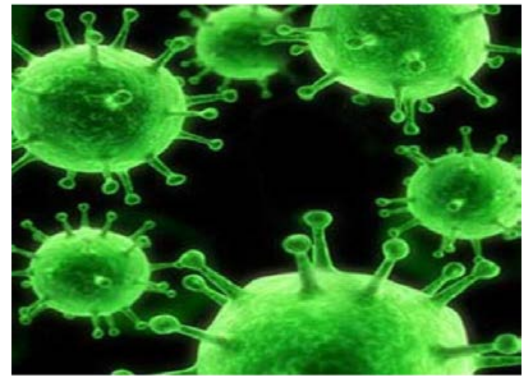
# Institutional Biosafety Manual



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IMAGINE THE UNIVERSITY OF UTAH

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# Policy

## A. Purpose

The purpose of this manual is to specify controls and safe handling practices for microorganisms (viruses, bacteria, fungi, rickettsia, mycoplasma, protozoans, multicellular parasites, and prions), biological toxins, recombinant or synthetic nucleic acid molecules, human blood or tissues, and animal cell cultures.

## B. Scope

This policy applies to University of Utah premises owned, operated and leased and all persons on the premises. All staff and volunteers of the University have individual responsibilities to take reasonable care for their own health and safety and for that of others who might be affected by their acts of omissions. They must cooperate with those persons who are responsible for health and safety to enable them to carry out their duties.

## C. Roles and Responsibilities

### 1. President

a. The University President has ultimate responsibility for establishing and maintaining environmental health and safety programs and establishing a system for assessing safety performance for the University.

### 2. University Administration

All Vice-Presidents, Deans and Department Heads are responsible to:

a. Ensure that facilities and equipment provided meet requirements for a safe work environment and activities being conducted or modified are in compliance with applicable rules, regulations and standards.

b. Ensure individuals under their management have the authority and support to implement health and safety policies, practices and programs.

c. Ensure areas under their management are in compliance with University, local, state and national environmental health and safety policies, practices and programs.

d. Establish priorities and committing resources for correction of safety deficiencies.

e. Establish procedures for dissemination of policies and other safety-related information.

f. Establish procedures for implementation of policies.

g. Establish a system for assessing safety performance.

h. Immediately notify the University of Utah's Occupational and Environmental Health and Safety (OEHS) department when they become aware of a violation of any University, local, state or national environmental health or occupational safety rule or regulation. This includes any contact with the local, state, and federal regulatory agencies.

i. The Assistant Vice President for Research Integrity serves as the Administrator of the Institutional Biosafety Committee (IBC).

**3. Principal Investigators and/or Laboratory Supervisors:**

The Principal Investigator or Laboratory Supervisor (referred to as the PI forthwith) has full responsibility for the health and safety of all personnel working in their laboratory. The PI may delegate the safety duties for which they are responsible, but must ensure that the delegate is sufficiently experienced to conduct this role and that the delegated duties are adequately performed. **However, delegation of these duties does not remove or limit the responsibility of the PI**. Specific responsibilities include:

a. The PI is responsible for full compliance with IBC approved research protocols, trainings required by the University, the University Biosafety Manual, the National Institute of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids (the NIH Guidelines), the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogen Standard (human-derived materials, including human cell lines: codified in 29 CFR 1910.1030) and other local, state and federal regulations that apply to research. Laboratories working with blood or other potentially infectious material must follow the requirements described in the University of Utah Exposure Control Plan.

b. The PI must enforce compliance with the approved standards and policies of the University.

c. PIs must conduct a risk assessment to identify potentially hazardous procedures involving biological agents, develop Standard Operating Procedures (SOPs), instruct and train all personnel and students working in the lab on safe work practices, keep the lab space clean and up-to-date, and follow regulations for disposal of infectious waste. The PI must provide appropriate personal protective equipment (PPE) to their staff, provide training in its proper use, ensure staff wear PPE whenever they are in the lab, and facilitate cleaning and/or disposal of PPE.

d. PIs must register research projects that require review by the IBC and/or OEHS, such as the generation and/or use of Recombinant or Synthetic Nucleic Acid Molecules (rsNA), work requiring BSL-2 or ABSL-2 containment, work with or storage of Select Agents, injection of human cells into animals, work with acute biological toxins, and other work with infectious agents, as needed.

e. PIs are responsible for hands on training for all laboratory procedures. Examples of hand on training include the donning and doffing of personal protective equipment, the correct and safe use of biological safety cabinets, techniques to eliminate or reduce the generation of aerosols, correct disposal of waste, including sharps, and spill cleanup. They must ensure that all laboratory personnel and students have fulfilled University training requirements and are current in all required training.

f. The PI shall complement initial safety training with ongoing actions and activities to encourage safety and promote a strong, positive safety culture in the laboratory.

g. The PI must maintain and annually review laboratory specific SOPs (e.g. bloodborne pathogen exposure control plans, biosafety manuals, lab specific training protocols, etc).
   - The PI must ensure their research laboratory staff and students are trained on the contents of this University Biosafety manual and relevant laboratory specific standard operating procedures and follow said requirements. Training shall be completed annually or as frequently as determined by IBC or OEHS.

h. The PI must periodically survey their laboratories for compliance with standards and policies regarding safe handling and use of biological agents and toxins.

i. PIs are responsible for maintaining good working order for equipment in their laboratories, including annual certifications of biological safety cabinets.

j. The PI must avail the laboratory to periodic OEHS inspections. The PI must respond in writing to any deficiencies noted during the inspection with a corrective action plan and date of implementation, within the time specified by the OEHS inspector.

k. The PI must ensure employees report any changes in their health status to an Occupational Medicine physician. Suspected infections or exposure to biohazardous materials, including all recombinant or synthetic nucleic acid molecules, must be reported immediately to the Biosafety officer.

l. As applicable, the PI must advise the IBC, Institutional Review Board for Research with Human Subjects (IRB), Institutional Animal Care and Use Committee (IACUC), and OEHS of any significant changes in approved protocols involving use of

biological agents and/or toxins.

m. The PI must comply with shipping requirements for biohazardous agents and toxins and materials shipped on dry ice.

n. The PI must maintain an accurate inventory of all acute biological toxins.

o. The PI and/or lab personnel are responsible for initiating cleanup and disinfection in the event of a biohazard spill in a laboratory. If assistance is required, contact OEHS. The PI is responsible for ensuring that all corrective actions and emergency procedures are followed in accordance with applicable University procedures and regulations. Any spills or accidents that result in exposure to biohazardous agents must be reported to the Biosafety Officer.

p. The PI must serve as a role model by exhibiting good safety behavior.

q. The PI must encourage open and on-going dialog about lab safety. Regular self-assessment inspections shall be conducted by employees.

r. Build and facilitate a partnership with Occupational and Environmental Health and Safety.

**4. Lab Personnel and Students:**

a. Lab personnel and students must adhere to the established policies, Standard Operating Procedures (SOP's), and guidelines for biological safety as trained and following the PI's instructions.

b. Lab personnel and students working with biohazardous agents must adhere to University training requirements and the University Biosafety Manual. Additionally, laboratory personnel and students must adhere to the approved research protocols, the NIH Guidelines, and the OSHA Bloodborne Pathogen Standard (codified in 29 CFR 1910.1030). Employees and students working with blood or other potentially infectious material must follow the requirements described in the University of Utah Exposure Control Plan.

c. Lab personnel and students must demonstrate understanding of how to safely work with potentially infectious agents, be provided and wear appropriate personal protective equipment (PPE), keep their laboratory space clean and up-to-date, and follow regulations regarding the disposal of infectious and/or hazardous waste.

d. Lab personnel and students must inform immediate supervisor of any unsafe practices or conditions in the work area.

e. Lab personnel and students must report any change in health status to the supervisor if there is a possibility it may be work related.

f. Lab personnel and students must report all biological spills and incidents, including exposures to biohazardous agents, to their supervisor, who must file a report with OEHS.

g. Lab personnel and students shall seek immediate medical attention following exposure to

biohazardous agents.

h. Lab personnel and students must be proactive in regards to obtaining annual training, certification and medical clearance necessary to perform job description and duties.

**5. Institutional Biosafety Committee (IBC):**

a. The IBC is authorized by the Vice President for Research to formulate policy and procedures related to the use of biohazardous agents, including: human pathogens, oncogenic viruses, other infectious agents, recombinant or synthetic nucleic acids (rsNA), including human gene transfer and transgenic animal protocols, acute biological toxins and the injection of human cells into research animals.

b. The IBC is responsible for review and approval of projects involving rsNA research and human gene transfer protocols, in accordance with the NIH Guidelines. Additionally, the IBC reviews work with human and non-human primate blood and tissue samples, Select Agents, biohazardous agents that are animal or human pathogens requiring Biosafety Level 2 (BSL-2) or higher or Animal Biosafety level-2 (ABSL-2) containment, and acute biological toxins, as well as work with other potentially infectious agents on an as needed basis.

c. The IBC sets containment levels in accordance with NIH and Centers for Disease Control and Prevention (CDC) guidelines, and adopts emergency plans and procedures covering accidental spills and personnel contamination.

d. The IBC periodically reviews this Biosafety Manual and the current inventory of rsNA activity. In the event of any significant violations or accidents, the IBC shall report the incident to the NIH Office of Science Policy (OSP).

e. The IBC shall determine the necessity for health surveillance and pre-exposure prophylaxis for research projects.

f. Reviews reports of violations of the NIH Guidelines, such as failure to register non-exempt work with the IBC, exposures to recombinant or synthetic nucleic acid molecules, or any significant research-related accidents and illnesses, prepared by the Biosafety officer. The IBC reviews corrective action plans submitted by the Biosafety officer.

**6. Institutional Review Entity (IRE)**

On September 24, 2015 the US Government issued the policy entitled "United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern." All institutions subject to this Policy must now have a mechanism in place to evaluate research that is potentially Dual Use Research of Concern (DURC). Institutions subject to the Policy must also have an established Institutional Review Entity (IRE). The IRE assesses the risks and benefits associated with research conducted by the institution, identifies DURC as described in the Policy, and works with the principal investigator and

funding agency to develop a risk mitigation plan, when appropriate. If the IRE determines that Federally-funded research has DURC potential, the institution must notify the funding Agency listed on the award within 30 days. The University of Utah Institutional Biosafety Committee (IBC) will serve as the DURC Institutional Review Entity (IRE) and will identify and recruit additional ad hoc members to meet the requirements established by the NIH. See Appendix H for details of the DURC policy.

**7. Occupational and Environmental Health and Safety (OEHS) and Biosafety Officer:**

a. Provide consultation and technical information on the safe handling of biological agents and toxins.
b. Periodically review, maintain, and update the University of Utah Biosafety Manual, as well as the University of Utah Exposure Control Plan, which specifically addresses requirements for work with human blood or other potentially infectious material (OPIM), including human cell lines.
c. Coordinate and provide oversight for the annual certification of biological safety cabinets by an outside contractor.
d. Review and recommend purchases of biological safety cabinets and other related safety equipment.
e. Advise in the disinfection of facilities and equipment.
f. Assist in the development of safety and exposure control plans and training programs.
g. Assist with incident investigations.
h. Submit incident reports to the National Institutes of Health Office of Science Policy following violations of the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids, such as failure to register non-exempt work with the IBC, exposures to recombinant or synthetic nucleic acid molecules, or any significant research-related accidents and illnesses.
i. Submit an annual report to the Vice President for Research and Associate Vice President detailing activities of the IBC, including details of incidents occurring in laboratories registered with the IBC.

# Procedures

## A. General

1. Eating, drinking, chewing gum, smoking, applying cosmetics and contact lenses, or storage of foods is not permitted in the laboratory. Cell phones and headphones must not be used while conducting work.

2. Personnel must wash their hands and wrists for at least 20 seconds with soap and warm running water after handling infectious material, removal of gloves or other PPE, and before leaving the laboratory.
3. Control access to restricted biohazard areas.
4. Keep laboratory doors and windows closed while work is in progress.
   A. Post a warning sign, such as the universal biohazard symbol, when infectious material is present in the area. This warning sign must identify the agent and indicate the requirements for entry.
   B. Limit access to the laboratory during procedures involving biohazardous agents. Make sure doors to laboratory are secured and locked at the end of each day.

## B. Biological Risk Assessment

Adapted from the **"Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories:** Recommendations of a CDC-convened, Biosafety Blue Ribbon Panel"

A risk assessment must always be conducted prior to initiating any work in a laboratory. For work that needs to be registered with and approved by the Institutional Biosafety Committee (IBC), a risk assessment is a key part of the registration process. The Principal Investigator (PI)/Laboratory Director is responsible for identifying potential hazards, assessing risks associated with those hazards, and establishing precautions and standard procedures to minimize employee exposure to those risks. These must be documented in a laboratory-specific Biosafety manual and made available to all staff working in the laboratory. The risk assessment conducted by the PI will be reviewed by the IBC who may require changes prior to the approval of the work.

Qualitative biological risk assessment is a subjective process that involves professional judgments. Because of uncertainties or insufficient scientific data, risk assessments sometimes are based on incomplete knowledge or information. Inherent limitations of and assumptions made in the process also exist, and the perception of acceptable risk differs for everyone. The risk is never zero, and potential for human error always exists.

Identifying potential hazards in the laboratory is the first step in performing a risk assessment. A comprehensive approach for identifying hazards in the laboratory will include information from a variety of sources. No one standard approach or correct method exists for conducting a risk assessment; However, several strategies are available, such as using a risk prioritization matrix, conducting a job hazard analysis; or listing potential scenarios of problems during a procedure, task, or activity. The process involves the following five steps:

A. Identify the hazards associated with an infectious or biohazardous agent or material, including human pathogens, recombinant viral vectors, and acute biological toxins.
B. Identify the activities that might cause exposure to the agent or material.
C. Consider the training, competencies and experience of laboratory personnel.
D. Evaluate and prioritize risks (evaluate the likelihood that an exposure would cause a laboratory-acquired infection [LAI] and the severity of consequences if such an infection occurs).
E. Develop, implement, and evaluate controls to minimize the risk for exposure and establish plans for how to deal with an exposure, should it occur.

**Step 1. Identify the hazards associated with an infectious or biohazardous agent or material.**

A. The potential for infection, as determined by the most common routes of transmission (i.e., ingestion by contamination from surfaces/fomites to hands and mouth; percutaneous inoculation from cuts, needle sticks, nonintact skin, or bites; direct contact with mucous membranes; and inhalation of aerosols) (Table 1);
B. The volume and concentration of organisms handled;
C. Intrinsic factors (if agent is known):
   i. Pathogenicity, virulence, and strain infectivity/communicability;
   ii. Mode of transmission (mode of laboratory transmission may differ from natural transmission);
   iii. Infectious dose (the number of microorganisms required to initiate infection can vary greatly with the specific organism, patient, and route of exposure) or LD50 for toxic materials;
   iv. Genetic modifications that alter the risk, such as expression of toxins, oncogenes or siRNAs to knockdown tumor suppressors, or pseudotyping that expands susceptible host range;
   v. The risk of the formation of replication competent viruses when using recombinant viral vectors;
   vi. Form (stage) of the agent (e.g., presence or absence of cell wall, spore versus vegetation, conidia versus hyphae for mycotic agents);
   vii. Invasiveness of agent (ability to produce certain enzymes);
   viii. Origin of the material being handled. For example human tissues or cell lines may harbor pathogens (Table 2);
   ix. Availability of vaccines and/or prophylactic interventions; and
   x. Resistance to antibiotics.

**Step 2. Identify activities that might cause exposure to the agent or material.**

A. The facility (e.g., BSL-2, BSL-3, open floor plan [more risk] versus separate areas or rooms for specific activities [less risk], sufficient space versus crowded space, workflow, equipment present);

B. The equipment (e.g., uncertified Biological Safety Cabinets [BSCs], cracked centrifuge tubes, improperly maintained autoclaves, overfilled sharps containers, Bunsen burners);

C. Potential for generating aerosols and droplets. Aerosols refer to liquid droplets or solid particulates dispersed in air. Aerosols are too small to be seen by the unaided eye and remain suspended in air for a period of time. The production of aerosols while handling infectious agents historically has accounted for the greatest source of laboratory-acquired infections (LAIs).

D. Aerosols can be generated from most routine laboratory procedures but often are undetectable. The following procedures have been associated with generation of infectious aerosols.

1. Manipulating pipets, needles, syringes and sharps
    - Subculturing positive blood culture bottles, making smears
    - Expelling air from tubes or bottles
    - Withdrawing needles from stoppers
    - Separating needles from syringes
    - Aspirating and transferring body fluids
    - Harvesting tissues

2. Manipulating inoculation needles, loops, and pipettes
    - Flaming loops
    - Cooling loops in culture media
    - Subculturing and streaking culture media
    - Expelling last drop from a pipette

3. Manipulating specimens and cultures
    - Centrifugation
    - Setting up cultures, inoculating media
    - Mixing, blending, grinding, shaking, sonicating, and vortexing specimens or cultures
    - Pouring, splitting, or decanting liquid specimens
    - Removing caps or swabs from culture containers, opening lyophilized cultures, opening cryotubes
    - Spilling infectious material

- Filtering specimens under vacuum
- Preparing smears, performing heat fixing, staining slides
- Performing serology, rapid antigen tests, wet preps, and slide agglutinations
- Throwing contaminated items into biohazardous waste
- Cleaning up spills

E. To avoid aerosols;

1. Perform activities in a biological safety cabinet (BSC); or chemical fume hood when appropriate.
2. Keep tubes stoppered when vortexing or centrifuging.
3. Allow aerosols to settle prior to opening centrifuges, blenders, or mixed tubes.
4. Place cloth soaked with disinfectant over work surface to deactivate possible spills or droplets of biohazard agents. Soaked gauze can be wrapped around ampoules while breaking, needles while being removed from a vial or stoppers being removed from tubes.
5. When reconstituting or diluting contents of an ampoule do so slowly and carefully.
6. Mix solutions by discharging the secondary fluid down the side of the container or as close as possible to the surface of the primary solution.
7. Allow inoculating needle to cool before touching biological specimens.

F. Pipetting;

1. Mouth pipetting is not permitted.
2. No infectious mixture shall be prepared by bubbling air through the liquid with the pipet.
3. No infectious materials shall be forcibly discharged from pipets.

G. Use of animals;

1. Restrain animals by physical or chemical (anesthesia) means.
2. Inject animals inside in a biological safety cabinet (BSC); or chemical fume hood when appropriate.
3. Select gloves that provide added protection with larger animals, if appropriate.
4. Wear PPE to avoid exposure to animal body fluids, including feces.

