- Ascaris including *Ascaris lumbricoides* suum
- Babesia including *B. divergens*, *B. microti*
- Brugia filaria worms including *B. malayi*, *B. timori*
- Coccidia
- Cryptosporidium including *C. parvum*
- Cysticercus cellulosa (hydatid cyst, larva of *T. solium*)
- Echinococcus including *E. granulosus*, *E. multilocularis*, *E. vogeli*
- Entamoeba histolytica
- Enterobius
- Fasciola including *F. gigantica*, *F. hepatica*
- Giardia including *G. lamblia*
- Heterophyes
- Hymenolepis including *H. diminuta*, *H. nana*
- Isospora
- Leishmania including *L. braziliensis*, *L. donovani*, *L. ethiopia*, *L. major*, *L. mexicana*, *L. peruviana*, *L. tropica*
- Loa loa filaria worms
- Microsporidium
- Naegleria fowleri
- Necator human hookworms including *N. americanus*
- Onchocerca filaria worms including, *O. volvulus*
- Plasmodium including simian species, *P. cynomolgi*, *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax*
- Sarcocystis including *S. sui hominis*
- Schistosoma including *S. haematobium*, *S. intercalatum*, *S. japonicum*, *S. mansoni*, *S. mekongi*
- Strongyloides including *S. stercoralis*
- Taenia solium
- Toxocara including *T. canis*
- Toxoplasma including *T. gondii*
- Trichinella spiralis
- Trypanosoma including *T. brucei brucei*, *T. brucei gambiense*, *T. brucei rhodesiense*, *T. cruzi*
- Wuchereria bancrofti filaria worms

Risk Group 2 (RG2) - Viruses

- Adenoviruses, human - all types
- Alphaviruses (Togaviruses) - Group A Arboviruses
  - Eastern equine encephalomyelitis virus
  - Venezuelan equine encephalomyelitis vaccine strain TC-83
  - Western equine encephalomyelitis virus
- Arenaviruses
  - Lymphocytic choriomeningitis virus (non-neurotropic strains)
  - Tacaribe virus complex

--Other viruses as listed in the reference source: CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, 1999.

#### Bunyaviruses

--Bunyamwera virus

--Rift Valley fever virus vaccine strain MP-12

--Other viruses as listed in the reference source: CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, 1999.

#### Caliciviruses

#### Coronaviruses

#### Flaviviruses (Togaviruses) - Group B Arboviruses

--Dengue virus serotypes 1, 2, 3, and 4

--Yellow fever virus vaccine strain 17D

--Other viruses as listed in the reference source: CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, 1999.

#### Hepatitis A, B, C, D, and E viruses

Herpesviruses - except Herpesvirus simiae (Monkey B virus) (see, Risk Group 4 (RG4) – Viral Agents)

--Cytomegalovirus

--Epstein Barr virus

--Herpes simplex types 1 and 2

--Herpes zoster

--Human herpesvirus types 6 and 7

#### Orthomyxoviruses

--Influenza viruses types A, B, and C

--Other tick-borne orthomyxoviruses as listed in the reference source: CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, 1999.

#### Papovaviruses

--All human papilloma viruses

#### Paramyxoviruses

--Newcastle disease virus

--Measles virus

--Mumps virus

--Parainfluenza viruses types 1, 2, 3, and 4

--Respiratory syncytial virus

#### Parvoviruses

--Human parvovirus (B19)

#### Picornaviruses

--Coxsackie viruses types A and B

--Echoviruses - all types

--Polioviruses - all types, wild and attenuated

--Rhinoviruses - all types

Poxviruses - all types except Monkeypox virus (see, Risk Group 3 (RG3) - Viruses and Prions)

and restricted poxviruses including Alastrim, Smallpox, and Whitepox ) (see CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, 1999, Footnotes and References of Sections I through IV)

Reoviruses - all types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)

#### Rhabdoviruses

--Rabies virus - all strains

--Vesicular stomatitis virus - laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow

Togaviruses (see Alphaviruses and Flaviviruses)

--Rubivirus (rubella)

### 13.6.4 Risk Group 3 (RG3) Agents

RG3 agents are associated with serious or lethal human disease for which preventive or therapeutic

interventions may be available.

