VAPORHCS R&D Service Biosafety Manual

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Purpose

To educate laboratory personnel at the VA Portland Health Care System (VAPORHCS) in the use of safe laboratory practices and of the risks associated with hazardous biological agents, thereby reducing the chance of exposure or illness.

Scope and Policy

VAPORHCS maintains a program of continuous education to prevent unsafe practices and protect against health risks associated with use of hazardous biological agents in the laboratory. The program is designed to ensure compliance with pertinent Federal, State, and local regulations, including Occupational Safety and Health Administration (OSHA) Regulations 29 CFR 1910, the U.S. Dept. of Health & Human Services Biosafety in Microbiological and Biomedical Laboratories (BMBL) and the NIH Guidelines. The medical center establishes this Biosafety Manual to protect employees, patients, visitors, property, and the environment from potential health hazards associated with the handling, use, and disposal of biohazardous materials in laboratories.

Principle of Containment

A fundamental objective of any biosafety program is the containment of potentially harmful biological agents. The term “containment” is used in describing safe methods, facilities and equipment for managing infectious materials in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.

General Laboratory Biosafety Practices and Responsibilities

Although this manual outlines the basic practices and principles of biosafety, each laboratory working with biohazardous materials should also develop a set of standard operating procedures (SOPs) that identifies the specific hazards that may be encountered in their research program and outlines practices and procedures designed to minimize or eliminate exposures to these hazards. The Principal Investigator (PI) is responsible for the conduct of work with any infectious agents or materials within their laboratory. Before beginning new work with infectious agents, the PI, together with the VA biosafety officer, should conduct an initial risk assessment of the work. See p.10 for instructions on conducting a risk assessment. The PI must then submit appropriate paperwork for review and approval by the Subcommittee for Research Safety (SRS).

Persons working with infectious agents or potentially infected materials must be aware of possible hazards, and must be trained and proficient in the practices and techniques required for handling such material safely. Trainings include general safety and laboratory safety training modules. If handling blood, human biospecimens, or infectious materials, blood borne pathogen training is also required. Refer to the training website for all up-to-date training requirements:

https://www.va.gov/PORTLANDRESEARCH/training/index.asp

The PI is responsible for providing or arranging for the appropriate training of personnel. The PI is also required to document blood borne pathogen training and

notify the research office of updated trainings annually.

Appropriate facility design and engineering features, safety equipment, and management practices must supplement laboratory personnel, safety practices, and techniques.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

Safety equipment including biosafety cabinets (BSC), enclosed containers, and other engineering controls are primary barriers designed to minimize exposures to hazardous biological materials. The BSC is the principal device used to provide containment of infectious droplets or aerosols generated by many microbiological procedures. Only Class II BSCs are used in VAPORHCS laboratories. These open-fronted BSCs offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques. They also provide protection from external contamination of the materials (e.g., cell cultures, microbiological stocks) being manipulated inside the cabinet.

An example of another primary barrier is the safety centrifuge cup, an enclosed container designed to prevent aerosols from being released during centrifugation. To minimize aerosol hazards, containment controls such as BSCs or centrifuge cups may be required when handling an infectious agent, depending on the risks of the agent and procedures conducted.

Safety equipment also may include items for personal protection, such as gloves, lab coats, gowns, shoe covers, respirators, face shields, or safety goggles. Personal protective equipment (PPE) is often used in combination with BSCs and other devices that contain the agents, animals, or materials being handled. In some situations, in which it is impractical to work in BSCs, PPE may form the primary barrier between personnel and the infectious materials. Examples include certain animal studies, animal necropsy, agent production activities, and use of certain pieces of equipment too large to fit in a BSC.

Biosafety Levels: Introduction and Definitions

A biosafety level (BSL) is a set of containment practices designed to prevent the spread of biohazardous agents outside of a designated laboratory space. There are four biosafety levels recognized by the U.S. Dept. of Health and Human Services, though VAPORHCS is not authorized to use any agents requiring the highest level of containment, BSL-4.

Biosafety Level 1 (BSL-1) practices, safety equipment, and facility design are appropriate for undergraduate and secondary educational teaching laboratories, and for research laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans (known as “Risk Group 1” agents). An example of a Risk Group 1 agent is the K12 strain of *E. coli*, used commonly for the propagation of recombinant DNA such as plasmids. Many agents not ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals. BSL-1 represents a basic level of containment that relies on standard microbiological practices with no

special engineering barriers or equipment recommended, other than a sink for hand washing. Personal protective equipment (PPE) may include gloves, lab coat, eye protection, and/or a face mask, depending on the procedures to be performed.

Biosafety Level 2 (BSL-2) practices, equipment, and facility design and construction are appropriate for research laboratories in which work is done with the broad spectrum of indigenous, moderate-risk agents that are present in the community and associated with human disease of varying severity ("Risk Group 2" agents). Examples of Risk Group 2 agents are Hepatitis B virus, *Bordetella pertussis*, and *Toxoplasma gondii*. BSL-2 handling is also appropriate when work is done with any human-derived blood, body fluids, tissues, or cell lines, as the absence of infectious agents in those materials may not be conclusively established.