H. Use of sharps, syringes and needles;

1. Avoid the use of syringes and needles if possible. Use the needle-locking type or a disposable syringe needle unit.
2. Needles must not be re-sheathed, bent, broken or removed from disposable syringes. Needles and syringes must be discarded in biohazard labeled sharps containers. Do not discard needles into disinfectant pans containing pipets or other glassware.

I. Production of large volumes or concentrations of potential pathogens or agents;

J. Improper disposal of hazardous waste;

K. Improperly used or maintained equipment;

L. Examples of possible hazards are decreased dexterity or reaction time for workers wearing gloves, reduced ability to breathe when wearing N95 respirators, or improperly fitting personal protective equipment (PPE).

M. Working alone in the laboratory.

   1. No inherent biologic danger exists to a person working alone in the laboratory; however, the supervisor is responsible for knowing if and when a person is assigned to work alone. Because assigning a person to work alone is a facility-specific decision, a risk assessment shall be conducted that accounts for all safety considerations, including type of work, physical safety, laboratory security, emergency response, potential exposure or injury, and other laboratory-specific issues.

**Step 3. Consider the competencies and experience of laboratory personnel.**

A. Experience (Less experienced employees might be at higher risk);

B. Genetic predisposition and nutritional deficiencies, immune/medical status (e.g., underlying illness, receipt of immunosuppressive drugs, chronic respiratory conditions, pregnancy, nonintact skin, allergies, receipt of medication known to reduce dexterity or reaction time);

C. Education, training, competence;

D. Stress, fatigue, mental status, excessive workload;

E. Perception, attitude, adherence to safety precautions; and

F. The most common routes of exposure or entry into the body (i.e., skin, mucous membranes, lungs, and mouth) (Table 1).

**Step 4. Evaluate and prioritize risks.**

Risks are evaluated according to the likelihood of occurrence and severity of consequences.

A. Likelihood of occurrence:

- Almost certain: expected to occur
- Likely: could happen sometime
- Moderate: could happen but not likely
- Unlikely: could happen but rare
- Rare: could happen, but probably never will

B. Severity of consequences:

Consequences may depend on duration and frequency of exposure and on availability of vaccine and appropriate treatment. Following are examples of consequences for

individual workers:

- Colonization leading to a carrier state
- Asymptomatic infection
- Toxicity, oncogenicity, allergenicity
- Infection, acute or chronic
- Illness, medical treatment
- Disease and sequelae
- Death

**Step 5. Develop, implement, and evaluate controls to minimize the risk for exposure.**

Controlling exposures to occupational hazards is the fundamental method of protecting workers. Traditionally, a hierarchy of controls has been used as a means of determining how to implement feasible and effective control solutions.

A. Elimination and Substitution:
Elimination and substitution, while most effective at reducing hazards, also tend to be the most difficult to implement in an existing process. If the process is still at the design or development stage, elimination and substitution of hazards may be inexpensive and simple to implement. For an existing process, major changes in equipment and procedures may be required to eliminate or substitute for a hazard.

B. Engineering controls:
If possible, first isolate and contain the hazard at its source.
- Primary containment: BSC, sharps containers, centrifuge safety cups, splash guards, safer sharps (e.g., auto-retracting needle/syringe combinations, disposable scalpels), and pipette aids
- Secondary containment: building design features (e.g., directional airflow or negative air pressure, hand washing sinks, closed doors, double door entry)

C. Administrative and work practice controls:
- Strict adherence to standard and special microbiological practices
- Adherence to signs and standard operating procedures
- Frequently washing hands
- Wearing PPE only in the work area
- Minimizing aerosols
- Prohibiting eating, drinking, smoking, chewing gum
- Limiting use of needles and sharps, and banning recapping of needles
- Minimizing splatter (e.g., by using lab "diapers" on bench surfaces, covering tubes with gauze when opening)

- Monitoring appropriate use of housekeeping, decontamination, and disposal procedures
- Implementing "clean" to "dirty" work flow
- Following recommendations for medical surveillance and occupational health, immunizations, incident reporting, first aid, post-exposure prophylaxis
- Training
- Implementing emergency response procedures

D. PPE:

- Gloves for handling all potentially contaminated materials, containers, equipment, or surfaces
- Face protection (face shields, splash goggles worn with masks, masks with built-in eye shield) if BSCs or splash guards are not available. Face protection, however, does not adequately replace a BSC. At BSL-2 and above, a BSC or similar containment device is required for procedures with splash or aerosol potential.
- Laboratory coats and gowns to prevent exposure of street clothing, and gloves or bandages to protect nonintact skin
- Additional respiratory protection if warranted by risk assessment

Job safety analysis (JSA)

A job safety analysis is required for procedures, tasks, or activities performed at each workstation or specific laboratory. A JSA is accomplished by listing the steps involved in a specific protocol and identifying the hazards associated with them, then determining the necessary controls to minimize the hazard, on the basis of the agent/organism. Precautions beyond the standard and special practices for BSL-2 may be indicated in the following circumstances:

- Organisms transmitted by inhalation
- Work with vectors expressing oncogenes or toxins
- Work with large volumes or highly concentrated cultures
- Compromised immune status of staff
- Training of new or inexperienced staff
- Technologist preference

Monitoring effectiveness of controls

Risk assessment is an ongoing process that requires at least an annual review because of changes in new and emerging pathogens and in technologies and personnel.

- Identify causes and problems; make changes, provide follow-up training.
- Conduct routine laboratory inspections.
- Repeat risk assessment routinely.

| TABLE 1. Laboratory activities associated with exposure to infectious agents | |
|---|---|
| **Routes of exposure/transmission** | **Activities/practices** |
| **Ingestion/oral** | • Pipetting by mouth<br>• Splashing infectious material<br>• Placing contaminated material or fingers in mouth<br>• Eating, drinking, using lipstick or lip balm |
| **Percutaneous inoculation/nonintact skin** | • Manipulating needles and syringes<br>• Handling broken glass and other sharp objects<br>• Bites and scratches from animals and insects<br>• Using scalpels to cut tissue for specimen processing<br>• Waste disposal (containers with improperly disposed sharps ) |
| **Direct contact with mucous membranes** | • Splashing or spilling infectious material into eye, mouth, nose<br>• Splashing or spilling infectious material onto intact and nonintact skin<br>• Working on contaminated surfaces<br>• Handling contaminated equipment (i.e., instrument maintenance)<br>• Inappropriate use of loops, inoculating needles, or swabs containing specimens or culture material<br>• Bites and scratches from animals and insects<br>• Waste disposal<br>• Manipulation of contact lenses |
| **Inhalation of aerosols** | • Manipulating needles, syringes, and sharps<br>• Manipulating inoculation needles, loops, and pipettes<br>• Manipulating specimens and cultures<br>• Spill cleanup |
| **Source:** Sewell DL. Laboratory-associated infections and biosafety. Clin Micobiol Rev 1995;8:389–405 (*18*). | |

| TABLE 2. Selected adventitious agents associated with cell cultures, organs and tissues that could be used to generate cell cultures, and cell culture reagents | |
|---|---|
| **Infectious agent** | **Source** |
| Adenovirus | Human kidney, pancreas, some adenovirus transformed cell lines, rhesus monkey kidney cells |
| Bovine viruses:<br>Bovine rhinotracheitis virus<br>Bovine diarrhea virus<br>Parainfluenza type 3<br>Bovine enterovirus<br>Bovine herpesvirus<br>Bovine syncytial virus | Bovine serum, fetal bovine serum (substantially lower risk today due to ultrafiltration of bovine serum) |
| Cytomegalovirus | Kidney, human foreskin, monkey kidney cells |
| Epstein-Barr virus (EBV) | Some lymphoid cell lines and EBV-transformed cell lines, human kidney |
| Hepatitis B virus | Human blood, liver |
| Herpes simplex virus | Human kidney |
| Herpesvirus group | Monkey kidney cells |
| Human or simian immunodeficiency virus | Blood cells, serum, plasma, solid organs from infected humans or monkeys |
| Human papilloma virus (HPV) | HeLa cell lines |
| HTLV-1 | Human kidney, liver |
| Lymphocytic choriomeningitis virus | Multiple cell lines, mouse tissue |
| Mycoplasmas | Many cell cultures |
| Myxovirus (SV5) | Monkey kidney cells |
| Porcine parvovirus | Fetal porcine kidney cells, trypsin preparations |
| Rabies virus | Human cornea, kidney, liver, iliac vessel conduit |
| Simian adenoviruses | Rhesus, cynomologous, and African green monkey kidney cells |
| Simian foamy virus | Rhesus, cynomologous, and African green monkey kidney cells |
| Simian virus 40 (SV40) | Rhesus monkey kidney cells |
| Simian viruses 1–49 | Rhesus monkey kidney cells |
| Swine torque teno virus | Trypsin, swine-origin biological components |
| Squirrel monkey retrovirus | Multiple cell lines, commercial interferon preparations |
| West Nile virus | Human blood, heart, kidney, liver, lung, pancreas |

## C. Classification of Agents on the Basis of Hazard

These agents, as listed by the Centers for Disease Control and Prevention (CDC), are those biological agents known to infect humans as well as select 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 is a list of the more commonly encountered agents and is not meant to be all inclusive. They are divided into Risk Groups that usually correspond to the equivalent Biosafety Level. The CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) and Pathogen Safety Data Sheets provided by the Public Health of Canada are excellent sources of information that can assist in the risk assessment of an agent. Work with some agents classified as risk group 1 (such as recombinant Adeno-Associated Virus (AAV)) and all agents classified as Risk Group 2 or higher requires registration with the IBC.

### 1. 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, Escherichia coli*-K12, and recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a biological toxin, or is targeted to knockdown a tumor suppressor, and are produced in the absence of a helper virus.

Those agents not listed in Risk Groups (RG's) 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.

### 2. 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.

#### A. Bacterial Agents

*Acinetobacter baumannii* (formerly *Acinetobacter calcoaceticus*)
*Actinobacillus*
*Actinomyces pyogenes* (formerly *Corynebacterium pyogenes*)
*Aeromonas hydrophila*
*Amycolata autotrophica*
*Archanobacterium haemolyticum* (formerly *Corynebacterium haemolyticum*)

*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 asRG3)
*Campylobacter coli, C. fetus, C. jejuni*
*Chlamydia psittaci*, *C. trachomatis*, *C. pneumoniae*
*Clostridium botulinum*, *C. chauvoei, C. haemolyticum, C. histolyticum, C. novyi, C. septicum, C. tetani*
*Coxiella burnetii* – specifically the Phase II, Nine Mile strain, plaque purified, clone 4
*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
**Francisella tularensis* specifically **F. tularensis* subspecies *novicida* [aka *F. novicida*], strain Utah 112; **F. tularensis* subspecies *holarctica* LVS; **F. tularensis* biovar *tularensis* strain ATCC 6223 (aka strain B38)

*For research involving high concentrations, BL3 practices should be considered.

*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 as 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*
*Pseudomonas aeruginosa*
*Rhodococcus equi*
*Salmonella* including *S. arizonae, S. choleraesuis, 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. parahaemolyticus, V. vulnificus*
*Yersinia enterocolitica*
*Yersinia pestis* specifically $pgm^{(-)}$ strains (lacking the 102 kb pigmentation locus) and $lcr^{(-)}$ strains (lacking the LCR plasmid)

**B. 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 marneffei*
*Sporothrix schenckii*
*Trichophyton*

**C. 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 cellulosae* (hydatid cyst, larva of *T. solium*)
*Echinococcus* including *E. granulosis, 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

**D. Viruses**

Adenoviruses, human - all types

Alphaviruses (Togaviruses) - Group A Arboviruses
- Chikungunya vaccine strain 181/25
- Eastern equine encephalomyelitis virus
- Venezuelan equine encephalomyelitis vaccine strains TC-83 and V3526
- Western equine encephalomyelitis virus

Arenaviruses
- Junin virus candid #1 vaccine strain
- Lymphocytic choriomeningitis virus (non-neurotropic strains)
- Tacaribe virus complex
- Other viruses as listed in the reference source (see Section V-C of the NIH Guidelines, *Footnotes and References of Sections I through IV*)

Bunyaviruses

Bunyamwera virus

Rift Valley fever virus vaccine strain MP-12

Other viruses as listed in the reference source (see Section V-C of the NIH Guidelines, *Footnotes and References of Sections I through IV*)

Caliciviruses

Coronaviruses

Flaviviruses - Group B Arboviruses

Dengue virus serotypes 1, 2, 3, and 4

Japanese encephalitis virus strain SA 14-14-2

Yellow fever virus vaccine strain 17D

Other viruses as listed in the reference source (see Section V-C of the NIH Guidelines, *Footnotes and References of Sections I through IV*)

Hepatitis A, B, C, D, and E viruses

Herpesviruses - except Herpesvirus simiae (Monkey B virus) (see Appendix B-IV-D of the NIH Guidelines, *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 (except those listed in Appendix B-III-D of the NIH Guidelines, *Risk Group 3 (RG3) - Viruses and Prions*)

Tick-borne orthomyxoviruses

Papilloma viruses

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 Appendix B-III-D of the NIH Guidelines, *Risk Group 3 (RG3) - Viruses and Prions*) and restricted poxviruses including Alastrim, Smallpox, and Whitepox (see Section V-L of the NIH Guidelines, *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 non exotic strains: VSV-Indiana 1 serotype strains (*e.g.* Glasgow, Mudd-Summers, Orsay, San Juan) and VSV-New Jersey serotype strains (*e.g.* Ogden, Hazelhurst)

Rubivirus (Togaviruses)
Rubella virus

## 3. Risk Group 3 (RG3) Agents

RG3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.

### A. Bacterial Agents Including Rickettsia

*Bartonella*
*Brucella* including *B. abortus, B. canis*, *B. suis*

*Burkholderia (Pseudomonas) mallei, B. pseudomallei*
*Coxiella burnetii (*except the Phase II, Nine Mile strain listed as RG2)
*Francisella tularensis (*except those strains listed as RG2)
*Mycobacterium bovis* (except BCG strain, listed as RG2)
*Orientia tsutsugamushi* (was *R. tsutsugamushi*)
*Pasteurella multocida* type B -"buffalo" and other virulent strains
*Rickettsia akari*, *R. australis*, *R. canada*, *R. conorii*, *R. prowazekii*, *R. rickettsii*, *R, siberica*, *R. typhi* (*R. mooseri*)
*Yersinia pestis (*except those strains listed as RG2)

**B. Fungal Agents**

*Coccidioides immitis* (sporulating cultures; contaminated soil)
*Histoplasma capsulatum, H. capsulatum var. duboisii*

**C. Parasitic Agents**

None

**D. Viruses and Prions**

Alphaviruses (Togaviruses) - Group A Arboviruses

- Chikungunya virus (except the vaccine strain 181/25 which is RG2)
- Semliki Forest virus
- St. Louis encephalitis virus
- Venezuelan equine encephalomyelitis virus (except the vaccine strains TC-83 and V3526, which are RG2)
- Other viruses as listed in the reference source (see Section V-C of the NIH Guidelines, *Footnotes and References of Sections I through IV*)

Arenaviruses

- Flexal
- Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)

Bunyaviruses

- Hantaviruses including Hantaan virus
- Rift Valley fever virus

Coronaviruses

- SARS-associated coronavirus (SARS-CoV)
- Middle East respiratory syndrome coronavirus (MERS-CoV)

Flaviviruses - Group B Arboviruses

Japanese encephalitis virus (except those strains listed as RG2)
West Nile virus (WNV)
Yellow fever virus
Other viruses as listed in the reference source (see Section V-C of the NIH Guidelines, *Footnotes and References of Sections I through IV*)

Orthomyxoviruses

Influenza viruses 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968), and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1).

Poxviruses

Monkeypox virus

Prions

Transmissible spongiform encephalopathies (TSE) agents (Creutzfeldt-Jacob disease and kuru agents) (see Section V-C of the NIH Guidelines, *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 (except those strains as RG2)

## 4. 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. The University of Utah does not have containment facilities that support RG4 research.

### A. Bacterial Agents

None

**B. Fungal Agents**

None

**C. Parasitic Agents**

None

**D. Viral Agents**

Arenaviruses

Guanarito virus

Lassa virus

Junin virus (except the candid #1 vaccine strain listed asRG2)

Machupo virus

Sabia

Bunyaviruses (Nairovirus)

Crimean-Congo hemorrhagic fever virus

Filoviruses

Ebola virus

Marburg virus

Flaviruses - 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 (Hendra virus)

**Hemorrhagic fever agents and viruses as yet undefined**

**5. 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

- Papilloma viruses
  - Bovine papilloma virus
  - Shope papilloma virus

- Polyoma viruses
  - Polyoma 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

## 6. Murine Retroviral Vectors

Murine retroviral vectors (MSCV) to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent virus can be maintained, handled, and administered under RG1 containment.

## 7. Acute Biological Toxins

The University of Utah Institutional Biosafety Committee (IBC) reviews registrations for work with, possession of, use of, and transfer of acute biological toxins (mammalian LD50 <100 µg/kg body weight) or toxins that fall under the Federal Select Agent Guidelines, as well as the organisms, both natural and recombinant, which produce these toxins

The following is a list of toxins that are required to be registered with the IBC. However, the list is not comprehensive and principal investigators can confirm that toxins they propose to work with do not require IBC registration (LD50 >100 µg/kg body weight and not on Select Agent list) by contacting the Biosafety Office (biosafety@oehs.utah.edu or 801-581-6590).

- Abrin
- Aflatoxin
- *Bacillus anthracis* edema factor
- *Bacillus anthracis* lethal toxin
- Botulinum neurotoxins
- Brevetoxin
- Cholera toxin
- *Clostridium difficile* toxin
- *Clostridium perfringens* toxins
- Conotoxins
- Dendrotoxin (DTX)
- Diacetoxyscirpenol (DAS)
- Diphtheria toxin
- Domoic acid
- Pertussis toxin
- *Pfeisteria* spp. toxin(s)
- Ricin
- Saxitoxin

- Shiga-like ribosome inactivating proteins
- Shigatoxin
- *Staphylococcal* enterotoxins
- T-2 toxin
- Tetanus toxin
- Tetrodotoxin (TTX)

Guidelines for working with biological toxins can be found in Appendix I of the *Biosafety in Microbiological and Biomedical Laboratories* (http://www.cdc.gov/biosafety/publications/bmbl5/index.htm). Routine operations with dilute toxin solutions are conducted using **Biosafety Level 2 (BSL-2)** practices: see below).