Risk Group 3 (RG3) - Bacterial Agents Including Rickettsia

- Bartonella
- Brucella including *B. abortus*, *B. canis*, *B. suis*
- Burkholderia (*Pseudomonas mallei*, *B. pseudomallei*)
- Coxiella burnetii
- Francisella tularensis
- Mycobacterium bovis (except BCG strain, see Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia), *M. tuberculosis*
- Pasteurella multocida type B - "buffalo" and other virulent strains
- Rickettsia akari, *R. australis*, *R. canada*, *R. conorii*, *R. prowazekii*, *R. rickettsii*, *R. siberica*, *R. tsutsugamushi*,  
*R. typhi* (*R. mooseri*)
- Yersinia pestis

Risk Group 3 (RG3) - Fungal Agents

- Coccidioides immitis (sporulating cultures; contaminated soil)
- Histoplasma capsulatum, *H. capsulatum* var. *duboisii*

Risk Group 3 (RG3) - Parasitic Agents

None

Risk Group 3 (RG3) - Viruses and Prions

Alphaviruses (Togaviruses) - Group A Arboviruses

- Semliki Forest virus
- St. Louis encephalitis virus
- Venezuelan equine encephalomyelitis virus (except the vaccine strain TC-83, see RG2)
- Other viruses as listed in the reference source: CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, 1999.

Arenaviruses

- Flexal
- Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)

Bunyaviruses

- Hantaviruses including Hantaan virus
- Rift Valley fever virus

Flaviviruses (Togaviruses) - Group B Arboviruses

- Japanese encephalitis virus
- Yellow fever virus
- Other viruses as listed in the reference source: CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, 1999.

Poxviruses

- Monkeypox virus

Prions

- Transmissible spongiform encephalopathies (TME) agents (Creutzfeldt-Jacob disease and kuru agents) CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, 1999. Footnotes and References of Sections I through IV, for containment instruction)

Retroviruses

- Human immunodeficiency virus (HIV) types 1 and 2
- Human T cell lymphotropic virus (HTLV) types 1 and 2
- Simian immunodeficiency virus (SIV)

Rhabdoviruses

--Vesicular stomatitis virus

### **13.6.5 Risk Group 4 (RG4) Agents**

RG4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.

Risk Group 4 (RG4) - Bacterial Agents

None

Risk Group 4 (RG4) - Fungal Agents

None

Risk Group 4 (RG4) - Parasitic Agents

None

Risk Group 4 (RG4) - Viral Agents

Arenaviruses

--Guanarito virus

--Lassa virus

--Junin virus

--Machupo virus

--Sabia

Bunyaviruses (Nairovirus)

--Crimean-Congo hemorrhagic fever virus

Filoviruses

--Ebola virus

--Marburg virus

Flaviruses (Togaviruses) - Group B Arboviruses

--Tick-borne encephalitis virus complex including Absetterov, Central European encephalitis, Hanzalova, Hypr,

Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses

Herpesviruses (alpha)

--Herpesvirus simiae (Herpes B or Monkey B virus)

Paramyxoviruses

--Equine morbillivirus

Hemorrhagic fever agents and viruses as yet undefined

### **13.6.6 Animal Viral Etiologic Agents in Common Use**

The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents is associated with disease in healthy adult humans; they are commonly used in laboratory experimental work.

A containment level appropriate for RG1 human agents is recommended for their use. For agents that are infectious to human cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.

Baculoviruses

Herpesviruses

--Herpesvirus ateles

--Herpesvirus saimiri

- Marek's disease virus
- Murine cytomegalovirus

Papovaviruses

- Bovine papilloma virus
- Polyoma virus
- Shope papilloma virus
- Simian virus 40 (SV40)

Retroviruses

- Avian leukosis virus
- Avian sarcoma virus
- Bovine leukemia virus
- Feline leukemia virus
- Feline sarcoma virus
- Gibbon leukemia virus
- Mason-Pfizer monkey virus
- Mouse mammary tumor virus
- Murine leukemia virus
- Murine sarcoma virus
- Rat leukemia virus