Primary hazards to personnel working with these agents relate to accidents with sharps such as needles, mucous membrane exposures such as a splash in the eyes, or accidental ingestion of infectious materials. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures such as vortexing, centrifuging, shaking, sonicating, or pipetting may nevertheless increase the risk of personnel exposure through the production of small droplets of infectious material. Thus, this work is conducted in containment equipment such as a BSC and, in some instances, using additional equipment such as safety centrifuge cups or splash shields. Personal protective equipment (PPE) worn with BSL-2 level work includes gloves and gown or lab coat, plus eye protection, face shields, and/or face masks as determined by a risk assessment of the procedures to be performed.

Biosafety Level 2+ (BSL-2+) is the term frequently used to describe handling practices conducted with microorganisms in a BSL-2 laboratory with some additional practices normally found in a BSL-3 facility. This enhanced containment level is used for biological agents that may pose a higher risk than standard BSL-2 agents due to changes to the agent at the molecular level that render it more infectious, the use of procedures that create a higher droplet or aerosol risk, or the amount of agent being propagated. While BSL-2+ is not a standard containment level in the Centers for Disease Control's (CDC) Biosafety in Microbiological and Biomedical Laboratories (BMBL),it is recognized by the American Biological Safety Association as well as by many research universities and facilities as a practical way to develop an appropriate containment plan for some infectious agents. There is no standardized list of agents that should be conducted at BSL-2+. This level of containment and associated practices will be determined by the SRS and/or the OHSU Institutional Biosafety Committee (IBC).

Biosafety Level 3 (BSL-3) practices, safety equipment, and facility design and construction are appropriate for research laboratories in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection ("Risk Group 3" agents). An example of a Risk Group 3 agent is *Mycobacterium tuberculosis*. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols. At BSL-3, more emphasis is placed on primary and secondary barriers to protect lab personnel, the community, and the environment

from exposure to potentially infectious aerosols. For example, all laboratory manipulations should be performed in a BSC or other equipment with built-in aerosol containment. Secondary barriers for this level include controlled access to the laboratory and ventilation requirements that minimize the release of infectious aerosols from the laboratory.

Biosafety Levels: Standard Safety Practices

Biosafety Level 1

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment.

The following standard practices, safety equipment, and facility requirements apply to BSL-1:

BSL-1: Standard Microbiological Practices

1. The PI must ensure that lab personnel receive specific training in the procedures conducted in the laboratory, including precautions to prevent exposures and steps to take in case of exposures or spills. Refer to the training website for all up-to-date training requirements:

   https://www.va.gov/PORTLANDRESEARCH/training/index.asp

   The PI is also responsible for providing adequate supervision of personnel conducting these procedures.
2. The laboratory director must enforce the institutional policies that control access to the laboratory.
3. Personnel must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
4. 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.
5. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
6. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented in the lab. Whenever practical, the PI should encourage improved engineering and work practice controls, such as the substitution of plasticware for glassware or the use of needleless, blunt-tipped, or self-sheathing injection devices. These measures for safe sharps handling must be strictly followed:

   a. 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 placed in puncture-resistant sharps disposal containers placed close to the area where the work will occur. Employees should not place their hands inside the

container opening to dispose of sharps. Containers should be mounted or secured away from the main traffic stream to prevent spillage, must be closed when not in use, and must be replaced when ¾ full.

c. Non-disposable sharps must be placed in a hard-walled container if they must be transported to another location for decontamination. Autoclaving is the preferred method for this decontamination, whenever feasible.

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps.

7. Perform all procedures to minimize the creation of splashes and/or aerosols.

8. Decontaminate laboratory equipment and work surfaces after completion of work and after any spill or splash of potentially infectious material. Equipment must be decontaminated before repair or removal from the lab. The disinfectant type, dilution, and contact time must be appropriate for the infectious agents in use in the laboratory. Refer to Appendix A for disinfectant options.

9. Liquid cultures and stocks of potentially infectious materials must either be autoclaved or inactivated with the correct concentration of an appropriate disinfectant before discarding into the municipal sewer system. See Appendix A for disinfectant options. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak-proof container and secured for transport.

10. Dry specimens and potentially contaminated supplies such as gloves and plastics must be disposed of in biohazard waste bins:

a. Biohazard waste bags must be placed in rigid outer containers such as heavy gauge plastic. These containers must have a tightly fitted lid, which must be in place whenever the bin is not in active use.

b. Bins should be located away from the traffic stream and placed in a manner to prevent spillage.

c. The bins must not be overfilled, and minor compaction of contents should only be done by mechanical means (do not use hands).

d. Biohazardous waste must be removed from labs weekly and discarded in designated collection rooms. Housekeepers will transport biohazard waste containers from the collection areas for disposal by incineration.

e. No biohazard waste containers should be left unprotected in corridors.

f. Spills occurring during transport must be contained, cleaned, and the area disinfected immediately.

11. Personal health status may impact an individual’s susceptibility to infection, or ability to receive immunizations or prophylactic interventions. Some of the conditions that may increase the risk of an individual are pre-existing health conditions, use of certain medications, compromised immunity, and pregnancy or breast-feeding that may increase exposure of infants to certain

agents. Therefore, all laboratory personnel should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals should contact VA Employee Health at x55165 for appropriate counseling and guidance if warranted.