## D. Biosafety Levels (BSL)

In general, the four biosafety levels correspond directly to the four risk groups of microorganisms listed in Section C. However, in some cases the BSL may be adjusted based on the risk assessment of the PI and the IBC. The agents of minimal hazard are Biosafety Level 1 (BSL-1), with the more dangerous microorganisms at Biosafety Level 4 (BSL-4). Each Biosafety level has different recommended practices, containment and facilities (Table 3) that build upon the recommendations for the level below: table taken from the Biosafety in Microbiological and Biomedical Laboratories, 5th Edition (http://www.cdc.gov/od/ohs/biosfty/biosfty.htm).

**Table 3: Summary of recommended Biosafety Levels for Infectious Agents**

| Level | Practices and Techniques | Safety Equipment | Facilities |
|---|---|---|---|
| BSL-1 | Standard Microbiological practices. | No primary barriers required. Lab coats and gloves; eye, face protection as needed. | Lab bench and sink required |
| BSL-2 | Level 1 practices plus: limited access; biohazard warning signs; sharps precautions; decontamination of all infectious wastes. | Class I or II Biological safety cabinets for manipulations of agents that cause splashes or aerosols of infectious materials. Lab coats, gloves, and eye protection; face protection as needed. | Level 1 plus: Autoclave; eyewash |
| BSL-3 | Level 2 practices plus: controlled access; decontamination of all waste; decontamination of all clothing before laundering. | Class I or II Biological safety cabinets or other physical containment devices used for all open manipulation of agents. Solid front lab coats, double gloves, face, eye and face protection; respiratory protection as needed. | Level 2 plus: Physical separation; self-closing, double door access; exhausted air not recirculated; negative airflow into lab. |
| BSL-4 | Level 3 practices plus: clothing change before entering; shower on exit; all material decontaminated on exit from the facility. | All procedures conducted in biological safety cabinet in combination with full-body, air-supplied, positive-pressure suit. | Level 3 plus: Separate building or isolated zone; dedicated supply, exhaust, and decontamination |

| | | | system |
|---|---|---|---|

1. **Biosafety Level 1 (typically Risk Group 1 Agents)**

Biosafety Level 1 is suitable for work involving agents of known or of minimal potential hazard to laboratory personnel and the environment. The laboratory is not separated from the general traffic patterns in the building. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science. Non-student minors may be allowed to work in BSL-1 laboratories, but only after prior approval from OEHS: more information can be found on the OEHS website.

The following standard and special practices, safety equipment, and facilities apply to agents assigned to Biosafety Level 1:

**A. Standard Microbiological Practices**

1. Access to the laboratory is limited or restricted at the discretion of the Principal Investigator/Supervisor when experiments are in progress.
2. Work surfaces are decontaminated with an appropriate disinfectant after completion of work and after any spill of viable material.
3. Decontaminate all cultures, stocks, other potentially infectious materials, and materials containing recombinant or synthetic nucleic acid molecules, before disposal using an effective method. Depending on where the decontamination will be performed, the following methods shall 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.
4. Technical pipetting devices are used; mouth pipetting is prohibited.
5. Eating, drinking, smoking, chewing gum, and applying cosmetics are not permitted in the work area. Food must be stored in cabinets or refrigerators located OUTSIDE of the work area.
6. Do not put items (e.g., pens and pencils) in the mouth.
7. Persons wash their hands after they handle viable materials and animals and before leaving the laboratory.
8. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

9. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors must 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 must be substituted for glassware whenever possible.
10. All procedures are performed carefully to minimize the creation of aerosols.
11. Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before leaving the laboratory for non-laboratory area (e.g., cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.
12. 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 shall also wear eye protection.
13. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection must be based on an appropriate risk assessment (vinyl gloves are not appropriate). Alternatives to latex gloves must be available. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers must:
   a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
   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.
14. An insect and rodent control program is in effect.
15. Animals not associated with the work being performed are not be permitted in the laboratory. Except for service animals, no animals are permitted in University of Utah buildings or facilities (University of Utah Policy 3-231). Requests to permit the entry of

service animals into laboratories must be made to the Office of Equal Opportunities and OEHS.

16. 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 shall be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions shall be encouraged to self-identify to the Occupational Medicine Clinic for appropriate counseling and guidance.

**B. Special Practices**

1. None required.

**C. Containment Equipment**

1. Special containment equipment is generally not required for manipulation of agents assigned BSL-1.

**D. Laboratories Facilities**

1. The laboratory is designed so that it can be easily cleaned. Carpets and rugs are not appropriate in laboratories.
2. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
3. Laboratory furniture is sturdy with surfaces for easy cleaning and decontamination. No cloth chairs. Spaces between benches, cabinet and equipment are accessible for cleaning.
4. Each laboratory contains a sink for hand washing.
5. If the laboratory has windows that open, they are fitted with fly screens.

**2. Biosafety Level 2 (typically Risk Group 2 Agents)**

Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited while work is being conducted and lab doors are secured and

locked at the end of each day; and (3) certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment. Non-student minors are not allowed to work in BSL-2 laboratories.

The following standard and special practices, equipment and facilities apply to agents assigned to BSL-2.

**A. Standard Microbiological Practices**

1. Access to the laboratory is limited or restricted by the Principal Investigator/Supervisor when work with infectious agents is in progress. Lab doors are secured and locked at the end of the day.
2. Work surfaces are decontaminated with an appropriate disinfectant after completion of work and after any spill of viable material.
3. Decontaminate all cultures, stocks, other potentially infectious materials, and materials containing recombinant or synthetic nucleic acid molecules, before disposal using an effective method. Depending on where the decontamination will be performed, the following methods shall 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.
4. Mechanical pipetting devices are used; mouth pipetting is prohibited.
5. Eating, drinking, smoking, chewing gum, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only. Food storage cabinets or refrigerators must be located OUTSIDE of the work area.
6. Do not put items (e.g., pens and pencils) in the mouth.
7. Persons wash their hands after handling infectious materials and animals when they leave the laboratory.
8. All procedures are performed carefully to minimize the creation of aerosols.
9. Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before leaving the BSL-2 laboratory for non-BSL-2 laboratory area (e.g., BSL-1 laboratory, cafeteria, library, administrative offices), this protective clothing is removed and left in the BSL-2 laboratory.
10. Eye and face protection (safety glasses, goggles, mask, face shield or other splatter 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 shall also wear eye protection.

11. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection shall be based on an appropriate risk assessment: vinyl gloves are not appropriate. Alternatives to latex gloves shall be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers must:
    a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    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.
12. When the infectious or hazardous agent(s) in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign, incorporating the universal biohazard symbol, is posted on the access door to the laboratory work area. The hazard warning sign identifies the infectious/hazardous agent, the laboratory's biosafety level, lists the name and telephone number of the Principal Investigator/Supervisor or other responsible person(s) for entering the laboratory and required procedures for entering and exiting the laboratory.
13. An insect and rodent control program is in effect.
14. 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 must be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions shall be encouraged to self-identify to the Occupational Medicine Clinic for appropriate counseling and guidance.

**B. Special Practices**

1. The Principal Investigator/Supervisor limits access to the laboratory. In general, persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. The Principal Investigator/Supervisor has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.
2. Laboratory personnel must be provided medical surveillance, as appropriate, and offered

available immunizations for agents handled or potentially present in the laboratory.

3. The Principal Investigator/Supervisor establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet any specific entry/exit requirements (e.g., immunization) may enter the laboratory or animal rooms.
4. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
5. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
6. Laboratory equipment must 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.
7. Animals and plants not associated with the work being performed are not be permitted in the laboratory. Except for service animals, no animals are permitted in University of Utah buildings or facilities (University of Utah Policy 3-231). Requests to permit the entry of service animals into laboratories must be made to the Office of Equal Opportunities and OEHS.
8. Special practices are taken to avoid skin contamination with infectious materials; gloves must be worn when handling infected animals and when contact with infectious materials is unavoidable.
9. Hypodermic needles and syringes are 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., needle is integral to the syringe) are used for the injection or aspiration of infectious fluids. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles must 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 decontaminated, preferably by autoclaving, before discarding.
10. Contaminated sharps are discarded immediately or as soon as possible in containers that are closable, puncture-resistant, leakproof on sides and bottoms, and labeled or color-coded appropriately. Sharps disposal containers are available through the OEHS on-line pick up request at www.OEHS.utah.edu. All serological pipets (1ml, 5ml, 10ml etc.), either glass or disposable plastic, as well as pipet tips, whether used to manipulate potentially infectious materials or not, **CANNOT** be disposed in any plastic bags. All

disposable pipets and pipet tips used for handling potentially infectious materials are considered to be contaminated sharps and must either be decontaminated prior to disposal in a broken glass container OR must be disposed in a rigid, closable, constructed to contain all contents and prevent leakage, appropriately labeled sharps container.

11. Spills and accidents which result in overt exposures to infectious materials are immediately reported to the Principal Investigator/Supervisor and to the IBC/OEHS. Medical evaluation, surveillance and treatment are provided as appropriate and written records are maintained.
12. When appropriate, considering the agent(s) handled baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.
13. A laboratory-specific biosafety manual is prepared or adopted. The Biosafety manual must, at a minimum, provide a risk assessment for the agents used, describe the physical containment and PPE requirements, describe appropriate disinfectants, waste disposal, and describe spill and post exposure procedures. Personnel are advised of special hazards and are required to read instructions on practices and procedures and how to follow them.
14. All procedures involving the manipulation of infectious materials that may generate an aerosol shall be conducted within a BSC or other physical containment devices.
15. Eye, face and respiratory protection shall be used in rooms containing infected animals as determined by the risk assessment.

**C. Containment Equipment**

1. Biological safety cabinets (Class I or II) or other appropriate personal protective or physical containment devices are used whenever:
   a. Procedures with a high potential for creating infectious aerosols are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intra-nasally, and harvesting infected tissues from animals or eggs.
   b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed heads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

See Section K and Appendix A for instruction on the use of a Class II Biosafety Cabinet.

**E. Laboratory Facilities**

1. The laboratory is designed so that it can easily be cleaned. Carpets and rugs are not permitted in laboratories.
2. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
3. Laboratory furniture is sturdy with surfaces for easy cleaning and decontamination. No cloth chairs. Spaces between benches, cabinet and equipment are accessible for cleaning.
4. Laboratory doors must be self-closing and have locks: doors must remain closed.
5. Each laboratory contains a sink for hand washing. It must be located near the exit door.
6. An eyewash station must be readily available.
7. There are no specific requirements for ventilation systems. However, planning of new facilities shall consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
8. HEPA filtered exhaust air from a Class II BSC can be safely recirculated back 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.
9. A method for decontaminating all laboratory wastes must be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).
10. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.
11. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs shall be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
12. Vacuum lines must be protected with liquid disinfectant traps and in-line HEPA filters.

**3. Biosafety Level 2 Enhanced (typically Risk Group 2 or 3 Agents)**

Biosafety Level 2 enhanced (BSL-2 enhanced, sometimes referred to as BSL-2+) is a term frequently used to describe laboratories where work with microorganisms is conducted in a BSL-2 laboratory with biosafety practices and procedures that are typically found at BSL-3. BSL-2 enhanced is not a recognized

containment level in biosafety guidance documents such as the Centers for Disease Control and Prevention's (CDC) Biosafety in Microbiological and Biomedical Laboratories (BMBL) or the National Institutes of Health's (NIH) Guidelines for Recombinant DNA Research. However, the NIH's Biosafety Considerations for Research with Lentiviral Vectors refer to "enhanced BL2 containment".

The use of BSL-3 practices and procedures in a BSL-2 laboratory allows for research work with microorganisms, including viral vectors, to take place in an environment where the safety practices are enhanced over and above the practices required at BSL-2. BSL-enhanced is not appropriate for RG3 pathogens that are infectious via the inhalation route. At a minimum, such pathogens must be utilized in a BSL-3 laboratory with BSL-3 practices.

There is no standardized list of microorganisms, viral vectors or research projects that must be conducted at BSL-2 enhanced. Each decision to use BSL-3 practices in a BSL-2 laboratory must be made via the risk assessment process. The risk assessment serves to guide the selection of appropriate biosafety levels and microbiological practices, safety equipment, and facility safeguards that will contribute to preventing a laboratory exposure. Non-student minors are not allowed to work in BSL-2 enhanced laboratories.

The risk assessment process must be applied to every new or revised research project. Examples of when BSL-2 enhanced may be appropriate include:

- Viral vectors encoding oncogenes, toxins or genes of unknown function, or that produce products that reduce or knockout the expression of tumor suppressors.
- Drug-resistant Risk Group Two (RG2) bacteria such as methicillin resistant Staphylococcus aureus (MRSA).
- Low titer and small volumes of Human Immunodeficiency Virus (HIV), an RG3 agent.
- High concentrations (>$10^6$ PFU/mL) of RG2 viruses.
- Work with greater than 10 liters of an RG2 agent.

Significant differences from standard BSL-2 practices can include:

1. A sharps policy is implemented and sharps (e.g., glass Pasteur pipettes, needles) are not allowed. Plasticware is substituted for glassware.
2. All work is performed in a BSC.
3. Lab personnel must participate if medical surveillance is required per direction of IBC and Occupational Medicine physician.
4. The PI must provide training to lab personnel who may not have experience working with the materials to be used with BSL-3 practices, e.g., an apprentice program may be established for personnel where they shadow more experienced personnel and are not allowed to work independently until they demonstrate proficiency.

5. Create a “Spill Kit” and store within the lab. Consider a yearly “shut down” for a few days to accommodate servicing and maintenance activities.
6. Disposable, solid-front, fluid-resistant gowns rather than front opening gowns. The sleeves must be cuffed or disposable sleeve covers must be worn.
7. Two pairs of gloves.
8. Safety glasses with side-shields must be worn at all times while in the lab.
9. Other BSL-3 practices, based on the risk assessment by the PI and IBC.

**A. Black Box Warnings**

*“Black Box Warnings” for agents that would typically require BSL-2 enhanced containment must be included in the laboratory Biosafety manual. This phrase is required by the Food and Drug Administration as the strictest warning put in the labeling of prescription drugs or drug products when there is reasonable evidence of an association of a serious hazard with the drug. It is also used by agencies to highlight a higher than standard risk. If the laboratory is proposing BSL2-enhanced containment, the following statements (as applicable) must be added to the Biosafety Manual, using a bolded and enlarged font, ensuring it is clearly identifiable by laboratory personnel:*

1. ***Black box warning (lentivirus expressing oncogene):** The viral vectors used in this research pose a greater risk to laboratory workers than typical viral vectors. This research includes one or more lentivirus/retrovirus vectors that infect human cells efficiently, and that express proven or potential oncogenic proteins or other proteins that alter the cell cycle. Accidental human exposure to these vectors may increase the risk of tumor formation.*
2. ***Black box warning (lentivirus expressing small RNA molecule):** The viral vectors used in this research pose a greater risk to laboratory workers than typical viral vectors. This research includes one or more lentivirus/retrovirus vectors that infect human cells efficiently, and that express small RNA molecules intended to reduce or eliminate expression by targeted genes. These molecules may inhibit expression of tumor suppressors through a targeted or off-target effect. Accidental human exposure to these vectors may increase the risk of tumor formation.*
3. ***Black box warning (HIV pseudovirus):** The viral vectors used in this research pose a greater risk to laboratory workers than typical viral vectors. This research includes a pseudovirus vector system with a low genetic barrier to the generation of replication-competent HIV virus. Accidental human exposure to these vectors could result in HIV infection.*
4. ***Black box warning (HIV infectious molecular clone):** The viral vectors used in this research pose a greater risk to laboratory workers than typical viral vectors. This research*

*includes a plasmid capable of producing replication-competent HIV virus if expressed in a human cell. Accidental human exposure to these vectors could result in HIV infection.*

5. ***Black box warning (VSV-G pseudotyped HIV infectious molecular clone):*** *The viral vectors used in this research pose a greater risk to laboratory workers than typical viral vectors. This research includes a viral vector capable of producing HIV infection in the event of an accidental exposure. Furthermore, the viral vector has the VSV-G envelope protein on its surface. This protein makes the vector capable of infecting a wider range of human cells, so may make it more likely to result in infection in the event of an accidental exposure. Accidental human exposure to these vectors could result in HIV infection.*
6. ***Black box warning (CRISPR/Cas system on a single vector):*** *The viral vectors used in this research pose a greater risk to laboratory workers than typical viral vectors. This research includes a CRISPR (clustered regularly interspaced short palindromic repeat)/Cas system capable of editing human chromosomal DNA. Both components of the system (gRNA and Cas9) are expressed on the same viral vector. This increases the risk for damage to chromosomal DNA with potential oncogenic effects. Accidental human exposure to these vectors may increase the risk of tumor formation.*

### 4. Biosafety Level 3 (Risk Group 3 Agents)

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or with other physical containment devices by personnel wearing appropriate personal protective clothing and devices. The laboratory has special engineering and design features. Minors are not allowed to work in BSL-3 laboratories.

It is recognized, however, that many existing facilities may not have all the facility safeguards recommended for BSL-3 (e.g., access zone, sealed penetrations, and directional airflow, etc.). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in laboratories where facility features satisfy BSL-2 recommendations provided the recommended "Standard Microbiological Practices," "Special Practices," and "Containment Equipment" for BSL-3 are rigorously followed. The decision to implement this modification of Biosafety Level 3 recommendations must be made only by the Principal Investigator and approved by the IBC.

The following practices, equipment, and facilities apply to agents assigned to BSL-3:

**A. Standard Microbiological Practices**

1. Access to the laboratory is limited or restricted by the Principal Investigator/Supervisor when work with infectious agents is in progress. Lab doors are secured and locked at the end of the day.
2. Work surfaces are decontaminated with an appropriate disinfectant after completion of work and after any spill of viable material.
3. All liquid or solids wastes are decontaminated and properly labeled before disposal. Lab waste must be disposed at the end of each day. Depending on where the decontamination will be performed, the following methods shall 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.
4. Mechanical pipetting devices are used; mouth pipetting is prohibited.
5. Eating, drinking, smoking, chewing gum, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only. Food storage cabinets or refrigerators must be located OUTSIDE of the work area.
6. Do not put items (e.g., pens and pencils) in the mouth.
7. Persons wash their hands after handling infectious materials and animals when they leave the laboratory.
8. All procedures are performed carefully to minimize the creation of aerosols.
9. Workers in the laboratory where protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. The sleeves must be cuffed or disposable sleeve covers must be worn. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.
10. Safety glasses with side-shields must be worn at all times while in the lab. 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.
11. Two pairs of gloves must be worn to protect hands from exposure to hazardous materials. Glove selection shall be based on an appropriate risk assessment; vinyl gloves are not acceptable. Alternatives to latex gloves should be available. Gloves

must not be worn outside the laboratory. In addition, BSL-3 laboratory workers must:

a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
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.

12. When the infectious or hazardous agent(s) in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign, incorporating the universal biohazard symbol, is posted on the access door to the laboratory work area. The hazard warning sign identifies the infectious/hazardous agent, the laboratory's biosafety level, lists the name and telephone number of the Principal Investigator/Supervisor or other responsible person(s) for entering the laboratory and required procedures for entering and exiting the laboratory.
13. An insect and rodent control program is in effect.
14. 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 must be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions shall be encouraged to self-identify to the Occupational Medicine Clinic for appropriate counseling and guidance.

## B. Special Practices

1. The Principal Investigator/Supervisor limits access to the laboratory. In general, persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.
2. Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
3. The Principal Investigator/Supervisor establishes policies and procedures whereby only

persons who have been advised of the potential hazards and meet any specific entry/exit requirements (e.g., immunization) may enter the laboratory or animal rooms.

4. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with Risk group 3/BSL-3 agents.
5. Potentially infectious materials must be placed in a durable, leak-proof container during collection, handling, processing, storage, or transport within a facility.
6. Laboratory equipment shall 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.
    c. Pre-assemble a spill kit.
7. Animals and plants not associated with the work being performed are not be permitted in the laboratory. Except for service animals, no animals are permitted in University of Utah buildings or facilities (University of Utah Policy 3-231). Requests to permit the entry of service animals into laboratories must be made to the Office of Equal Opportunities and OEHS.
8. A sharps policy is implemented and sharps (e.g., glass Pasteur pipettes, needles) are not allowed. Plasticware is substituted for glassware.
9. Spills and accidents which result in overt exposures to infectious materials are immediately reported to the Principal Investigator/Supervisor and to the IBC/OEHS. Medical evaluation, surveillance and treatment are provided as appropriate and written records are maintained.
10. When appropriate, considering the agent(s) handled baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.
11. A laboratory-specific biosafety manual is prepared or adopted. The Biosafety manual must, at a minimum, provide a risk assessment for the agents used, describe the physical containment and PPE requirements, describe appropriate disinfectants, waste disposal, and describe spill and post exposure procedures. Personnel are advised of special hazards and are required to read instructions on practices and procedures and how to follow them.
12. All procedures involving the manipulation of infectious materials that may generate an aerosol must be conducted within a BSC or other physical containment devices.
13. Eye, face and respiratory protection must be used in rooms containing infected animals

as determined by the risk assessment.

### C. Containment Equipment

Biological safety cabinets (Class I, II, or III) or other appropriate combination of personal protective or physical containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals) are used for all activities with infectious materials which pose a threat of aerosol exposure. These include: manipulation of cultures and of those clinical or environmental materials which may be a source of infectious aerosols; the aerosol challenge of experimental animals; harvesting of tissues or fluids from infected animals and embryonated eggs, and necropsy of infected animals.

See Section K and Appendix A for instruction on the use of a Class II Biosafety Cabinet.

### D. Laboratory Facilities

1. The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. Physical separation of the high containment laboratory from access corridors or other laboratories of activities may also be provided by a double- doored clothes change room (showers may be included), airlock, or other access facility which requires passage through two sets of doors before entering the laboratory.
2. The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces must be sealed. Spaces around doors and ventilation openings must be capable of being sealed to facilitate space decontamination.
    a. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration shall be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.
    b. Walls must be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
    c. Ceilings must be constructed, sealed, and finished in the same general manner as walls.

    Decontamination of the entire laboratory must 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.

3. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
4. Laboratory furniture is sturdy with surfaces for easy cleaning and decontamination. No cloth chairs. Spaces between benches, cabinet and equipment are accessible for cleaning.
5. Each laboratory contains a sink for hand washing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.
6. Windows in the laboratory are closed and sealed.
7. An eyewash station must be readily available.
8. Access doors to the laboratory or containment module are self-closing and locking
9. An autoclave for decontaminating laboratory wastes is available, preferably within the laboratory.
10. A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry areas. The exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from occupied areas and air intakes. 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 must be considered to notify personnel of air flow disruption. The exhaust air from the laboratory room can be discharged to the outside without being filtered or otherwise treated. HEPA filter housings must have gas-tight isolation dampers, decontamination ports, and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing must allow for leak testing of each filter and assembly. The filters and the housing must be certified at least annually.
11. 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 must 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. 11.
12. A method for decontaminating all laboratory wastes must be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, or other validated decontamination method).
13. 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 must be tested and/or replaced at least annually.

14. Facility design consideration must be given to means of decontaminating large pieces of equipment before removal from the laboratory.
15. 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.
16. 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.

**Note the University of Utah currently has one BSL-3 laboratory.**

## 5. Biosafety Level 4 (Risk Group 4 Agents): ***There are no BSL-4 facilities at the University of Utah***

Biosafety Level 4 is required for work with dangerous and exotic agents which pose a high individual risk of life-threatening disease. Members of the laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents, and fully understand the primary and secondary containment functions of the standard and special practices, the containment equipment, and the laboratory design characteristics. They must be supervised by competent scientists who are trained and experienced in working with these agents.

Access to the laboratory is strictly controlled. The facility is either in a separate building or in a controlled area within a building, which is completely isolated from all other areas of the building. A specific facility operations manual must be prepared or adopted.

***The University of Utah does not have containment facilities that support BSL-4 research***.

## 6. Animal Biosafety Level 1

Animal Biosafety Level 1 is suitable for work in animals involving well-characterized agents that are not known to cause disease in immunocompetent adult humans, and present minimal potential hazard to

personnel and the environment.

ABSL-1 facilities must be separated from the general traffic patterns of the building and restricted as appropriate. Special containment equipment or facility design may be required as determined by appropriate risk assessment. (See Section 2, Biological Risk Assessment)

Personnel must have specific training in animal facility procedures and must be supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures.

The following practices, equipment, and facilities apply to agents assigned to ABSL-1:

**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 also be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) 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. The safety manual must be available and accessible. Personnel are advised of potential hazards and are required to read and follow instructions on practices and procedures.
5. 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 exposures, and hazard/ exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.).
    a. Personnel must receive annual updates and additional training when procedures or policies change.
    b. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.
6. An appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program shall be considered.
7. Facility supervisors must ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.
8. 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 must be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions shall be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

9. Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.
10. A sign incorporating safety information must be posted at the entrance to the 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 being used within an animal room. Security-sensitive agent information must be posted in accordance with the institutional policy. Advance consideration must be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.
11. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the facility.
12. 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.
    a. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
    b. Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials, and when handling animals.
    c. Gloves and personal protective equipment must be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.
    d. Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.
    e. Eye and face and respiratory protection must be used in rooms containing infected animals, as dictated by the risk assessment.
13. Eating, drinking, chewing gum, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory and other work areas. Food must be stored outside of the laboratory in cabinets or refrigerators designed and used for this purpose.
14. Do not put items (e.g., pens and pencils) in the mouth.

15. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
16. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
17. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.
18. When applicable, laboratory supervisors must adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, 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 for such procedures 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 must 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. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware shall be substituted for glassware whenever possible.
    e. Equipment containing sharp edges and corners must be avoided.
19. 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.
20. 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.
21. An effective integrated pest management program is required.
22. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional, local and state requirements.
    a. Decontaminate all potentially infectious materials before disposal using an effective method.

**B. Special Practices**

None required.

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

1. A risk assessment will determine the appropriate type of personal protective equipment to be utilized.
2. Special containment devices or equipment may not be required as determined by appropriate risk assessment.
3. Protective laboratory coats, gowns, or uniforms may be required to prevent contamination of personal clothing.
4. Protective eyewear is worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses must also wear eye protection when entering areas with potentially high concentrations or airborne particulates. Persons having contact with NHPs must assess risk of mucous membrane exposure and wear protective equipment (e.g., masks, goggles, face shields, etc.) as appropriate for the task to be performed.
5. Gloves are worn to protect hands from exposure to hazardous materials.
6. A risk assessment will be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.
7. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary. Gloves must not be worn outside the animal rooms.
8. Gloves and personal protective equipment must be removed in a manner that prevents transfer of infectious materials.
9. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.
10. 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 must occur after the removal of gloves.

**D. Laboratory Facilities**

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. Access to the animal facility is restricted.
2. 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 must never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
3. The animal facility must have a sink for hand washing.
4. Sink traps are filled with water, and/or appropriate liquid to prevent the migration of

vermin and gases.

5. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant. Floors must be slip resistant, impervious to liquids, and resistant to chemicals.
6. It is recommended that penetrations in floors, walls and ceiling surfaces be sealed, including openings around ducts, doors and doorframes, to facilitate pest control and proper cleaning.
7. 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 must be accessible for cleaning.
8. Chairs used in animal area 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. Sharp edges and corners must be avoided.
9. External windows are not recommended; if present windows must be resistant to breakage. Where possible, windows should be sealed. If the animal facility has windows that open, they are fitted with fly screens. The presence of windows may impact facility security and therefore must be assessed by security personnel.
10. Ventilation must be provided in accordance with the Guide for Care and Use of Laboratory Animals. No recirculation of exhaust air may occur. It is recommended that animal rooms have inward directional airflow.
11. Ventilation system design must consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
12. 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.
13. If floor drains are provided, the traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
14. Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washer must have a final rinse temperature of at least 180°F. If manual cage washing is utilized, ensure that appropriate disinfectants are selected.
15. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
16. Emergency eyewash and shower are readily available; location is determined by risk assessment.

## 7. Animal Biosafety Level 2

Animal Biosafety Level 2 builds upon the practices, procedures, containment equipment, and facility

requirements of ABSL-1. ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure. In addition, work with animal pathogens that pose a risk to the animal colony may require ABSL-2 (or higher) containment even if they are not a human pathogen.

ABSL-2 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 pathogenic agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and 4) BSCs or other physical containment equipment is used when procedures involve the manipulation of infectious materials, or where aerosols or splashes may be created.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Implementation of employee occupational health programs shall be considered.

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

**A. Standard Microbiological Practices**

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies.
    a. Each organization must assure that worker safety and health concerns are addressed as part of the animal protocol review.
    b. Prior to beginning a study, animal protocols must also be reviewed and approved by the IACUC and the Institutional Biosafety Committee.
2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals.
    a. The safety manual must be available and accessible. Personnel are advised of potential hazards, and are required to read and follow instructions on practices and procedures.
    b. Consideration must be given to specific biohazards unique to the animal species and protocol in use.
3. The supervisor must ensure that animal care, laboratory, and support personnel receive appropriate training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes,

aerosolization, etc.).

a. Personnel must receive annual updates or additional training when procedures or policies change.

b. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.

4. An appropriate medical surveillance program is in place, as determined by risk assessment.

a. The need for an animal allergy prevention program shall be considered.

b. Facility supervisors must ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.

c. 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 must be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions shall be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

d. Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.

5. 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 when infectious agents are present

a. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or names of other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of all infectious agents is necessary when more than one agent is being used within an animal room.

b. Security-sensitive agent information and occupational health requirements must be posted in accordance with the institutional policy.

c. Advance consideration must be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.

6. Access to the animal room is limited. 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 manipulated.

a. All persons including facility personnel, service workers, and visitors are advised of the potential hazards (physical, naturally occurring, or research pathogens,

allergens, etc.) and are instructed on the appropriate safeguards.

7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
    a. Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials and when handling animals.
    b. Gloves and personal protective equipment must 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.
    c. Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.
    d. Eye, face and respiratory protection shall be used in rooms containing infected animals, as dictated by the risk assessment.
8. Eating, drinking, chewing gum, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside of the laboratory in cabinets or refrigerators designated and used for this purpose.
9. Do not put items (e.g., pens and pencils) in the mouth.
10. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
11. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
12. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. When applicable, laboratory supervisors must 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. The 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 must 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 must 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 must be avoided.
13. 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.
14. 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 manipulated.
15. An effective integrated pest management program is required.
16. 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.
    a. 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. When appropriate, a base line serum sample shall be stored.
2. Procedures involving a high potential for generating aerosols must be conducted within a biosafety cabinet or other physical containment device. When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used.
3. Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications) must be used whenever possible.
4. Decontamination by an appropriate method (e.g. autoclave, chemical disinfection, or other approved decontamination methods) is necessary for all potentially infectious materials and animal waste before movement outside the areas where infectious materials and/or animals are housed or are manipulated. This includes potentially infectious animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse.
5. A method for decontaminating routine husbandry equipment, sensitive electronic and medical equipment must be identified and implemented.
    a. Materials to be decontaminated outside of the immediate areas where infectious materials and/or animals are housed or are manipulated must be placed in a durable, leak proof, covered container and secured for transport. The outer surface of the container is disinfected prior to moving materials. The transport container must have a universal biohazard label.

    b. Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements. Autoclaving of content prior to incineration is recommended.

6. Equipment, cages, and racks must be handled in a manner that minimizes contamination of other areas.
    a. Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated.
7. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
8. 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 must be provided as appropriate and records maintained.

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

1. Properly maintained BSCs, personal protective equipment (e.g., gloves, lab coats, face shields, respirators, etc.) and/or other physical containment devices or equipment, are used whenever conducting procedures with a potential for creating aerosols, splashes, or other potential exposures to hazardous materials. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals.
    a. When indicated by risk assessment, animals are housed in primary biosafety containment equipment appropriate for the animal species, such as solid wall and bottom cages covered with filter bonnets for rodents or other equivalent primary containment systems for larger animal cages.
2. A risk assessment will determine the appropriate type of personal protective equipment to be utilized.
    a. Scrub suits and uniforms are removed before leaving the animal facility. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing must never be taken home.
    b. Gowns, uniforms, laboratory coats and personal protective equipment are worn while in the areas where infectious materials and/or animals are housed or manipulated and removed prior to exiting. Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.

3. Eye and face protection (mask, goggles, face shield or other splatter guard) are used for manipulations or activities that may result in splashes or sprays from infectious or other hazardous materials and when the animal or microorganisms must be handled outside the BSC or containment device.
    a. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse.
    b. Persons who wear contact lenses must also wear eye protection when entering areas with potentially high concentrations or airborne particulates.
4. Persons having contact with NHPs must assess risk of mucous membrane exposure and wear protective equipment (e.g., masks, goggles, face shields) appropriate for the task to be performed. Respiratory protection is worn based upon risk assessment.
5. Gloves are worn to protect hands from exposure to hazardous materials. A risk assessment will be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.
    a. Gloves are changed when contaminated, glove integrity is compromised, or when otherwise necessary.
    b. Gloves must not be worn outside the animal rooms.
    c. Gloves and personal protective equipment must be removed in a manner that prevents transfer of infectious materials.
    d. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste. 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 must 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. 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 must never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
3. 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 must be located in other appropriate locations within the facility.
4. If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink must also be available for hand washing at the exit from

each segregated area. Sink traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.

5. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant.
6. Penetrations in floors, walls and ceiling surfaces are sealed, including openings around ducts, doors and doorframes, to facilitate pest control and proper cleaning.
7. Floors must be slip-resistant, impervious to liquids, and resistant to chemicals.
8. 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 must be accessible for cleaning.
9. Furniture should be minimized. Chairs used in animal area 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. Sharp edges and corners must be avoided.
10. External windows are not recommended; if present, windows must be sealed and resistant to breakage. The presence of windows may impact facility security and therefore must be assessed by security personnel.
11. Ventilation must be provided in accordance with the Guide for Care and Use of Laboratory Animals. 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. Ventilation system design must consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
12. 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.
13. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
14. Cages shall be autoclaved or otherwise decontaminated prior to washing. Mechanical cage washer must have a final rinse temperature of at least 180°F. The cage wash area shall be designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures during the cage/equipment cleaning process.
15. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
16. If BSCs are present, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs must be located away from doors,

heavily traveled laboratory areas, and other possible airflow disruptions.

17. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back 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 to the outside through an independent, hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs must be recertified at least once a year to ensure correct performance.
18. All BSCs shall be used according to manufacturer's specifications to protect the worker and avoid creating a hazardous environment from volatile chemicals and gases.
19. If vacuum service (i.e., central or local) is provided, each service connection must be fitted with liquid disinfectant traps and an in-line HEPA filter placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement.
20. An autoclave must be present in the animal facility to facilitate decontamination of infectious materials and waste.
21. Emergency eyewash and shower are readily available; location is determined by risk assessment.

## 8. ABSL2 to ABSL1 Stepdown

Inoculating non-permissive mice or rats with some replication deficient viral vectors are eligible for "step-down" from ABSL-2 to ABSL-1, if approved by the Institutional Biosafety Committee (IBC). These are BSL-2 agents that cannot reproduce in rodents and may therefore be classified as ABSL-1 once inoculated into rodents. Examples include third generation lentiviral vectors. Vector systems have variable periods after inoculation during which the animals are handled using ABSL-2 practices in ABSL-1 housing rooms. These time periods are determined by the IBC for each vector to ensure no viable vector remains present on animal fur or is shed in excreta post-inoculation.

Similarly, animals injected with acute biological toxins will initially be handled using ABSL2-proctices with stepdown to ABSL-1 after a period determined by the IBC.

After this holding period animals are transferred to a new cage and managed using ABSL-1 practices. The cage from which animals are transferred is managed using ABSL-2 practices as outlined below.

"Humanized rodents," those in which human xenografts are present, may be permissive hosts for some viral vectors. Step down procedures do not apply to permissive hosts.

## A. Procedure

### 1. Vector or Toxin Inoculation

a. Research staff will inform the animal care staff at least 24 hours in advance that ABSL-2 vectors/acute toxins will be used for animals housed in ABSL-1 rooms.
b. Only vectors approved by the IBC for step-down practice may be used.
c. Vectors/toxins are administered in a Biosafety Cabinet (BSC) on absorbent pad.

### 2. Cage Management During the Step Down Period

a. Investigators place a "Viral Vector Step Down" or "Toxin Step Down" Card on the cage, noting the vector/toxin, date inoculated, and date of step down on the card.
b. Animals are housed in microisolator caging, opened only in biosafety cabinets.
c. Investigators provide required care (i.e. supplementing feed, water, etc.) until the step down occurs. The investigator's staff perform any required cage changes during the step down period. Cage changes occur only in cage changing stations or biosafety cabinets.
d. On the day of the step down the investigator's staff place the cage in a biosafety cabinet and move the animals to a clean cage. Do not transfer the "Viral Vector/Toxin Step Down" Card to the new cage.
e. The new cage is returned to the rack and managed at the ABSL-1 level.