BSL-1: Special Practices

None required.

BSL-1: Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment such as BSCs or special facility design is not required but may be used as determined by a risk assessment of the work to be performed.
2. Lab coats or gowns are recommended to prevent contamination of personal clothing.
3. Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should 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. For example, chemical-impermeable gloves should be worn if caustic or carcinogenic chemicals are also in use. Alternatives to latex gloves should be available. In addition, workers should:
    a. Check expiration dates on boxes of gloves regularly and do not use if expired.
    b. Change gloves when contamination is suspected or glove integrity is compromised.
    c. Dispose of used gloves as biohazardous waste. Do not wash or reuse disposable gloves.
    d. Wash hands when work with hazardous material has been completed and also before leaving the laboratory.

Biosafety Level 2

Biosafety Level 2 builds upon BSL-1. BSL-2 containment is suitable for work involving Risk Group 2 agents that pose moderate hazards to personnel and the environment.

Note that VAPORHCS wet labs are considered BSL-2 labs, based on facility design and safety equipment located in those spaces.

The following standard and special practices, safety equipment, and facility requirements apply to BSL-2:

BSL-2: Standard Microbiological Practices

All of the Standard Microbiological Practices listed on pp. 5-6 for BSL-1 containment must also be followed for BSL-2 containment.

BSL-2: Special Practices

1. Access to BSL-2 laboratories is restricted by proximity card access.
2. All persons entering the laboratory while BSL-2 work is active must be advised of the potential hazards and meet specific entry/exit requirements, such as wearing appropriate PPE. Note that when BSL-1 and BSL-2 work is occurring simultaneously in a room, the effective containment level for the entire room will be BSL-2.
3. Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory. Contact VA Employee Health at x55165 with any medical surveillance/immunization questions.
4. A BSL-2 manual or set of standard operating procedures (SOPs) for laboratory-specific practices must be prepared and adopted as policy. These SOPs must be accessible to anyone working in the lab. If BSL-2+ procedures are required or utilized, a separate SOP will be developed detailing these procedures and available within the laboratory.
5. The Principal Investigator must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
6. Materials that are potentially infectious to humans must be placed in a durable, leak-proof container during collection, handling, and processing. A secondary durable and leak-proof container must be used for storage or transport of the agent within a facility.
7. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described below in the Bloodborne Pathogen and Needle-stick Exposures section, page 13. All such incidents must be reported to the Principal Investigator and to the Research office. Medical evaluation, surveillance, and treatment will be provided where necessary by Employee Health and appropriate records maintained.
8. Animal and plants not associated with the work being performed must not be permitted in the laboratory.
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 agent in use, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. See the last page of this document for an example of such a sign. This sign should be printed on a color printer to preserve the required orange/red color of the biohazard

symbol.

BSL-2: Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. All procedures must be conducted within a BSC (or using other physical containment measures approved by the SRS):
   a. The BSC must be certified annually. This is generally completed automatically by a VA contractor, but users should check the date on their BSC occasionally to ensure that its certification is current.
   b. Work in the BSC should be conducted in the operationally effective zone, generally at least 6" from the front.
   c. For the BSC to act as an effective safety barrier, air flow must not be impeded. Never block the air vents at the front and back of the cabinet. Do not clutter the cabinet with excess equipment.
   d. If the germicidal UV light in the cabinet will be used, it must be cleaned occasionally with a Kimwipe soaked in isopropanol, and it must be changed after 6 months of regular use in order to remain effective. Never substitute the UV light for good microbiological technique.
   e. Disinfect the surface of the BSC at the end of every session. See Appendix A for appropriate disinfectants for different infectious agents. Use correct dilutions and contact times. If bleach is used on the stainless-steel surface, follow with a rinse of distilled water or 70% ethanol to prevent corrosion.
2. Laboratory coats or disposable gowns must be worn while working with hazardous materials. Note that disposable gowns, not lab coats, must be used when working with infected animals. Remove PPE before leaving for non-laboratory areas, (e.g., cafeteria or office areas). Dispose of protective clothing appropriately. It is recommended that laboratory clothing not be taken home for washing.
3. Eye and face protection (goggles, mask, face shield or other splatter guard) must be used if splashes of infectious or other hazardous material is possible, particularly if the material must be handled outside the BSC. Safety goggles and face shields must be decontaminated before reuse, and face masks must be disposed of. Persons who wear contact lenses in laboratories should also wear eye protection.
4. Gloves must be worn at all times. See page 7, item 4 under "BSL-1 Safety Equipment" for glove guidelines.
5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by a risk assessment of work to be performed.

Biosafety Level 2+

Biosafety Level 2+ builds upon BSL-2. BSL-2+ containment may be required for work with agents whose inherent risks require one or more safety measures beyond those normally used for standard BSL-2 work. However, the risks and consequences of infection via the inhalation route are not considered great enough that a dedicated

BSL-3 facility and full BSL-3 procedures must be used. Examples of individual safety measures that may be added to the standard list of BSL-2 procedures are the required use of aerosol safety cups for centrifugation, use of an N95 respirator if inhalation hazards are suspected, or a strict ban on the use of sharps if the consequences of bloodborne transmission of the agent are serious. Work with the agent may need to occur in a dedicated tissue culture room or at times of the day when other researchers are not present.

Biosafety Level 3

Biosafety Level 3 is applicable to research 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.

For work requiring BSL-3 level containment, please contact the Facility BSL-3 Director. A separate VAPORHCS SOP covers work practices, PPE, training, and biosecurity of the BSL-3 facility.

Biological Risk Assessment and Selection of Appropriate Safeguards

Biological risk assessment is a subjective process requiring consideration of many hazardous characteristics of agents and procedures, with judgments based often on incomplete information. There is no standard approach for conducting a biological risk assessment, but the four-step approach described below can be helpful in guiding the process. The lab PI should conduct this assessment together with the biosafety officer, and then present the assessment to the Subcommittee for Research Safety (SRS) for review and approval.

1. Identify agent hazards and perform an initial assessment of risk.

Consider the principal hazardous characteristics of the agent, which include its capability to infect and cause disease in a susceptible human host, severity of disease, and the availability of preventive measures and effective treatments.

Several excellent resources provide information and guidance for making an initial risk assessment. The Biosafety in Microbiological and Biomedical Laboratories manual (BMBL) provides agent summary statements for some agents of increased public concern. Agent summary statements also identify known and suspected routes of transmission of laboratory infection and, when available, provide information on infective dose, host range, agent stability in the environment, protective immunizations, and attenuated strains of the agent. The Public Health Agency of Canada also provides useful Pathogen Safety Data Sheets for a wide range of agents. See Reference section for web links to these two sites.

Although a summary statement for one agent may provide helpful information for assessing the risk of a similar agent, it should not serve as the only resource for making the risk determination for that agent. Differences between the agents in such characteristics as infective dose or changes made at the molecular level that affect

virulence may alter the risk profile. Agents that are not thoroughly characterized may need to be treated with greater caution.

Using the description of the biosafety levels described earlier in the manual, make a preliminary determination of the biosafety level that best correlates with the initial risk assessment of the agent hazards.

2. Identify laboratory procedure hazards.

Agent summary statements typically provide information on the hazards associated with use of the agent during standard or routine procedures. When proposed laboratory procedures differ from the general conditions of the agent summary statement, or where an agent summary statement is not available, the risk assessment should consider how proposed procedures may affect the hazards of the agent.

Specific laboratory procedures can increase the hazard profile of an infectious agent by increasing the chance of exposure. These procedures include those that concentrate the agent or increase the suspension volume (leading to greater splash hazard), those that use equipment or procedures that generate small particle aerosols and larger airborne droplets, and those that use sharps. Complex procedures requiring numerous steps or a high level of dexterity may also increase the risk of accidental exposure.

Procedures involving animals can present a number of hazards such as bites and scratches, exposure to zoonotic agents, and generation of infectious aerosols.

3. Determine the appropriate biosafety level and select additional precautions indicated by the risk assessment.

The evaluation of the agent's basic characteristics plus a consideration of all lab procedures to be performed with the agent should allow for the selection of an appropriate biosafety level, which is then approved by the SRS. This biosafety level may be higher than that suggested by the characteristics of the agent alone and may require additional laboratory precautions. Selection of containment devices and PPE can be made using the descriptions of biosafety levels earlier in the manual. Remember that aerosol and droplet routes of agent transmission also are important considerations in specification of safety equipment. It is also important to recognize that individuals in the laboratory may differ in their susceptibility to disease and may need special safeguards.

4. Evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment.

The protection of laboratory workers, other persons who may enter the laboratory (such as maintenance workers), and the public will depend ultimately on the laboratory workers themselves. In conducting a risk assessment, the PI should ensure that the laboratory workers chosen to perform the work have acquired the technical proficiency in the use of microbiological practices and equipment operation required for the safe handling of the agent and have developed good habits that sustain excellence in the performance of those practices. A lab worker's training, experience in handling infectious agents, proficiency in the use of sterile techniques and BSCs,

ability to respond to emergencies, and willingness to accept responsibility for protecting oneself and others may all be considered during review of the project. Refer to the training website for all up-to-date training requirements:

https://www.va.gov/PORTLANDRESEARCH/training/index.asp

The PI should also ensure that the necessary safety equipment is available and operating properly. For example, a BSC that is not certified represents a potentially serious hazard to the laboratory worker using it and to others in the laboratory. The director should have all equipment deficiencies corrected before starting work.

Work with Human, Non-Human Primate, and Mammalian Cells and Tissues

Individuals who will be working with human cells or tissues, or who will be exposed to infectious materials, need to complete the required blood borne pathogen training. Refer to the training website for all up-to-date training requirements:
https://www.va.gov/PORTLANDRESEARCH/training/index.asp

Potential Laboratory Hazards

Potential laboratory hazards associated with human cells and tissues include exposure to bloodborne pathogens such as Hepatitis B virus (HBV), Hepatitis C virus (HCV), and Human Immunodeficiency Virus (HIV), as well as agents such as *Mycobacterium tuberculosis* that may be present in human lung tissue. Tumorigenic human cells also are potential hazards if accidently self-inoculated. Non-human primate (NHP) blood, cells, and tissues should always be considered potentially hazardous for agents such as *Cercopithecine herpesvirus 1* (B virus) that can infect humans as well. Mammalian cells immortalized with viral agents such as SV-40, Epstein-Barr virus (EBV), adenovirus or human papillomavirus (HPV), as well as cells carrying viral genomic material, also present potential hazards to laboratory workers due to the possible presence of replication-competent virus.