### 3. Contaminated Cage Management

a. Place the contaminated cage in an autoclavable bag. Seal the bag and spray the bag with disinfectant.
b. Transport the closed bag to the dirty cage rack and the animal care staff will process the cage using ABSL-2 practices.
c. All waste generated during the procedures, must be handled using ABSL-2 practices.

## 9. Injection of Human Cells into Animals

The potential hazards associated with the handling of human/nonhuman primate cell culture are mainly the contamination of the cells with pathogenic agents and/or the tumorigenicity of the cells. Agents such as bacteria, fungi, and mycoplasmas generally cause some kind of visual effect on the cells or culture media allowing for detection of contamination. However, many viruses do not cause cytopathic effect (CPE), can be latent or are undetectable with current technology.

Primate and other mammalian cell lines can harbor viruses with a broad host range. Human cell lines are most likely to be contaminated with the highly pathogenic viruses including hepatitis B virus and HIV

(human immunodeficiency virus). However, primate cells can contain dangerous pathogens, most notably herpes B virus and Marburg virus both of which have caused fatal infections in humans. Rodent cell lines can carry lymphocytic choriomeningitis virus (LCMV), Reo-3 virus and hantavirus with documented cases of human disease and death.

In 1994, OSHA issued an interpretation of the applicability of the Bloodborne Pathogen (BBP) Standard towards human cell lines. According to the interpretation, human cell lines are considered to be potentially infectious and within the scope of the BBP Standard unless the specific cell line has been characterized to be free of recognized bloodborne pathogens. The American Type Culture Collection (ATCC) recommends that all human cell lines be accorded the same level of biosafety consideration as a line known to human risk group 2 pathogens (BSL-2) unless they have been screened for human pathogens.

In addition, the 5th Edition of the NIH/CDC publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) recommends that human and other primate cells be handled using Biosafety Level 2 (BSL2) practices and containment.

Based on these recommendations, the University of Utah Institutional Biosafety Committee requires Animal Biosafety level 2 (ABSL-2) practices to be followed when animals are injected/implanted with human cell lines (primary or established), human tissues or human tumors. This work must be registered with the IBC through BioRAFT.

If a PI wishes to use lower containment (ABSL-1), the PI must submit a request to the IBC as part of their registration in BioRAFT. The IBC Chair and/or Biosafety Officer (BSO) will review the application, with review by the full IBC if deemed appropriate by the Chair or BSO. In general reduced containment will be considered if:

1) The experiments involve primary cells, new cell lines, and tissues that have been screened for human pathogens (at a minimum HCV, HBV, HIV) and LCMV by established methods. The IBC suggests the h-IMPACT II screen (HIV, HTLV, HAV, HBV, HCV, mycoplasma) plus the LCMV individual test from IDEX Biosciences, but other companies, such as Charles River, offer comparable products. Assays chosen must have lower limits of detection comparable to FDA approved assays.
2) The experiments involve established cell lines that have been certified free of human pathogens, such as from ATCC.
3) The experiments involve established cell lines that do not support the replication of human pathogens. In this case, the IBC may require proof of the identity of the cells lines, such as through STR profiling.

## 10. Animal Biosafety Level 3: *There are no ABSL-3 facilities at the University of Utah*

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 University of Utah does not have containment facilities that support Animal Biosafety Level 3 (ABSL-3) research.***

## 11. Animal Biosafety Level 4: *There are no ABSL-4 facilities at the University of Utah*

Animal Biosafety Level 4 is required for work with animals infected with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments; or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring ABSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or to re-designate the level. Animal care staff must have specific and thorough training in handling extremely hazardous, infectious agents and infected animals. Animal care staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All animal care staff and supervisors must be competent in handling animals, agents and procedures requiring ABSL-4 containment. The animal facility director and/or laboratory supervisor control access to the animal facility within the ABSL-4 laboratory in accordance with institutional policies.

***The University of Utah does not have containment facilities that support Animal Biosafety Level 4 (ABSL-4) research.***

**Table 4. Summary of Recommended Biosafety Levels for Activities in Which Experimentally or Naturally Infected Vertebrate Animals are Used.**

| Level | Practices and Techniques | Safety Equipment | Facilities |
|---|---|---|---|
| 1 | Standard animal care and management practices. | None | Basic |
| 2 | Laboratory coats; decontamination of all infectious wastes and of animal cages prior to washing; limited access; protective gloves and hazard warning signs as indicated. | Partial containment equipment and/or personal protective devices used for activities and manipulations of agents or infected animals that produce aerosols. | Basic |
| 3 | Level 2 practices plus: special laboratory clothing; controlled access. | Containment and/or personal protective devices used for all activities and manipulations of agents or infected animals. | Containment |
| 4 | Level 3 practices plus: entrance through clothes change room where street clothing is removed and laboratory clothing is put on; shower on exit; all wastes are decontaminated before removal from the facility. | Maximum containment equipment (i.e., Class III biological safety cabinet or partial containment equipment in combination with full-body, air-supplied positive-pressure personnel suit) used for all procedures and activities. | Maximum Containment |

## 12. Arthropod Containment Levels (ACL)

An *ad hoc* committee of concerned vector biologists including members of the American Committee Medical Entomology (ACME), a subcommittee of the American Society of Tropical Medicine and Hygiene (ASTMH), and other interested persons drafted the "Arthropod Containment Guidelines (ACG)." The

ACG provide principles of risk assessment for arthropods of public health importance. The risk assessment and practices are designed to be consistent with the NIH Guidelines for recombinant or synthetic nucleic acid molecules research and the BMBL. Arthropods included are those that transmit pathogens; however, those arthropods that cause myiasis, infestation, biting, and stinging are not included. The ACG also specifically exclude most uses of Drosophila spp. The ACG were updated in 2018 and can be found here.

When arthropods are used, facilities, trained staff and established practices must be in place to ensure appropriate safety, and the protection of health and well-being of workers and the environment. This publication provides guidelines for laboratory work with arthropod vectors of pathogenic agents, and has been prepared in response to concerns related to the consequences of an accidental release of arthropods. These consequences (risk factors) are basically answering the question "What happens if the arthropod escapes?" and the suggested containment levels address the question "How do we prevent escape?" If working with a vector in a particular set of circumstances (see Table 5), a certain containment level may be recommended. The IBC is an essential component in establishing the appropriate ACL. It is responsible for reviewing a research protocol and decides at what level of containment the experiments must be performed.

**Table 5. Summary of Arthropod Containment Levels**

| **Arthropod containment level:** | **1** | | **2** | **3** | **4** |
|---|---|---|---|---|---|
| **Arthropod distribution, escaped arthropod fate** | Exotic, inviable or transient | Indigenous | Exotic with establishment, indigenous, and transgenic | | |
| **Infection status** | Uninfected or infected with non- | | Up to BSL-2 | Up to BSL-3 | BSL-4 |
| **Active VBD cycling** | No | Irrelevant | | | |
| **Practices** | ACL-1 Standard Arthropod-Handling Practices | | ACL-1 plus more rigorous disposal, signage, and limited access | ACL-2 with more highly restricted access, training and record-keeping | ACL-3 with high access restriction, extensive training, full isolation |

| Arthropod containment level: | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| **Primary Barriers** | Species-appropriate containers | Species-appropriate containers | Escape-proof arthropod containers, glove boxes, BSC | Escape-proof arthropod containers handled in cabinet or suit laboratory |
| **Secondary Barriers** | | Separated from laboratories, double doors sealed electrical/ plumbing openings. Breeding containers and harborages minimized | BSL-3 | BSL-4 |
| Three fates of arthropods upon accidental escape are classified here: (1) Inviable; conditions are sufficiently unfavorable to the arthropod that reproduction does not occur. (2) Transient; conditions vary either seasonally or annually such that the arthropod could reproduce upon escape but would be eliminated during a typical climatic year. (3) Establishment; the conditions found in the range of the arthopod are sufficiently similar to those of the laboratory location that escaped arthropods could reasonably be expected to persist through a typical climatic year. Active Local VBD Cycling means that transmission of vector-borne diseases of public health importance that are known to be or probably transmitted by the arthropod are cycling in the locale. Indigenous species are those biological species whose current range includes the research location. All others are considered exotic. | | | | |

Where an arthropod is infected with an agent, the containment level required is automatically increased to at least that required for the agent, regardless of factors such as the competence of that arthropod for the particular pathogen. An example is the use of male mosquitoes to propagate dengue viruses. Although they cannot transmit by bite, the presence of the agent requires that they be held at BSL-2 level. Furthermore, in recognition of the fact that escape of uninfected exotic arthropods is to be prevented by all reasonable means, unless unusual measures are taken to reduce risks, these are also handled at the ACL-2 level or higher.

One advantage of working with certain arthropods is that the risk of release can be effectively manipulated by, e.g., performing relatively high-risk experiments during the winter when any escaped arthropods would quickly be killed by adverse environmental conditions. For example, the IBC might use such biological considerations to “downgrade” a particular protocol from ACL-3 to ACL-2, providing that experiments are performed during a particular period. Documentation of the justification for this decision-making process shall be prepared to ensure careful consideration of the risks.

It is impossible to prescribe universal levels of containment for a particular species since the risks associated with its accidental release from a laboratory are determined by several factors e.g., the

climate at the facility and history of transmission in that location. The accidental release of an uninfected anthropophilic tropical vector species during the winter in Wisconsin, might be considered as significantly less of a "risk" than the release of the same species in a tropical area in which it could become permanently established and act as a bridging vector of an established zoonotic pathogen to humans. Furthermore, the existence of zoonoses means that we have to consider certain pathogens that are predominantly an animal health issue. USDA guidelines must therefore be considered when assigning a containment level to a particular vector species.

Although specific details are not covered here, it is important to develop a response procedure that is appropriate in case of an accidental release. The ideal response would be one in which all released arthropods are killed almost immediately after the escape. This may be impossible if the escaped arthropods get outside of the laboratory, hence the use of several barrier levels are recommended to maximize the opportunities for location and destruction of the escapees.

***The University of Utah does not have containment facilities that support ACL-2, 3 or 4 research***.

## 13. Arthropod Containment Level 1 (ACL-1)

Arthropod Containment Level 1 (ACL-1) is suitable for work with uninfected arthropod vectors or those infected with a non-pathogen including: 1) arthropods that are already present in the local geographic region regardless of whether there is active vector borne disease transmission in the locale, and 2) exotic arthropods that upon escape would be inviable or become only temporarily established in areas not having active vector borne disease transmission. This category would include most educational use of arthropods. A summary of the containment levels is provided in Table 5.

### A. Standard Practices

1. *Location of Arthropods*. Furniture and incubators containing arthropods are located in such a way that accidental contact and release is minimized. This may be achieved by locating arthropods out of the flow of general traffic, avoiding hallways, or placing them in closets.

2. *Supply Storage*. The area is maintained to allow detection of escaped arthropods. For example, materials unrelated to arthropod rearing and experimentation (e.g., plants, unused containers, clutter) that provide breeding sites and harborages are minimized.

3. *General Arthropod Elimination*. Accidental sources of arthropods from within the insectary are eliminated. This may be accomplished by cleaning work surfaces after a spill of materials, including soil or water that might contain viable eggs. Pools of water are mopped up immediately.

4. *Primary Container Cleaning and Disinfestation*. Practices must be in place such that arthropods do not escape by inadvertent disposal in primary containers. Cages and other culture containers are appropriately cleaned to prevent arthropod survival and escape (e.g., heated to over the lethal temperature or killed by freezing).

5. *Primary Container Construction*. Cages used to hold arthropods effectively prevent escape of all stages. Screened mesh, if used, is durable and of a size appropriate to prevent escape. Non-breakable cages are recommended. Bags, rearing trays and so on effectively prevent leakage and escape.

6. *Disposal of Arthropods*. Living arthropods are not to be disposed of. All wastes from the insectary (including arthropod carcasses, and rearing medium) are transported from the insectary in leak-proof, sealed containers for appropriate disposal in compliance with applicable institutional or local requirements. All stages of arthropods are killed before disposal. Autoclaving or incineration of material infected with a non-pathogen is recommended. Material may be killed with hot water or freezing before flushing down drains.

7. *Primary Container Identification and Labeling*. Arthropods are identified with descriptive labels to include the species, strain/origin, date of collection, responsible investigator, and so on; labels are firmly attached to the container (and cover if removable). Vessels containing stages with limited mobility (e.g., eggs, pupae, hibernating adults) are likewise labeled and (if applicable) housed or stored to prevent progression to, and escape of, a mobile life stage.

8. *Prevention of Accidental Dispersal on Persons or via Sewer*. Personnel take appropriate precautions to prevent transport or dissemination of live mobile arthropods from the insectary by practicing appropriate disposal methods and preventing escapees at every level of containment (primary container, environmental chamber, laboratory, etc) to prevent dispersal on persons.

9. *Pest Exclusion Program*. A program to prevent the entrance of wild arthropods (e.g., houseflies, cockroaches, spiders) and rodents effectively precludes predation, contamination, and possible inadvertent infection.

10. *Escaped Arthropod Monitoring*. Investigators assess whether escapes are occurring. An effective arthropod trapping program is recommended to monitor the escape prevention program.

11. *Source and Harborage Reduction*. Harborage and breeding areas are reduced as appropriate. Furniture and racks are minimized and can be easily moved to permit cleaning and location of escaped arthropods.

12. *Microbiological and Medical Sharps*. Syringes that re-sheath the needle, needle-less

systems, and other safe devices are used when appropriate. Plastic-ware is substituted for glassware whenever possible.

13. *Notification and Signage*. Persons entering the area are aware of the presence of arthropod vector species by signage if recommended by an institutional research oversight committee.

B. Special Practices

1. *IACUC and IBC Approval*. IACUC approval is required for use of vertebrate animals used as hosts. IBC approval is required for non-exempt recombinant or synthetic nucleic acid molecule protocols.

2. *Housing of Non-Arthropod Animals*. Animals used as hosts or blood sources should be housed according to institutional lab animal guidelines. If necessary, vertebrate animals may be housed within the insectary but need to be adequately protected from access by escaped arthropods. Animals not necessary for maintaining arthropods should not be accessible to hematophagous arthropods in the laboratory setting.

3. *Containment During Blood-Feeding*. Special considerations should be taken when hematophagous arthropods are fed on host animals. The primary container must be sufficiently robust to prevent escape during feeding. When handling/removing vertebrate animals after exposure to arthropods, precautions must be taken to prevent arthropod escape through screens, covers, and by flying. Host animals are inspected closely (e.g., concealment in fur, ears, axillae, or other possible hiding places). Finally, all precautions should be taken to prevent arthropods fed on host animals from accidental transfer to host cages and therefore dispersal outside of containment, if animals and their cages are returned to a holding room.

4. *Blood Source*. The blood source should be considered as a possible source of inadvertent arthropod infection and transmission. Whenever feasible, use of sterile blood or blood from sources known to be specific pathogen-free is recommended whereas use of blood from animals or humans whose disease status is uncertain should be avoided. In some instances, a vector colony is specifically adapted to and will not propagate without human blood acquired directly by feeding on a volunteer. Such arthropods should not be fed a second time on a different volunteer; those fed initially by membrane on animal or human blood should not be allowed to subsequently feed on a human volunteer.

5. *Escaped Arthropod Handling*. Escaped arthropods are killed or collected and properly disposed of.

6. *Accidental Release Reporting*. The insectary director is notified promptly of accidental release of vectors.

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

1. *Personal Protective Equipment*. Laboratory coats, gowns, and/or uniforms are worn at all times in the insectary when handling blood and vertebrate animals. Gloves are worn when handling host animals or blood used to feed the arthropods.

2. *Arthropod-Specific Personal Protective Equipment*. Personal protective equipment is worn as appropriate e.g., respirators for arthropod-associated allergies, particle masks, head covers, but local risk assessment and institutional policy may provide exceptions.

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

1. *Location of Insectary*. The insectary area is separated from areas that are used for general traffic within the building.

2. *Insectary Doors*. Doors openings, whether covered by rigid panels, glass, screens, plastic sheets or cloth, minimize escape and entrance of arthropods.

3. *Insectary Windows*. Windows, if present, effectively prevent escape of the smallest arthropods contained within.

4. *Lack of an insectary*. Arthropods may be maintained at ACL-1 in rooms other than those specifically designed as insectaries. If the facility does not have secondary barriers that would minimize escape or entry of pests, and is not separated from general traffic, specific operating procedures must be developed and tested to mitigate such risks. For example, mosquitoes might be held by a "cage within a larger cage", removal of adult mosquitoes accomplished by the aspirator manipulated through cage sleeves placed perpendicular to each other and the sample container loaded entirely within the outer cage. Alternatively, entire mosquito containers may be chilled before aspirating individual mosquitoes. Plexiglas glove boxes might also be used for manipulations, particularly if exotic species are maintained. Non-flying species may be manipulated on designated tables or benches in pans within moats of water, and housed in vials or other containers held within a secondary storage container such as a lidded plastic food container.

## 14. Plant Biosafety and Oversight

Policies and procedures have been established to safeguard against the accidental release of recombinant nucleic acid-containing plants, plant-associated microorganisms and plant associated animals to the environment outside of the Greenhouse facility, as required by federal, state, and local regulatory policies. All Principal Investigators (PIs), laboratory personal, and greenhouse staff must adhere to these policies and procedures in their research and the management of their laboratories.

## A. Regulations, Permitting Agencies, and Oversight

### 1. NIH Guidelines

Specific regulations for research involving modified whole plants can be found in Sections III-D-5 and III-E-2 of the *NIH Guidelines.* Appendix P of the *NIH Guidelines* "Physical and Biological Containment for Recombinant or Synthetic Nucleic Acid Molecule Research Involving Plants" outlines the physical and biological containment practices that must be employed for greenhouse research at the four plant biosafety levels (BL-P), BL1-P through BL4-P.

The use of genetic manipulation and other techniques to produce transgenic or genetically modified plants, recombinant plant pathogens, and transgenic arthropods is common in plant science research.

### 2. Permitting Agencies

Research involving transgenic or genetically modified plants, recombinant plant pathogens, or transgenic animals may require permits from a Federal agency. Prior to applying for a permit through any of the following agencies, PI's should contact the University of Utah Biosafety Officer for assistance to ensure that the appropriate biocontainment procedures are in place for the proposed research project.

a) <u>United States Department of Agriculture (USDA)/Animal and Plant Health Inspection Service (APHIS)</u>

Biological materials that may pose a risk to plants and/or animals or the environment are regulated by APHIS. APHIS permits for working with certain plants, plant pests, and plant-associated organisms are granted by agencies based on the biological material involved and the at-risk population (i.e. plants). These agencies are:

- Plant Protection and Quarantine (PPQ)
- Biotechnology Regulatory Services (BRS)

i. *PPQ*

Ensures that the appropriate protections are in place to protect agriculture and natural resources from the risks associated with the entry, establishment, or spread of plant pests, plant pathogens, and noxious weeds to ensure an abundant, high-quality, and varied food supply. Generally, PPQ purview includes only unmodified (i.e. non-transgenic) materials.

ii. *BRS*

BRS protects America's agriculture and environment through regulatory oversight that allows for the safe development, transport, and use of genetically modified organisms (GMOs), including plants, plant pests, and arthropods. BRS also regulates and oversees environmental releases of these GMOs (i.e. transgenic field releases).

APHIS permits are available as electronic permits (e-permits) through the APHIS website. Holders of APHIS permits assume all legal responsibility for the materials, their transport, and their security. Researchers are advised to contact the University of Utah Biosafety Officer or the appropriate agency if they have questions about the permits required for their research.

b) Environmental Protection Agency (EPA)

The EPA regulates two categories of GMOs: plants producing toxins (e.g., *Bacillus thuringiensis*) and novel microbes for commercial use (e.g., pollutant degrading bacteria). Information is available on these two categories through the *Biopesticides and Pollution Prevention Division* of the EPA.

c) Food and Drug Administration (FDA)

The FDA regulates GMO-derived commercial products for human and animal consumption, as well as human and veterinary pharmaceuticals. FDA's oversight does not apply to the research and development phases of the product(s).

d) Centers for Disease Control and Prevention (CDC)

The CDC and USDA APHIS jointly regulate certain plant pathogens that are recognized as potential bioterrorism agents as specified in the National Select Agent Registry. The National Select Agent Registry is charged with permitting and tracking agents and toxins that pose a threat to public health and agriculture. Currently, there are eight plant pathogens listed as Select Agents and the University of Utah is not registered to work with any of these pathogens.

## B. Plant Biosafety Levels (BL-P)

A plant biosafety level designation will dictate the physical and biological containment practices aimed at reducing the public health threat, and avoid an unintentional transmission or release of regulated

plant material into the environment. The BL-P levels of containment were specifically devised to describe containment for transgenic plants. There are four BL-P, and as the level increases, the level of protection and physical and/or biological containment practices also increase.

There are several issues to consider when determining the appropriate biosafety level:

- What is the source and nature of the introduced genetic material?
- Is it from an exotic infectious agent or pathogenic organism?
- Is it a fragment of DNA or a complete genome?
- What is the nature of the host organism?
- Can the host readily disseminate the genetic material? By what mechanism(s)?
- Is the recipient likely to be invasive to local ecosystems?
- Is the recipient a USDA APHIS-listed noxious weed or capable of interbreeding with noxious weeds?
- What is the potential for outcrossing between the recipient organism and related species?
- What is the potential for detrimental impact on natural or managed ecosystems?
- Are bioactive proteins expressed?
- What is the nature of expressed proteins?
- Are the proteins vertebrate toxins or potential/known allergens?
- Are the proteins toxic to other organisms in the local environment?
- What is the profile of the local environment?
- Are potentially affected important crops located nearby?
- Are sexually compatible wild or weed species capable of sustaining and/or spreading the genetic modification(s)?
- What experimental procedures may impact containment?
- Will it be necessary to transport sensitive materials to/from the greenhouse?
- Will arthropods or other potential vectors be used during the course of the project? How will these be contained to prevent or minimize the release of genetically modified materials?

**Note, the University of Utah does not have containment facilities to conduct BL-2P, BL3-P or BL4-P designated research.**

### C. BL1-P

BL1-P designation is for experiments that are deemed a low risk to the environment. This designation

also applies to plant associated microorganisms and arthropods that are considered to have a minimal impact on the environment. Some examples include: plants that are not noxious weeds, plants with no potential for out- crossing with related species, and *Agrobacterium*-mediated transfer of innocuous genetic material.

Work involving other organisms that require a containment level of BL1-P or lower may be conducted concurrently in a greenhouse bay as long as all work is conducted using BL1-P practices.

**1. Containment**

Greenhouse containment is essential in preventing the accidental release of transgenic research materials into the environment. When planning an experiment, all ways that an organism can breach containment must be considered. Traffic flow of personnel, air flow within the facility, prevention of cross- contamination, proper labeling, and permit requirements are important elements in this process.

**2. Access**

Only approved students, faculty, staff or other critical personnel may enter the University of Utah's greenhouse facility and must obey greenhouse facility rules (a single entrance to the facility is security card protected). Within the greenhouse facility, entry to greenhouse bays or growth chambers with ongoing experiments requiring BL1-P containment is restricted as specified in the greenhouse rules. All support staff and external contractors must be approved by the Greenhouse Manager to enter BL1-P active greenhouses or growth chambers, and by the PI for students or research staff deemed to critically require access to support experiments in progress (the PI will be responsible for providing the Greenhouse Director with a list of research personnel). The Greenhouse Manager must be notified when the plants contained within that greenhouse bay or growth chamber are under restricted access, and signage indicating restricted access must be posted on greenhouse or growth chamber doors. The Greenhouse Director and PI will be responsible for ensuring that all individuals with access to BL1-P active greenhouses or growth chambers are knowledgeable of special containment strategies, any required personal protective equipment, entry/exit procedures, and requirements for removal/disposal of recombinant biological materials.

**3. Records**

In BL1-P greenhouse bays, it is important that all users read the University of Utah Greenhouse Manual and follow applicable Standard Operating Procedures (SOPs) as established by the PI. A copy of the SOPs for experiments being performed in any given greenhouse bay must be maintained in the respective greenhouse bay by the PI. Record logs of all in-process experiments

in the greenhouse facility must be kept by the greenhouse manager (IBC registrations or a simple list of ongoing experiments are suitable records for BL1-P).

It is the responsibility of the PI to provide the Greenhouse Manager and Biosafety Officer access to record logs at the time of decommissioning or in the event of an audit by regulatory authorities.

Additionally, any specific federal/state permits required for greenhouse projects must be on file with the Biosafety Officer to ensure compliance with all procedural and containment expectations indicated in the permit(s).

### 4. Structural Containment

The greenhouse bay floors in BL1-P containment may be composed of gravel or other porous material, but concrete walkways are recommended. Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to contain or exclude pollen, microorganisms, or small flying animals (e.g., arthropods and birds); however, screens are recommended.

Regular inspections of the physical condition of the greenhouses are performed by the Biosafety Officer and/or Greenhouse Manager. All authorized greenhouse users are required to be vigilant for structural damage due to age related wear and tear, seasonal influences, extreme weather, vandalism, and other causes. Observations must be reported to the Greenhouse Coordinator, PI, and the Biosafety Officer. Items include, but are not limited to, the following:

- Doors that do not properly close;
- Damaged door sweeps;
- Cracks, breaks to glass;
- Damage to screens;
- Evidence of insects in the greenhouse; and/or
- Damaged or missing seals between structural components, around pipes and conduit.

### 5. Signs and Labeling

All transgenic seeds, plants, and materials must be clearly labeled and identified to distinguish them from other non-transgenic materials. Coding, such as numeric codes or color codes, may be used to identify materials as long as the PI maintains a list of codes that can be easily accessed by approved staff and the Biosafety officer. Note, if color coding is used avoid colors that may be indistinguishable to individuals who are color blind.

## 6. Proper Hygiene/Housekeeping

Good basic hygiene/housekeeping practices are important in preventing the accidental release and/or unintentional spread of plant pests and pathogens. Basic practices and procedures include:

- Keep greenhouse bay(s) clean and uncluttered;
- Do not eat or drink in greenhouse bays;
- Wash hands before leaving the greenhouse facility;
- Use personal protective equipment (PPE), as specified in the SOPs, when handling transgenic plant material, recombinant plant pathogens, and arthropods. Examples include disposable fluid-resistant gloves and facility-dedicated or disposable lab coats/smocks;
- Thoroughly inspect street clothes/shoes for transgenic material (especially seed and/or pollen) prior to leaving the greenhouse bay;
- Observe all special containment measures, such as footbaths, sticky mats, etc. when present;
- Change clothes prior to entering greenhouse if there is an increased potential to introduce unwanted plant pests/pathogens (e.g. working in an insect rearing facility before entering the greenhouse facilities);
- Eliminate any unnecessary equipment in greenhouse bays with transgenic plants, and recombinant plant pathogens or arthropods, and.
- Prohibit smoking throughout the Greenhouse Facility.

## 7. Pest Control

The NIH Guidelines specify that a weed and pest control program must be in place for all levels of greenhouse containment. In the Greenhouse Facility, PIs assigned to the bays are responsible for pest management. Plants must be regularly inspected for signs of insect infestation.

## 8. Transporting Transgenic Material

Experimental plants, seeds, and microorganisms that are transported to and from the greenhouse facility must be transported in a double walled, leak-proof, shatterproof container. The outside of the container must be sanitized prior to transport to ensure that transgenic pollen and seed are removed.

## 9. Biological Containment Techniques

Unless integral to the research project, the production/dissemination of transgenic pollen and

seed must be eliminated. There are several special practices that can be used to prevent the spread of transgenic material that include, but are not limited to, the following:

- Removing flower heads or bagging plants prior to flowering;
- Harvesting material before the reproductive stage;
- Using male sterile lines;
- Localizing engineered genes in the non-reproductive parts of the plant by expressing the transgene transiently rather than in stably transformed plants; or
- Conducting the experiment when pollination will not occur outside (e.g. winter months).

Transgenic or unmodifed insects or mites that are associated with transgenic plants must be housed in appropriate containment caging systems (e.g. BugDorms) to minimize escape from the greenhouse bay. A cost effective alternative can be constructed using plastic sheeting. Additional biocontainment techniques to be used when working with insects and mites include:

- Treatment or evaporation of runoff water to kill eggs and larvae;
- Destruction of pollinating insects in cages after pollen transfer.

Containment practices must be registered with, and approved by, the IBC.

Recombinant microbes such as bacteria, fungi, protozoa, viruses, and nematodes may be used during experiments. Additionally, unmodified microbes may be used in association with transgenic plants. In these cases, the goal of containment is to minimize dissemination of pollen and the microorganisms. Containment techniques that can be used when working with microorganisms include:

- Elimination of potential vectors;
- Genetic attenuation of the microorganism;
- Limiting production of aerosols during inoculation;
- Ensuring adequate distance between infected and susceptible hosts;
- Chemically treating runoff water to kill microorganisms;
- Using microorganisms that have an obligate association with the plant host.

**10. Disposal of Materials**

BL1-P experimental plants and soil must be rendered biologically inactive before final disposal. Viable non-seed plant tissue can be rendered inactive by desiccation, steam treatment, chemical treatment, freezing, or by a validated autoclave while seeds can be rendered inactive by steam or chemical treatment or by autoclaving. Transport of viable materials to the site of deactivation

must be conducted in double walled containers (such as an autoclave bag in an autoclave tub). If viable BL1-P transgenic materials must be transferred to another facility for inactivation, a transportation containment SOP must be reviewed and approved by the IBC. After plant materials are inactivated using validated parameters, they may be disposed of in the regular trash.

## 11. Containment Breach

Weather related incidents, vandalism, or human error can result in a containment breach. Seeds can become attached to clothing and/or shoes, especially if greenhouse containment practices are not rigorously followed. These seeds can be easily spread by the wind and could grow in the surrounding area, causing volunteers. Therefore, routine volunteer monitoring outside the greenhouse shall be conducted. There are several steps that must be taken if an accident results in the inadvertent release or spill of recombinant microorganisms, transgenic arthropods, and/or transgenic plants from physical containment:

- If known, seed/pollen dissemination distances must be considered when determining the monitoring area. If a known breach of containment has occurred, volunteer monitoring must be enhanced by increasing the monitoring zone and/or frequency of monitoring.
- Determine if any transgenic material has been removed from the greenhouse bay/facility (or other containment vessels within the greenhouse bay/facility) or is otherwise unaccounted for.
- Contain and recover all transgenic materials as best as possible.
- The PI must report the containment breach to the Greenhouse Manager, Biosafety Office, IBC chair, and other appropriate agencies within 24 hours, or as indicated by permit.

## 12. Standard Operating Procedures (SOPs)

SOPs need to be prepared for all transgenic experiments that will be conducted in the greenhouse facilities. SOPs must be stored in a notebook inside the respective greenhouse bay. SOPs must be included in the IBC registration. SOPs must include:

- Growth and management practices for the transgenic materials;
- Biocontainment techniques;
- Methods of inactivation of transgenic materials (including soil and pots);
- A written contingency plan to be implemented in the event of the unintentional release of transgenic material.

Copies of all SOPs as well as the contingency plan must be made available to the Greenhouse

Manager and the Biosafety Officer, and hardcopies of the SOPs and applicable contingency plans must be available within respective greenhouse bays at the entrance or nearby growth chamber doors.

Copies of any permits/performance standards associated with the greenhouse work must be included in the SOP notebook.

If applicable, copies of Safety Data Sheets (SDS) for chemicals and suitable descriptions of biological agents must also be accessible in the notebook.

## E. Working with Human Tissues and Cells

Biosafety Level 2 practices and procedures must be followed when handling human blood, blood products, body fluids and tissues, as well as primary and established human and non-human primate cell lines that have not been screened for human pathogens. These materials have the potential to contain bloodborne pathogens and must be handled according to the provisions set out in 29 CFR 1910.1030 – OSHA's bloodborne pathogens standard. This is consistent with the concept known as "Universal Precautions". Note work with human samples needs to be registered with the IBC as a Biological Materials Registration and containment measures compatible with the pathogen must be implemented.

### 1. Exposure Control Plan.

A site-specific (laboratory, clinic, etc.) Exposure Control Plan (ECP) must be developed and made readily available to all employees. To this end, OEHS has developed a campus-wide ECP, as well as templates that can be edited to address the specific procedures in laboratories and other facilities. The ECP addresses in detail the OSHA requirements for working with human blood, tissue and cell lines.

### 2. Vaccination

OSHA regulations (29 CFR 1910.1030(f)(1)(i)) require that all employee with the risk of occupational exposure to blood or other potentially infectious materials be provided with HBV vaccination. Employees may decline vaccination without cause or reason but must have a sign declination form on file. HBV vaccination is available by contacting OEHS at 801-581-6590.

### 3. Best Practices

Under no circumstances shall anyone work with autologous cells (cells derived from themselves) or from first-degree relatives. These cells will express the tissue type of the operator and could evade the normal immune responses that recognize and destroy foreign cells.

Also, it is best practice to avoid using one's own blood for any tissue culture experiments. No one shall work with their own blood samples or those of colleagues working in the lab, if the intention is to transform lymphocytes because in the event of an accidental exposure, their immune system will not challenge the transformed cells.

### 4. Training

OSHA regulations (29 CFR 1910.1030(g)(2)) require training on the safe handling of human blood and other potentially infectious materials. This training is required prior to work in the lab with human tissues and must be repeated annually for all personnel with the potential for exposure to blood and other potentially infectious materials. Courses satisfying the regulatory requirements are taught by OEHS. Registration for these courses is available through the Research Administration Training Series (RATS) web site.

Participation in work involving infectious agents will be allowed only after proficiency has been demonstrated to the satisfaction of the Principal Investigator or Laboratory Supervisor.

## F. Cell Culture

The following must be handled at BSL-2 or higher containment level:

1. All cell lines of human/primate origin, including human cell lines
2. Any cell lines derived from lymphoid or tumor tissue
3. All cell lines exposed to or transformed by any oncogenic virus
4. All cell lines exposed to or transformed by amphotropic packaging systems
5. All clinical material (e.g., samples of human tissues and fluids obtained after surgical resection or autopsy)
6. All cell lines new to the laboratory (until proven to be free of all adventitious agents)
7. All mycoplasma-containing cell lines – The cell line must be classified at the same level as that recommended for the agent when cell cultures are known to contain an etiologic agent, an oncogenic virus or amphotropic packaging system.

## G. Recombinant or Synthetic Nucleic Acid Molecules Research

Recombinant or synthetic nucleic acid molecules are defined as either: (1) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (2) DNA molecules that result from the replication of a molecule described in (1).

Synthetic DNA segments which are likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) are considered as equivalent to their natural DNA counterpart. If the synthetic DNA segment is not expressed *in vivo* as a biologically active polynucleotide or polypeptide product, it is exempt from the NIH Guidelines. Genomic DNA of plants and bacteria that have acquired a transposable element, even if the latter was donated from a recombinant vector no longer present, are not subject to the NIH Guidelines unless the transposon itself contains recombinant or synthetic nucleic acid.

1. Federal Guidelines and Registering Experimental Protocols – All research conducted at the University of Utah involving recombinant or synthetic nucleic acid molecules must meet current NIH guidelines. All experimental protocols must be approved by the IBC and in special instances by a committee at the NIH or USDA as well. The principal investigator is responsible for determining the status of his/her experiments and filing the proper documents if review is required.
2. Emergency Plans – The NIH Guidelines instruct a set of emergency plans covering accidental spills and resulting personnel contamination for work involving rsNA. Section Q of this document constitutes a basic spill plan. Research that is carried out at physical containment level BSL-2 or higher requires the principal investigator to prepare or adopt a laboratory-specific biosafety manual.

The IBC has established guidance documents for working with recombinant viral vectors to assist investigators with their Risk Assessment when registering their work with the IBC (Appendix B).

## H. Human Gene Transfer

All protocols involving the generation of rsNA for human gene transfer must be registered and approved by the IBC prior to submission to the University of Utah IRB, outside agencies and the initiation of experimentation. Prior approval by the IRB is required before commencing gene therapy in humans.

## I. Transgenic Animals

Investigators who create transgenic animals or animals with modifications to their germline must complete a Transgenic Animal Registration Form and submit it to the IBC for approval. In addition, the protocol must receive approval from the IACUC.

## J. Biological Toxins

Guidelines for working with biological toxins can be found in Appendix I of the *Biosafety in Microbiological and Biomedical Laboratories*. These are summarized below.

Routine operations with dilute toxin solutions are conducted using **Biosafety Level 2 (BSL2)** practices and these must be detailed in the IBC protocol and will be verified during the inspection by OEHS staff prior to IBC approval. BSL2 Inspection checklists can be found *here*. All personnel working with biological toxins or accessing a toxin laboratory must be trained in the theory and practice of the toxins to be used, with special emphasis on the nature of the hazards associated with laboratory operations and be familiar with the signs and symptoms of toxin exposure. This includes how to handle transfers of liquids containing toxin, where to place waste solutions and contaminated materials or equipment, and how to decontaminate work areas after routine operations, as well as after accidental spills. The worker must be reliable and sufficiently adept at all required manipulations before being provided with toxin. Laboratory work with toxins will be done only in designated rooms with controlled access and at pre-determined bench areas. When toxins are in use, the room must be clearly posted: "Toxins in Use—Authorized Personnel Only."