Hepatitis B Virus

This virus can be present in blood, urine, semen, cerebrospinal fluid, saliva and tissues. Transmission is typically via accidental inoculation or direct exposure of mucous membranes or compromised skin to infectious material. The virus is quite stable and has been shown to survive several days in dried blood. Symptoms of infection may or may not be noticeable; they can include fatigue, nausea, weakness, headache, chills, jaundice and liver disease. The Hepatitis B vaccine provides active, long-term immunization against HBV infection and is recommended for pre- and post-exposure prophylaxis. Pre-exposure vaccination is offered to all employees at no cost by Employee Health.

Hepatitis C Virus

HCV is very similar to HBV in potential transmission routes and symptoms. All human tissues or fluids should be handled under universal precautions, and, like HBV, the virus is very stable in dried blood. According to the CDC, hepatitis C is more prevalent in the U.S. than hepatitis B. Due to the fact that symptoms may not be noticeable, by the time most cases are diagnosed there may be irreversible liver damage. There is no

vaccine for HCV and no treatment for acute infection with HCV, though several treatments for chronic infections exist.

Human Immunodeficiency Virus (HIV)

HIV has been found in blood, semen, saliva, tears, urine, cerebrospinal fluid, amniotic fluid, breast milk, vaginal secretions, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, and a number of different tissues. The virus appears to be quite fragile and succumbs quickly to drying and chemical disruption. Over one million Americans are believed to be seropositive for this retrovirus, yet very few are believed to have seroconverted due to occupational exposure. Of those cases, the most common means of transmission appears to have been percutaneous inoculation, direct mucous membrane exposure, and direct exposure of non-intact skin to infected body fluids or tissues. Prophylactic antiretroviral treatment is available and effective, if started within 72 hours after HIV exposure.

Containment Practices

Human, NHP, and other hazardous mammalian cells must be handled using BSL-2 practices and containment. All work should be performed in a BSC unless approved by the SRS. Assays using these cells should be performed in a BSC unless potential pathogens have been completely inactivated by means such as heat or chemical treatment (e.g., homogenization of cells with Trizol). BSL-2 recommendations for personal protective equipment such as laboratory coats, gloves and eye protection should be rigorously followed. A biohazard standard operating procedure (SOP) specific for each lab must be posted near the BSC and followed. Every effort must be made to minimize the potential for spills, leakage, or generation of aerosols during the handling, transport, or storage of potentially infectious materials.

Bloodborne Pathogen and Needle-stick Exposures

Any needle-stick or sharp injury, mucous membrane contact, eye splash, or broken-skin contact with human, NHP, or other hazardous mammalian material is to be considered an emergency situation, due to potential for infection by bloodborne pathogens. For puncture wounds, immediately wash wound with soap and water for 5-10 minutes. There is no evidence to suggest that making a puncture wound bleed decreases risk of bloodborne pathogen transmission. Use of disinfectants such as hydrogen peroxide or alcohol is not recommended. Cover wound with sterile dressing. For eye/mucous membrane splash immediately rinse/flush area with water for 5-10 minutes. After washing/flushing the exposed area, immediately report to VA Employee Health (or the Emergency Department if after hours) for a confidential medical evaluation and possible prophylactic treatment. All employees, whether paid by the VA, OHSU, or PVARF, can receive this treatment at the VA. Every exposure must then be reported as soon as reasonably possible by the employee to the supervisor, who must investigate and document the incident, and report it to the Research office for documentation.

Work with Laboratory Animals

Recommendations for work with animals follows a similar biosafety classification system to that used for other research, with the levels ABSL-1 through ABSL-4 (i.e., “animal biosafety level) used to describe the practices, safety equipment, and facilities

required to protect workers from biohazards encountered in animal research. Only ABSL-1 through ABSL-3 work is permitted at the VA. Although research animals are capable of causing disease in humans through animal to human transmission of infectious agents (zoonotic pathogens), research work with rodents is generally considered to be low risk. Laboratory-reared rodents are specified pathogen-free and are typically accompanied by a health history when purchased from a VAPORHCS Veterinary Medical Unit (VMU)-approved source or when transferred from another institution. The VMU quarantines newly arrived animals and repeats health screenings before release of the animals to the general colony.

As a result, the risk of transmission of zoonotic agents from working with laboratory rodents is very low and often can be performed with ABSL-1 handling conditions. These requirements, which are taught to all users of VAPORHCS animal facilities by the VMU staff, include restrictions for animal room access, PPE to be worn, methods to be employed for cleaning and disposal of cages and bedding, and measures to be taken if an animal becomes ill.

However, animals infected with microorganisms as part of a research project may present an increased hazard to humans due to the infectious agents themselves, with new routes of transmission of these agents via animal bites and scratches or exposure to contaminated bedding. The same process described above for risk assessment of work with infectious agents should be undertaken for animal work using pathogens. If the infectious agent would normally be handled with BSL-2 precautions in a laboratory setting, then often the recommended animal biosafety level will be ABSL-2 as well. However, depending on the microorganism and its mode of transmission, work with infected animals may require additional safety precautions. Possible hazards to consider include:

- Inoculation from animal bites and scratches
- Exposure to animal excreta in cage bedding
- Self-inoculation from instruments and sharps
- Generation of aerosols during procedures
- Exposure to animal tissues during dissection of infected animals (including particular tissues that may be preferred sites of infection for a microorganism, such as the brains of *Toxoplasma*-infected animals)

Animal-to-animal transmission must also be considered when determining the type of facilities, animal housing, and safety practices required. Occasionally, animals may be able to move from higher to lower levels of containment if the agent is no longer considered infectious after a period of time. Researchers should always contact the Veterinary Medical Officer and/or VA Biosafety Officer when considering new animal work with pathogens. The VMU, Biosafety Officer, and PI together will provide necessary training to ensure that lab members are able to conduct the work safely.

Laboratory Animal Allergens

Allergic reactions associated with handling animals are common, and animal care workers with preexisting allergic conditions, such as hay fever, are more likely to develop sensitivity to animal-related allergens at work. If not properly managed, symptoms can potentially evolve into occupationally related asthma.

The major sources of allergens in rats and mice appear to be urine and saliva, and the primary exposure route for workers is through inhalation of allergens. Disturbance of contaminated litter and bedding results in the release of very small particles of litter containing the allergen. These particles are often small enough to stay airborne for extended periods of time and can easily be deposited in the airway.

Activities such as cage cleaning, weighing, shaving, injections, blood collection and surgery can release significant quantities of the allergens. Of these, cage cleaning and changing represent a major source of exposure. When cleaning and disinfecting cages in the cage wash facility, dirty bedding should be dumped using a certified 'dumping station' while wearing appropriate PPE. Cage changing stations should be used in the animal holding rooms. Both types of stations are equipped with HEPA filtration and direct airflow away from the user. Hands and exposed skin areas should be washed after all animal work. In addition, an N95 respirator or a PAPR (powered air-purifying respirator) should be worn at all times in the VMU by individuals with known animal-related allergies. Before using either type of respirator, you must be cleared by VA Employee Health and be fit tested. Any worker with questions about allergies and work with animals should contact VA Employee Health for guidance.

Work with Recombinant DNA or Recombinantly-Modified Organisms

Determining the biosafety risks associated with recombinantly-modified infectious agents starts with an assessment of the hazards of the agent itself, including any manipulations to that agent that may affect such things as virulence, environmental stability, route of spread (e.g., tissue tropism), and availability of a vaccine or treatment. Gene products expressed by infectious vectors are themselves evaluated for toxicity, physiological activity, and allergenicity. Any strain that has been manipulated to be more hazardous than the parent strain may require a higher containment level, while some attenuated strains that have lost virulence factors may qualify for a reduction in containment level, compared to the level assigned to the parent strain.

At VAPORHCS, review and approval for recombinant or synthetic nucleic acid work occurs via a collaborative effort between the SRS and the OHSU Institutional Biosafety Committee (IBC). Proposed work is first submitted to the IBC for review. In some cases, the proposed work will be considered extremely low risk and will be exempt from further IBC oversight. An example is the use of a non-viral based vector to express a non-toxic gene in a cultured cell line. If the work is subject to formal IBC review, the IBC approval letter will include a preliminary determination of the required biosafety level for conduct of the work. Infrequently, due to constraints such as available space or engineering controls, the SRS may determine that a different containment level must be used at the VA. Regardless of whether the work is determined to be exempt or subject to IBC oversight, all work with recombinant or synthetic nucleic acids must also be reviewed and approved by the SRS. Contact the VA Biosafety Officer with questions regarding the approval process.

Work with Select Agents and Toxins of Biological Origin

Select Agents and Toxins are a Federally designated group of highly infectious agents and potent biological toxins that are capable of posing a severe threat to public health and safety. This threat has resulted in legislation that strictly controls the possession and use of these materials. VAPORHCS does not permit the possession of any viable Select Agents, and only permits the use of Select Toxins, such as tetrodotoxin, in small quantities that are considered exempt from registration with the Federal Select Agents Registry. Use of even these small toxin quantities is highly regulated, with extra security measures, training, and inventory control required.

Other biological toxins used more frequently in laboratory work, such as pertussis toxin, diphtheria toxin, or the light chain (only) of botulinum toxin, are not considered Select Toxins as they are less hazardous to lab personnel and the community. Although toxins do not replicate and are not infectious, work with these agents should generally be performed using BSL-2 practices as a guideline, depending on a risk assessment of each specific lab operation. The main laboratory risks from toxins are accidental exposure by direct contamination of mouth, eyes or other mucous membranes; by inadvertent aerosol generation; and by needle-sticks or other accidents that may compromise the normal barrier of the skin.

Safety Equipment and Containment

Laboratory work with toxins should be done only in designated rooms with controlled access and at pre-determined bench areas. When toxins are in use, the room should be clearly posted: “Toxins in Use—Authorized Personnel Only.” Unrelated and nonessential work should be restricted from areas where stock solutions of toxin are used. Visitors or other untrained personnel granted laboratory access must be monitored and protected from inadvertently handling laboratory equipment used to manipulate the toxin.