Researchers working with a toxin must be vaccinated (or sign a declination form) if a vaccine is available (e.g. diphtheria toxin, tetanus toxin). Routine operations with dilute toxin solutions are conducted using **BSL2** practices and facilities shall be used for activities involving biological toxins. These include:

- Biohazard signs and labels must be displayed in areas and on equipment where biological toxins are used and stored. This includes, but is not limited to, laboratory entrance doors, biological safety cabinets, chemical fume hoods, refrigerators, and freezers.
- Use a biological safety cabinet (BSC) or a chemical fume hood for resuspension of biological toxins or manipulations of stock solutions of toxins that can generate aerosols, such as pipetting, harvesting, infecting cells, filling tubes/containers, and opening sealed centrifuge canisters. When using an open-fronted fume hood or BSC, workers must wear suitable laboratory PPE to protect the hands and arms, such as laboratory coats, smocks, or coveralls and disposable gloves.
- Whenever possible, use needle-free techniques to resuspend biological toxins.

- If a quantity of powder-form toxin must be weighed, then the scale must be located in a certified chemical fume hood.
- When conducting liquid transfers and other operations that pose a potential splash or droplet hazard in an open-fronted hood or BSC, workers must wear safety glasses and disposable facemask, or a face shield.
- Toxin may be removed from the hood or BSC only after the exterior of the closed primary container has been decontaminated and placed in a clean secondary container. Toxin solutions, especially concentrated stock solutions, must be transported in leak/spill-proof secondary containers labeled with a Biohazard sticker.

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 must be opened in a BSC, chemical fume hood, or other ventilated enclosure. Operations that expose toxin solutions to vacuum or pressure, for example sterilization of toxin solutions by membrane filtration, must always be handled in this manner, and the operator must also use appropriate respiratory protection. If vacuum lines are used with toxin, they must be protected with a HEPA filter to prevent entry of toxins into the line.

Centrifugation of cultures or materials potentially containing toxins shall only be performed using sealed, thick-walled tubes in safety centrifuge cups or sealed rotors. The outside surfaces of containers and rotors must be routinely cleaned before each use to prevent contamination that may generate an aerosol. After centrifugation, the entire rotor assembly is taken from the centrifuge to a BSC to open it and remove its tubes.

Experiments must be planned to eliminate or minimize work with dry toxin (e.g., freeze-dried preparations). Unavoidable operations with dry toxin may only be undertaken with appropriate respiratory protection and engineering controls.

To assist PIs in the development of SOPs for working with biological toxins, the IBC has developed a template that can be edited and submitted to the IBC as part of their registration (Appendix F).

### 1. Personal Protective Equipment (PPE)

Work with biological toxins shall be conducted using BSL2 PPE:

- Disposable gloves – consider the use of double gloves for enhanced protection. Ensure your gloves are compatible with any solvent your toxin may be dissolved in.
- Lab coat or back-closing disposable gown

- Eye protection (safety glasses or goggles) is recommended, but not required when working within a biosafety cabinet.

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

**2. 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. Literature values 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 inactivation caused by heating. In addition, the conditions for denaturizing toxins in aqueous solutions are not necessarily applicable for inactivating dry, powdered toxin preparations. Inactivation procedures should not be assumed to be 100% effective without validation using specific toxin bioassays.

General guidelines for laboratory decontamination of selected toxins are summarized in Tables 6 and 7, but inactivation procedures should not be assumed to be 100% effective without validation using specific toxin bioassays. 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 (Table 7). All disposable material used for toxin work must be placed in secondary containers, autoclaved and disposed of as toxic waste. Contaminated or potentially contaminated protective clothing and equipment must be decontaminated using suitable chemical methods or autoclaving before removal from the laboratory for disposal, cleaning or repair. If decontamination is impracticable, materials must be disposed of as toxic waste.

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

to the spill, beginning at the perimeter and working towards the center, and allow sufficient contact time to completely inactivate the toxin (Table 7).

Decontamination of buildings or offices containing sensitive equipment or documents poses special challenges. Large-scale decontamination is not covered explicitly here, but careful extrapolation from the basic principles may inform more extensive clean-up efforts.

**Table 6. Physical Inactivation of Selected Toxins**

| Toxin | Steam Autoclave | Dry Heat (10 min) | Freeze-thaw | Gamma Irradiation |
|---|---|---|---|---|
| Botulinum neurotoxin | Yes [a] | > 100º C [b] | No [c] | Incomplete [d] |
| Staphylococcal Enterotoxin | Yes [e] | > 100º C; refolds [f] | No [g] | Incomplete |
| Ricin | Yes [i] | > 100º C [i] | No [j] | Incomplete [k] |
| Microcystin | No [l] | > 260º C [m] | No [n] | ND |
| Saxitoxin | No [l] | > 260º C [m] | No [n] | ND |
| Palytoxin | No [l] | > 260º C [m] | No [n] | ND |
| Tetrodotoxin | No [l] | > 260º C [m] | No [n] | ND |
| T-2 mycotoxin | No [l] | > 815º C [m] | No [n] | ND |
| Brevetoxin (PbTx-2) | No [l] | > 815º C [m] | No [n] | ND |

**Notes:**

ND indicates "not determined" from available decontamination literature.

[a] Steam autoclaving should be at >121°C for 1 h. For volumes larger than 1 liter, especially those containing *Clostridium botulinum* spores, autoclave at >121°C for 2 h to ensure that sufficient heat has penetrated to kill all spores.

[b] Exposure to 100°C for 10 min. inactivates BoNT. Heat denaturation of BoNT as a function of time is biphasic with most of the activity destroyed relatively rapidly, but with some residual toxin (e.g., 1-5%) inactivated much more slowly.

[c] Measured using BoNT serotype A at -20°C in food matrices at pH 4.1 – 6.2 over a period of 180 days.

[d] Measured using BoNT serotypes A and B with gamma irradiation from a $^{60}Co$ source.

[e] Protracted steam autoclaving, similar to that described for BoNT, followed by incineration is recommended for disposal of SE-contaminated materials.

[f] Inactivation may not be complete depending upon the extent of toxin re-folding after denaturation.

Biological activity of SE can be retained despite heat and pressure treatment routinely used in canned food product processing.

[g] SE toxins are resistant to degradation from freezing, chilling or storage at ambient temperature. Active SEB in the freeze-dried state can be stored for years.

[i] Dry heat of >100ºC for 60 min in an ashing oven or steam autoclave treatment at >121ºC for 1 h reduced the activity of pure ricin by >99%. Heat inactivation of impure toxin preparations (e.g., crude ricin plant extracts) may vary. Heat-denatured ricin can undergo limited refolding (<1%) to yield active toxin.

[j] Ricin holotoxin is not inactivated significantly by freezing, chilling or storage at ambient temperature. In the liquid state with a preservative (sodium azide), ricin can be stored at 4ºC for years with little loss in potency.

[k] Irradiation causes a dose-dependent loss of activity for aqueous solutions of ricin, but complete inactivation is difficult to achieve; 75 MRad reduced activity 90%, but complete inactivation was not achieved even at 100 MRad. Gamma irradiation from a laboratory $^{60}$Co source can be used to partially inactivate aqueous solutions of ricin, but dried ricin powders are significantly resistant to inactivation by this method.

[l] Autoclaving with 17 lb pressure (121-132º C) for 30 min failed to inactivate LMW toxins. All burnable waste from LMW toxins should be incinerated at temperatures in excess of 815°C (1,500º F).

[m] Toxin solutions were dried at 150º C in a crucible, placed in an ashing oven at various temperatures for either 10 or 30 min, reconstituted and tested for concentration and/or activity; tabulated values are temperatures exceeding those required to achieve 99% toxin inactivation.

[n] LMW toxins are generally very resistant to temperature fluctuations and can be stored in the freeze-dried state for years and retain toxicity.

**Table 7. Chemical Inactivation of Selected Toxins**

| Toxin | NaOCl (30 min) | NaOH (30 min) | NaCOl + NaOH (30 min) | Ozone Treatment |
|---|---|---|---|---|
| Botulinum neurotoxin | > 0.1% [a] | > 0/25 N | ND | Yes [b] |
| Diphtheria Toxin | > 0.5% | ND | ND | ND |
| Staphylococcal Enterotoxin | > 0.5% [c] | > 0.25 N | ND | ND |
| Ricin | > 1.0% [d] | ND | > 0.1% + 0.25N [e] | ND |
| Saxitoxin | ≥ 0.1% [e] | ND | 0.25% + 0.25N [e] | ND |
| Palytoxin | ≥ 0.1% [e] | ND | 0.25% + 0.25N [e] | ND |
| Pertusis Toxin | > 0.5% | ND | ND | ND |
| Microcystin | ≥ 0.5% [e] | ND | 0.25% + 0.25N [e] | ND |
| Tetrodotoxin | ≥ 0.5% [e] | ND | 0.25% + 0.25N [e] | ND |

| Tetanus Toxin | > 0.5% | ND | ND | ND |
|---|---|---|---|---|
| T-2 mycotoxin | ≥ 2.5% [e, f] | ND | 0.25% + 0.25N [e] | ND |
| Brevetoxin (PbTx-2) | ≥ 2.5% [e, f] | ND | 0.25% + 0.25N [e] | ND |

**Notes:**

ND indicates “not determined” from available decontamination literature.

[a] Solutions of NaOCl (#0.1%) or NaOH (> 0.25 N) for 30 min inactivate BoNT and are recommended for decontaminating work surfaces and spills of *C. botulinum* or BoNT. Chlorine at a concentration of 0.3-0.5 mg/L as a solution of hypochlorite rapidly inactivates BoNT (serotypes B or E tested) in water.[20] Chlorine dioxide inactivates BoNT, but chloramine is less effective.[21]

[b] Ozone (> 2 mg/L) or powdered activated charcoal treatment also completely inactivate BoNT (serotypes A, B tested) in water under defined condition.

[c] SEB is inactivated with 0.5% hypochlorite for 10-15 mi.

[d] Ricin is inactivated by a 30 min exposure to concentrations of NaOCl ranging from 0.1-2.5%, or by a mixture of 0.25% NaOCl plus 0.25 N NaOH. In general, solutions of 1.0% NaOCl are effective for decontamination of ricin from laboratory surfaces, equipment, animal cages, or small spills.

[e] The minimal effective concentration of NaOCl was dependent on toxin and contact time; all LMW toxins tested were inactivated at least 99% by treatment with 2.5% NaOCl, or with a combination of 0.25% NaOCl and 0.25N NaOH.

[f] For T-2 mycotoxin and brevetoxin, liquid samples, accidental spills, and nonburnable waste should be soaked in 2.5% NaOCl with 0.25% N NaOH for 4 h. Cages and bedding from animals exposed to T-2 mycotoxin or brevetoxin should be treated with 0.25% NaOCl and 0.025 N NaOH for 4 h. Exposure for 30 min to 1.0% NaOCl is an effective procedure for the laboratory (working solutions, equipment, animal cages, working area and spills) for the inactivation of saxitoxin or tetrodotoxin.
Decontamination of equipment and waste contaminated with select brevetoxins has been reviewed.

Alternate methods of chemical decontamination: 1 N sulfuric or hydrochloric acid did not inactivate T-2 mycotoxin and only partially inactivated microcystin-LR, saxitoxin, and brevetoxin (PbTx-2). Tetrodotoxin and palytoxin were inactivated by hydrochloric acid, but only at relatively high molar concentrations. T2 was not inactivated by exposure to 18% formaldehyde plus methanol (16 h), 90% freon-113 + 10% acetic acid, calcium hypochlorite, sodium bisulfate, or mild oxidizing. Hydrogen peroxide was ineffective in inactivating T-2 mycotoxin. This agent did cause some inactivation of saxitoxin and tetrodotoxin, but required a 16 h contact time in the presence of ultraviolet light.

References are provided in Appendix I of the BMBL.

### 3. Exposures

Antitoxins are available for some biological toxins and immediate medical "first-aid" interventions may help prevent or lessen the severity of the reaction. If you know or suspect a biological toxin exposure:

- Irrigate the site of exposure
  - If exposure was by needle stick or other route which breaks the skin, wash with soap and water for 5-15 minutes and cover with a bandage.
  - If exposure was by splash to eyes or mucus membranes, irrigate thoroughly for 15 minutes at an appropriate eye wash station.
- Report to your Laboratory Supervisor and the Biosafety Officer IMMEDIATELY. Seek medical attention as outlined in your laboratory specific safety manual.

### 4. Export Controlled Toxins

Several biological toxins are restricted for export by the U.S. Department of Commerce and require an export license prior to any shipment out of the U.S. Many of the toxins on the export control list do not meet the definition of an acute toxin (LD50 <100 µg/kg body weight) and do not require registration with the IBC but the Principal Investigator is responsible for ensuring that they are in compliance with the export control laws and regulations. If you have questions about export controls, please visit the Office of Sponsored Projects webpage (http://osp.utah.edu/policies/export-controls.php) or contact OSP's Export Control Officer, Todd Nilsen.

## K. Biological Safety Cabinets

### 1. Selection

Biological Safety Cabinets serve as an effective primary barrier against biological or infectious agents by surrounding the immediate work area. It is the ideal complement to, not replacement for, careful work practices.

The cabinets are equipped with High Efficiency Particulate Air (HEPA) filters which have 99.97% efficiency against 0.3 micron particles. HEPA filters offer no protection against volatiles, such as ether, alcohol, etc. Selection of the correct biological safety cabinet is based on the classification of the agent, the associated biosafety level for the particular agent, and chemicals which will be used in the research.

**Table 8. Types of Cabinets**

| Cabinet | Operations and Uses |
|---|---|
| Horizontal Laminar Flow or Clean Bench | Filtered air flow across the work surface toward the operator, providing a protection for the product, but not the worker. Do not use for work with infectious materials, toxic chemicals, sensitizing agents, or radionuclides. |
| Class I | Only the exhaust air is filtered, therefore protection is provided to the user and to the environment, but not to the experiment. The operator's hands and arms may be exposed to hazardous materials inside the cabinet. This cabinet may be used with low to moderate risk biological agents. |
| Class II | These have vertical laminar air flow with HEPA filtered supply and exhaust air. They protect the worker, the product, and the environment. For use with low to moderate risk biological agents. |
| Class II, Type A | Recirculated 70% of the air inside the cabinet. Exhausts 30% into room after filtration. 75 fpm average face velocity. Do not use with volatile radionuclides or toxic chemicals. |
| Class II, Type B1 | Recirculated 30% of the air inside the cabinet and exhausts the rest to the outside of the building. Maintains 100 fpm average velocity. Contaminated ducts are under negative pressure. May be used with minute amounts of volatiles. |
| Class II, B2 | Referred to as Total Exhaust. No recirculation. 100% exhausted outside after filtration. Maintains 100 fpm average face velocity. All contaminated ducts are under negative pressure. Suitable where volatile toxic chemicals and radionuclides are required. |
| Class III, or Glove box | Is gas-tight and maintained under negative air pressure. Used to work with highly infectious, carcinogenic, or hazardous materials. All operations are conducted through rubber gloves attached to entry portals. |

## 2. Use of Class II Biological Safety Cabinets

**See Appendix A for a detailed SOP**

### A. Preparations

1. Turn blower on and purge air for at least ten minutes prior to use, to filter air inside.
2. Never work with the UV light illuminated. Skin and eye damage can occur from the direct and reflected light.

   *UV light is effective only for decontaminating clean, solid surfaces with which it comes in contact. It is not effective in decontaminating the cabinet air flow. UV*

*light is not effective against bacterial spores. UV germicidal light tubes must cleaned regularly and be replaced frequently (at least every 6 months for biosafety cabinets in use on a daily basis) to assure that they are emitting light at 254 nm and at an intensity appropriate for decontamination.* ***Due to concerns over the effectiveness of these lights and the risks to individuals in the room, some Institutions, such as the NIH, have banned their use in BSCs. The University of Utah strongly discourages the use of UV lights in BSCs.***

3. Wipe down the work surface with an appropriate disinfectant. Do not depend on the UV germicidal lamp to provide a sterile surface.
4. Everything needed to complete the particular procedure will be placed inside the cabinet prior to beginning work. Arrange in a logical manner to segregate clean and contaminated. Arm movements in and out of the cabinet may cause escape of aerosols.

**B. Use**

1. Always wear a laboratory coat and gloves.
2. Conduct work at least four inches inside the glass panel (sash). The further back in the cabinet, the better.
3. Minimize arm movements, keeping necessary movements slow and smooth.
4. Avoid use of a burner within the cabinet. Open flames are not permitted to be used in biosafety cabinets at the University of Utah.
5. Place a disinfectant soaked towel on the work surface to contain any splatters or small spills which may occur.

**C. Upon Completion of Procedures**

1. All contaminated equipment is segregated with container surfaces covered and decontaminated.
2. The cabinet blower must be left on for at least five minutes to purge the air.
3. Remove equipment from the cabinet and decontaminate the work surfaces.
4. Wash hands and arms thoroughly with soapy water.

**D. Certification**

Biological safety cabinets are not to be used with hazardous materials until certified as meeting minimum safety specifications (e.g., NIH-03-112 or National Sanitation Foundation Standard 49) on site. They are to be certified *in situ* by a trained technician:

1. When newly installed.
2. Any time the cabinet has been moved.
3. Annually.

4. After repair or maintenance (e.g., filter replacement, work on the blower, etc.)

OEHS will schedule the annual certification inspections through an outside contractor. For recertification after repair or after moving the cabinet, or for additional information please contact the Assistant Biosafety Specialist at (801) 585-3345 for more information.

## L. Personnel Exposure Control Plans/Procedures

1. Each lab area/space with the potential for exposure to blood or OPIM must have an Exposure Control Plan (ECP). OEHS has developed a comprehensive ECP, with which all employees in such labs or areas must be familiar. In addition, OEHS has developed templates that PIs/supervisors must edit to address laboratory or facility specific risks and procedures. At a minimum, the plan will list tasks and procedures, as well as job classifications, where occupational exposure may occur. This plan must be reviewed annually.
2. BSL-2, BSL2-enhanced and BSL-3 laboratories must develop laboratory-specific biosafety manuals. The Biosafety manual shall, at a minimum, provide a risk assessment for the agents used, describe the physical containment and PPE requirements, describe appropriate disinfectants, describe spill and post exposure procedures. OEHS has developed templates that PIs/supervisors can edit. These templates can also serve as laboratory-specific ECPs when appropriately edited.
3. Hepatitis B vaccinations must be made available to all employees who have the potential for an occupational exposure to blood or other potentially infectious materials within 10 days of assignment. Contact OEHS to coordinate the vaccination(s).
4. Following any exposure incident, the individual will immediately wash the affected area with soap and water and then seek counseling/testing at an Occupational Medicine clinic (RedMed or Redwood Road Health Clinic). The incident will be reported to the supervisor who will investigate: the supervisor will also report the incident to the University Biosafety Officer. Circumstances causing the occurrence and measures to prevent recurrence will be documented. A confidential medical evaluation and follow-up must be made available to the employee, at no cost to him/her. Contaminated clothing shall be spot disinfected or autoclaved and taken to the School of Medicine laundry facility on the A level of the SOM for laundering (See Section V): alternatively commercial companies may be used for cleaning.