Routine operations with toxin solutions should be conducted in a BSC. A certified chemical fume hood can also be used as a primary containment device. The interior of the hood or BSC should be decontaminated periodically, for example, at the end of a series of related experiments. Until thoroughly decontaminated, the hood or BSC should be posted to indicate that toxins remain in use, and access should remain restricted.

Personal protective equipment is similar to that used with BSL-2 handling. Personnel should wear a lab coat or disposable gown, gloves (selected for compatibility with the toxin and any solvents used to prepare a toxin solution) and face shield or safety glasses plus a face mask when conducting operations that pose a potential splash or droplet hazard. Measures to prevent sharps injuries should be used whenever possible; see page 5, item 6 under “BSL-1: Standard Microbiological Practices.”

An inventory control system should be in place to account for toxin use and disposition. If toxins are stored in the laboratory, containers should be sealed, labeled, and secured to ensure restricted access; refrigerators and other storage containers should be clearly labeled and provide contact information for laboratory staff.

Inadvertent Toxin Aerosols

Emphasis must be placed on evaluating and modifying experimental procedures to eliminate the possibility of inadvertent generation of toxin aerosols. Pressurized tubes or other containers holding toxins, such as tubes recently removed from the freezer, should be opened in a BSC or chemical fume hood. Operations that expose toxin solutions to vacuum or pressure, for example sterilization of toxin solutions by membrane filtration, should always be performed in a BSC or chemical fume hood. If vacuum lines are used with toxin, they should be protected with a HEPA filter to prevent entry of toxins into the line.

Centrifugation of cultures or materials potentially containing toxins should only be performed using sealed, thick-walled tubes in safety centrifuge cups or sealed rotors. The outside surfaces of containers and rotors should be routinely cleaned after each use to prevent contamination from aerosols.

Additional Precautions with Dry Toxin

Experiments should be planned to eliminate or minimize work with dry toxin (e.g., freeze-dried preparations). Dry toxin should be ordered in small quantities that can easily be reconstituted with solvent inside the primary container, rather than removing dry toxin to weigh on a balance. Unavoidable operations with dry toxin should only be undertaken with appropriate respiratory protection (contact a VA Industrial Hygienist to discuss) and engineering controls such as a disposable glove bag or glove box within a chemical fume hood or Class II BSC.

Decontamination and Spills

Toxin stability varies considerably outside of physiological conditions depending upon the temperature, pH, ionic strength, and availability of co-factors and other characteristics of the surrounding matrix. Values found in literature for dry heat inactivation of toxins can be misleading due to variations in experimental conditions, matrix composition, and experimental criteria for assessing toxin activity. Moreover, inactivation is not always a linear function of heating time: some protein toxins possess a capacity to re-fold and partially reverse the inactivation caused by heating. In addition, the conditions for denaturing toxins in aqueous solutions are not necessarily applicable for inactivating dry, powdered toxin preparations.

Many toxins are susceptible to inactivation with dilute sodium hydroxide (NaOH) at concentrations of 0.1-0.25N, and/or sodium hypochlorite (NaOCl) bleach solutions at concentrations of 0.1-0.5% (w/v). Use freshly prepared bleach solutions for decontamination; undiluted, commercially available bleach solutions typically contain 3- 6% (w/v) NaOCl.

Depending upon the toxin, contaminated materials and toxin waste solutions can be inactivated by incineration or extensive autoclaving, or by soaking in suitable decontamination solutions. All disposable material used for toxin work should be placed in a tightly closed biohazard bag and transported to a biowaste collection room for incineration.

Toxin solutions, especially concentrated stock solutions, should be transported in leak/spill-proof secondary containers. In the event of a spill, avoid splashes or generating aerosols during cleanup by covering the spill with paper towels or other disposable, absorbent material. Apply an appropriate decontamination solution (e.g,

bleach, diluted as above) to the spill, beginning at the perimeter and working towards the center, and allow sufficient contact time to completely inactivate the toxin.

Packaging Requirements for Shipping of Infectious Substances

Numerous accidents occur each year involving the transportation of infectious substances, including biological tissues and fluids. Transportation of infectious agents is regulated by the Dept. of Transportation and the International Air Transport Association (IATA), and individual couriers such as FedEx and UPS may have additional requirements that must be met for packages containing infectious materials. Only designated, trained individuals are authorized to prepare items classified as Dangerous Goods for shipment. Training is offered online through the OHSU Compass system and includes information on proper hazard identification, classification, packaging, labeling, and documentation. Training records must be retained and made available upon request to appropriate inspecting agencies. Refresher training must take place within 24 months of previous training to ensure that knowledge is current.

Some materials may not meet the definition of a Dangerous Good but may still require special packaging and shipping procedures. Examples of materials in this category include biological samples or diagnostic specimens that are not likely to cause disease in humans or animals. These materials still require proper packaging and should be brought to trained employees for shipping assistance.

Transport between the VA and OHSU

At no time may materials be transported between the VA and OHSU in a manner that requires or warrants the wearing of protective clothing or gloves by the person transporting the material. Containers must be securely closed and placed in a non-breakable secondary container such as a securely sealed plastic bin or cooler.

Personal vehicles can be used to transport biological materials only if the samples are placed in a secondary container (such as a sealed Ziploc bag) and then this in turn is placed in another sealed container, such as a hard-sided cooler. All containers must be labeled with a universal biohazard sign and positioned in the vehicle in such a way that the container remains upright and as far away as possible from the driver (e.g., trunk of the car).

References:

Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th Edition:
https://www.cdc.gov/labs/BMBL.html

Canadian Public Health Agency Pathogen Safety Data Sheets:
https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html

NIH Guidelines for Research Involving Recombinant DNA Molecules
https://osp.od.nih.gov/biotechnology/nih-guidelines/

Appendix A: Disinfectants

Disinfectants		Practical Requirements		Inactivation Efficacy					Potential Applications				Characteristics		
Category	Brand Examples	Effective Dilution	Shelf Life (dilute/open shelf life in parentheses)	Bactericidal	Virucidal	Fungicidal	Sporicidal	Tuberculocidal	Skin disinfectant	Hard surfaces	Stainless steel	Add to liquid waste	Leaves Residue	Inactivated by organic matter	Other
Quaternary Ammonia Compounds		0.1- 5.0% (1,000-50,000 ppm)													
	Quatricide TB	N/A (2100 ppm)		+	+	+		+	+	+	+			+	Parvovircidal
	Quatricide PV	1:64 (750 ppm)		+	+	+			+	+	+			+	Parvovircidal
	Quatricide	1:64 (780 ppm)		+	+	+			+	+	+			+	
	Cetylcide II	1:64 (740 ppm)	64 days	+	+	+	+			+	+			+	
	Lysol I.C. spray	N/A (1000 ppm)	2 years	+	+	+	+	+		+	+			+	
	Lysol I.C. Quat	1:256 (660 ppm)	2 years	+	+	+				+	+			+	
	Madacide-FD	N/A (3080 ppm)		+	+	+	+	+		+	+			+	
	Morning Mist	1:64 (780 ppm)		+	+					+	+				
	Roccal	1:256 (920 ppm)		+	+	+	+			+	+		+		
	Virex II 256	1:256	3 y	+	+	+	+			+	+			+	
Chlorine Compounds		50,000 ppm A												+	
	A-1 bleach	1:10 (5,000 ppm)	1 mo	+	+	+	+			+		+		+	Corrodes metal
	A-1 bleach	1:5 (10,000 ppm)	1 mo	+	+	+	+	+		+		+		+	Corrodes metal
Iodophors		25 –1600 ppm A												+	
	Wescodyne	1:100 (125 ppm)	3 years	+	+	+	+	+	+	+		+	+	+	
	Betadine		3 years	+	+	+	+		+	+			+	+	
	Iodine		3 years	+	+	+	+		+				+	+	
Phenolic Compounds		1.0 - 5.0% (10,000-50,000ppm)													
	Vesphene IIIse	1:128	(14 d)	+	+	+	+	+		+	+				
	Amphyl	5.0%		+	+	+	+	+		+	+				
	Sporicidin	N/A (15,600 ppm)	6 mos	+	+	+	+	+		+	+		+		
Alcohols		70 – 85% (700,000-850,000 ppm)													Flammable
	Ethyl & Isopropyl			+		+		+	+					+	
	Envirocide	N/A (202,000 ppm)	2 years	+	+	+		+		+	+				Biodegradable
	Opticide3	N/A (210,000 ppm)	years	+	+	+		+		+	+				
	Lysol brand spray - household	N/A (790,000 ppm)	2 years	+	+	+				+	+				
Aldehydes		0.2 – 8.0% (2,000-80,000 ppm)													
	Cetylcide G	1:16 (34,000 ppm)		+	+	+	+	+		+	+		+		
	Metricide Plus 30	N/A (34,000 ppm)		+	+	+	+	+		+	+		+		
	Cidex OPA	N/A (5,500 ppm)	2yr (14d)	+	+	+	+	+		+	+		+		
	Cidex Plus 28	N/A (< 50,000 ppm)	(28d)	+	+	+	+	+					+		
Hydrogen Peroxide															
	Hydrogen peroxide (L)	3-6% (30,000-60,000 ppm)		+	+	+	+ (18h)		+					+	
	Oxivir 1 RTU	Ready-to-use	2 years	+	+	+		+		+	+			+	
Hydrogen Peroxide + Peracetic Acid															High-level disinfectants
	Spor-Klenz	Ready-to-use	1 year	+	+	+	+ 32	+		+	+				
	Peridox	1:5	2 years	+	+	+	+ 32	+		+	+				
Chlorhexidine															
	Novalsan	1:50 (400 ppm)	1 year	+	+	+	+		+	+	+				Non-toxic

BSL-2 Laboratory

BIOHAZARD

Authorized Personnel Only

Biosafety Level 2 Biological Agents:	Enter agents here
Special Procedures, PPE, or Precautions for Entry/Exit:	Add information here

Principal Investigator		Emergency Contact (must be 24/7)	
Name	**Phone**	**Name**	**Phone**
Type name	Enter phone #	Type name	Enter phone #