## M. Training

Training must be accomplished prior to beginning duties, after changes in occupational exposure determinations or SOPs, and repeated at least annually. At a minimum, it will consist of methods to minimize exposure, proper shipping procedures, and if working with blood or blood containing products, access to a copy of the OSHA Bloodborne Pathogen Standard, explanation of its contents, and a general explanation of the Exposure Control Plan: Bloodborne Pathogen training provided by OEHS is offered through the Research Administration Training Series (RATS), which can be accessed here.

In addition, personnel in BSL2 or higher laboratories must attend annual BSL2 (and ABSL2 if animal work is performed) training provided by OEHS through RATS.

All personnel prior to working in BSL2 or higher laboratories, including those with exposures to blood or OPIM, and annually thereafter, must review, as applicable:

1. The University of Utah ECP
2. The University of Utah Biosafety Manual
3. Laboratory/Facilities-specific ECPs and Biosafety Manuals
4. Laboratory/Facility-specific Standard Operating Procedures (SOPs).

All BSL-2 (or higher) lab spaces that are shared by 2 or more PIs using different biohazardous agents, who are not co-PIs or co-investigators on an approved IBC protocol(s), must conduct a joint "Biohazards Awareness and Training" session for all the personnel working in the space. The training must be presented by each PI or delegate, outlining the agents, and risks associated with the agents used for their specific research, signs and symptoms of exposure, PPE while working in the BSL-2 suite and appropriate disinfectants. New employees must be trained as soon as possible after hire. The IBC recommends repeating the training annually. If you need assistance with these training sessions please contact the Biosafety Office at 801-581-9325 or biosafety@oehs.utah.edu.

## N. Medical Surveillance

All employees in research laboratories working with, or who may be exposed to, potentially infectious agents, including recombinant viral vectors, must be aware of signs or symptoms consistent with diseases caused by these agents and their parental strains. In some cases medical evaluations, vaccinations and/or other medical surveillance is required.

### 1. General Awareness

All employees in research laboratories must be aware of signs or symptoms consistent with diseases caused by the agents and materials present in their lab. For example. personnel working with recombinant lentiviral vectors must be aware of the signs and symptoms of human immunodeficiency virus (HIV) infection. Personnel exposed to these agents may or may not become sick; however, they may have the potential to transmit them to others outside the laboratory if proper biosafety practices have not been followed. Laboratory-specific training must include hazard communication related to the risks of these agents, anticipated signs/symptoms associated with these agents to facilitate recognition of potential occupational illnesses, and procedures to follow if a potential exposure has occurred.

For certain activities, medical surveillance must be undertaken prior to working with biological agents as designated by the Institutional Biosafety Committee (IBC). Examples include laboratories working with human pathogens, such as HIV or Zika virus, or with agents for which vaccination may offer protection, such as pertussis toxin (PT). In addition, all personnel must be made aware by their supervisors that certain medical conditions increase their risk of potential health problems when working with pathogenic microorganisms and/or animals. These conditions include pregnancy, immunosuppression, animal related allergies, and chronic skin conditions. All personnel must discuss their work with an Occupational Medicine physician or their personal physician/health care professional if any of these conditions apply.

Certain types of work may require the use of a respirator to protect against aerosol exposures. In such cases, personnel must get medical clearance from the Department of Occupational Medicine: for most personnel this can be achieved by completing and submitting an OSHA Respirator Medical Evaluation form that can be downloaded *here*. Once Occupational Medicine has provided clearance, call the Biosafety Officer (801-581-6590) to arrange an appointment for Respirator Fit Testing. A Respiratory Protection Plan must also be submitted to the Biosafety Officer: a template can be found on the OEHS website. Fit testing must be repeated on an annual basis.

**2. Vaccinations**

Personnel may be required by the IBC to be offered vaccinations to protect them from workplace hazards. Examples include the Hepatitis B vaccine for all workers with reasonable expectation of exposure to human blood or other potentially infectious materials (OPIM), which includes human and non-human primate cell lines, including those acquired from commercial sources.

Tdap vaccination, which is highly effective for the prevention of diphtheria, tetanus and pertussis, must be offered to personnel working with PT or handling animals dosed with PT. Vaccine recommendations can be found at http://www.cdc.gov/vaccines/hcp/acip-recs/vacc-specific/index.html and

http://www.cdc.gov/vaccines/hcp/vis/index.html?s_cid=cs_748.
Protective vaccines, if available and appropriate based on workplace hazards, will be provided by the University of Utah at no cost to the employee. In most cases, if there is limited public health concern, employees may choose to decline the recommended vaccinations after understanding their risks. In these circumstances, the University of Utah is obligated to document the offer and obtain a signed declination by the employee that they understand the risks, yet chose to decline the vaccination. If the employee changes his/her mind, the vaccination will be made available to them upon request.

### 3. Post Exposure Surveillance

Exposures or potential exposures shall be reported to the supervisor and the Biosafety Officer (801-581-6590), and affected individuals must report to the Occupational Medicine Clinic at the Redwood Health Center or to the RedMed Clinic at the Student Union building. In the event of a life threatening event call 911 immediately. Information about the University of Utah Health Care Occupational Medicine Clinics can be found here: http://healthcare.utah.edu/occmed/

Employees must also follow the Incident Reporting Policy described in their laboratory Exposure Control Plan or Biosafety Manual. The medical professionals at Occupational Medicine will determine the need for antibody testing, post-exposure prophylaxis, treatment, and continued medical surveillance at that time. Employees must notify the medical professionals if the agent involved is modified in any way to allow the medical professionals to treat the agent appropriately.

The University of Utah IBC requires plans to address how a biological exposure incident be developed by the PI: details must be incorporated into the laboratory IBC registration and be part of the laboratory-specific exposure control plan and/or Biosafety Manual. The IBC has developed a template for a post exposure SOP (see Appendix C). This shall include identification of any post-exposure prophylaxis options and/or medical monitoring plans for those who may have been exposed to the agents, documentation of important aspects of the experimental design and procedures, such as changes in drug sensitivity and/or genetic modifications, which may modify the risks of exposure of these agents. In the event of an exposure it is recommended that laboratory personnel reporting to the Occupational Medicine clinic after an exposure bring completed post exposure SOPs with them to the health care provider to ensure proper communication to those who may be providing care, particularly for agents which are genetically modified agents.

All exposures to biological agents must be reported by the PI to OEHS by completing the template in Appendix D.

## O. Personal Protective Equipment

**1.** Laboratory-specific Personal Protective Equipment (PPE) must be described in the laboratory Biosafety Manual, Exposure Control Plan and/or Standard Operating Procedures.

**2.** Principal Investigators/Supervisors must provide PPE to all staff at no cost.

**3.** Staff shall be trained on how to don and take off PPE using good aseptic technique.

**4.** Protective clothing designed to keep street clothes and forearms free of contamination must be worn when working in the laboratory. Protective clothing must never be worn outside the laboratory. Long sleeve, full-length lab coats are recommended. However, specific requirements are defined according to the Biosafety Level of the laboratory and those specified by the Institutional Biosafety Committee, if applicable.

**5.** Protective gloves must be worn when working with infectious material. Gloves shall be changed if damaged and removed before contact with clean surfaces, such as the telephone or doorknob. Disposable gloves shall be changed often because the integrity wanes with use. Wetting of the glove may also enhance permeability and should be avoided as much as possible. Two pairs of gloves must be worn at BSL2+ and BSL3 and whenever a spill is cleaned up, but is recommended at all times. Hands and wrists must be washed with soap and water as soon as gloves are removed.

**6.** Eye protection (safety glasses or goggles) must be worn. If a face mask is worn to prevent exposure to splash or splatter, then eye protection (safety glasses or goggles) must also be worn unless the facemask is impact resistant (ANSI Z87 Standard).

**7.** Surgical masks may be worn for product protection, not personal protection. Where personnel cannot be adequately protected via procedural or ventilation controls, respiratory protection may be required. Use of disposable respirators for personnel protection must follow procedures outlined in the University of Utah Respiratory Protection Program, see Section N.1.

**8.** Reusable PPE must be decontaminated (and then laundered, if applicable) periodically and whenever there is visible contamination.

## P. Documentation and Recordkeeping

### 1. Medical Recordkeeping

The University of Utah Occupational Medicine Clinic will establish and maintain an accurate record for each employee with occupational exposure, in accordance with 29 CFR 1910.20. The record shall

include:

1. The name and employee identification number of the employee.
2. 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.
3. A copy of all results of examinations, medical testing, and follow-up procedures required.
4. The copy of the healthcare professional's written opinion as required.
5. A copy of the information provided to the healthcare professional as required.

The University of Utah Occupational Medicine Clinic will ensure that employee medical records required are kept confidential and not disclosed or reported without the employee's express written consent to any person within or outside the workplace except as required by the standard or as may be required by law. The University of Utah Occupational Medicine Clinic will maintain the records required for at least the duration of employment plus thirty years in accordance with 29 CFR 1910.20.

**2. Sharps Injury Log**

The University of Utah is required to establish and maintain a sharps injury log (see Appendix D) 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 is maintained by each supervisor and a copy must be provided annually to OEHS Biosafety. The sharps injury log must contain the following information:

a. The type and brand of device involved in the incident.
b. The laboratory in which the exposure occurred.
c. An explanation of how the incident occurred and personnel involved.

**3. Documentation of Updated Safe Practices**

Consideration of changes in technology that reduce or eliminate exposure must be evaluated and documented annually, including solicitation of input from non-managerial staff.

**4. OSHA Recordkeeping**

Human resources will evaluate all incident reports to determine if cases meet OSHA's Recordkeeping Requirements (29 CFR 1904). All percutaneous injuries from contaminated sharps are also recorded in the Sharps Injury Log (Appendix D).

## Q. Biological Waste Disposal

This section describes procedures for the proper handling and disposal of biological waste from research, instructional, and clinical laboratories at the University of Utah. These procedures are based on state and federal law, requirements from the Occupational Safety and Health Administration (OSHA), Centers for Disease Control (CDC) and National Institutes of Health (NIH), and good laboratory practice. Failure to manage biological waste properly could result in personal injury, disruption to research, fines, or criminal prosecution.

Infectious waste is regulated by the Salt Lake City-County Health Department. The key requirements with regard to infectious waste are proper labeling with subsequent disposal in a safe manner. For waste that has not been decontaminated, incineration, burial at an infectious waste landfill or in some cases discharge into the sanitary sewer system are acceptable disposal procedures. Waste which has been autoclaved can be disposed of with regular garbage only if it is obviously marked "autoclaved", and all biohazard labeling defaced so appropriate disposal is not questioned. Contact the OEHS Associate Environmental Specialist at 801-581-6590 for specific instructions.

### 1. Biowaste Disposal – Solids

a. The Occupational and Environmental Health and Safety (OEHS) Lab Management System (LMS) allows research investigators to request hazardous material pickups by OEHS staff and request empty containers. Please visit the LMS website for information.

b. Waste containers obtained from OEHS are solid sided, leak proof, lined with red biohazard bags, and labeled with a biohazard symbol. Keep the container lid closed unless someone is working nearby and regularly adding waste to the container.

c. When the red bag is ¾ full, loosely tie or tape the bag closed. Secure the lid on the waste container and move it to a convenient storage location or transport it to a biohazardous waste storage room, if available. Biohazardous waste must be moved or transported inside a rigid, leak- resistant, labeled container with the lid closed. Request a pickup from your lab using the LMS.

d. If you have an autoclave available for disinfection of biohazardous waste, place a red biohazard bag in a solid puncture resistant container. Place a Ziploc bag or balloon containing water in the bag when it is about half full to generate steam during autoclaving. When the red bag is full, tie or tape the bag closed. Secure the lid on the waste container and move it to the autoclave room.

e. The bag shall be removed and placed in a solid autoclave resistant tray: the bag must **NEVER** be placed directly on the floor. After the cycle, the bag may be disposed of as regular trash: indicators that the contents have been autoclaved must be present.

2. **Biowaste Disposal – Liquids**

a. Blood, aspirated tissue culture media, or other liquid waste generated from BSL-2 enhanced experiments must be disinfected and then disposed. Bleach is typically used to disinfect liquids, but other agents, such as Wescodyne, may be used if effective.

b. If you use bleach:
- Ensure the final concentration exceeds 0.5% sodium hypochlorite (no less than one part bleach to 9 parts liquid).
- Ensure the bleach is fresh: in tissue culture media traps change at least twice weekly. Undiluted bleach shall be replaced every 6 months.
- Ensure the media is exposed to disinfectant for at least 20 minutes prior to disposal.
- Dispose down the sink

c. If you use Wescodyne:
- Ensure the final concentration exceeds 1% (no less than one part Wescodyne to 99 parts liquid).
- In tissue culture media traps change at least every 3 months (indicate the date of the last change on the flask). Check the expiration date on the disinfectant stock bottle.
- Ensure the media is exposed to disinfectant for at least 20 minutes prior to disposal.
- Collect waste into containers marked "Unwanted Materials" and date when you start collecting. When full or 6 months after your start date (whichever happens first), arrange pickup by OEHS through the LMS website. **NO DRAIN DISPOSAL.**
- If the container will be unattended (outside of your immediate control) then label it with the date, time and the words "Biohazardous liquid" and keep it in a secondary container (for example, a plastic tub) while it is disinfecting.

d. If you use other disinfectants to decontaminate liquid cultures follow the instructions on the packaging. Contact the Biosafety Officer (801-581-6590) for advice on appropriate disinfectants and procedures for disposal of treated waste.

e. Mixed liquid and solid waste must be separated in a biosafety cabinet (decant the liquid from the solid). Manage the liquids and solids separately as detailed above.

3. **Use and Disposal of Sharps**
- Do not recap needles by hand. RECAPPING OF NEEDLES IS PROHIBITED.
- Do not remove needles from syringes by hand.
- Do not bend, break, or otherwise manipulate needles by hand.
- Avoid using needles whenever possible.
- Replace glass materials with plastic (such as Pasteur pipettes)

- Immediately after use, discard needle and syringe (whether contaminated or not) into puncture resistant sharps containers. RECAPPING OF NEEDLES IS PROHIBITED.
- Use a Food and Drug Administration (FDA)-cleared sharps container if you generate sharps waste (pictured below). A description of FDA-Cleared Sharps containers can be found here. FDA-cleared sharps disposal containers are made from rigid plastic, come marked with a line that indicates when the container shall be considered full, which means it's time to dispose of the container, and have the Universal Biohazard symbol.

- Never discard sharps into regular trash.
- Never discard sharps into bags of biological waste.
- Use care and caution when cleaning up after procedures that require the use of syringes and needles.
- Do not overfill sharps containers. Close completely when 3/4 full, request pickup from the OEHS through the Lab Management System (LMS) webpage.
- Locate sharps containers in areas in which needles are commonly used. Make containers easily accessible.
- Replacement sharps containers may be obtained through the LMS or can be from laboratory supply distributors, such as VWR and ThermoFisher. Be sure to select sharps containers that withstand autoclaving.

### 4. Contaminated Serological Pipets and Pipet Tips

Serological pipets (glass and plastic) and disposable pipet tips are considered puncture hazards and shall be disposed of as sharps. Contaminated pipets and tips must be discarded in approved sharps containers, as described above.

Due to the large size of serological pipets, investigators disposing of large numbers of these can request 20 gallon hard-sided biohazard waste containers (pictured below) from OEHS through the LMS. These

will be picked up by OEHS staff as for other biohazardous waste.

**20 Gallon Waste Container**

**5. Decontaminated Serological Pipets and Pipet Tips**

It is possible to decontaminate serological pipets and tips prior to disposal. Ensure that both the inside and outside of the pipets or tips are exposed to the approved disinfectant (e.g. a freshly prepared 1:10 dilution of bleach) for at least 20 minutes. However, serological pipets and disposable tips are still considered puncture hazards. Therefore, after removing the disinfectant, they can be disposed of in a Broken Glass box (rigid puncture resistant boxes lined with a plastic bag and labeled "Broken Glass": pictured below), which can be obtained from your custodial staff or from OEHS. Once they are 2/3 full they shall closed with tape and disposed as regular trash by your custodians.

**Broken Glass Box**

## R. Disinfection and Sterilization

1. Frequently disinfect floors, cabinet tops, and equipment where biohazard material is stored.
2. Sterilize all infectious materials and contaminated equipment prior to being washed, stored, or discarded.
3. Use autoclave or disposable materials whenever possible. Keep reusable and disposable items separate.
4. Mark holding containers as "NON-INFECTIOUS - TO BE CLEANED" or "BIOHAZARDOUS - TO BE AUTOCLAVED"
5. Disinfectants-reduce the number of pathogenic organisms. See **Appendix E** for more details.
   - A. Alcohols: Ethyl or isopropyl alcohol at 70-80% concentration is a good all-purpose disinfectant. It is not effective against bacterial spores and non-enveloped viruses, such as adenoviruses or enteroviruses. Alcohol solutions **must not** be used to disinfect blood or other potentially infectious material, as defined in the OSHA bloodborne pathogen standard.
   - B. Phenolic compounds: Effective against vegetative bacteria, fungi, and lipid-containing viruses. Less effective versus spores. Unpleasant odor (e.g., Amphyl, Vesphene 2).
   - C. Formaldehyde: At a concentration of 5-8% formalin, good disinfectant properties against vegetative bacteria, spores, and viruses. Irritant sensitizer and animal carcinogen.
   - D. Quaternary Ammonium Compounds (QUATs): Acceptable as disinfectant to control vegetative bacteria and non-lipid-containing viruses.
   - E. Chlorine: Low concentration (50-500 ppm) active against vegetative bacteria and most viruses. Higher concentrations (≥2500 ppm) are required for bacterial spores. Strong irritant. Corrosive to metal surfaces. Must be made up fresh. 10 ml laundry bleach per liter of water yields approximately 525ppm, while 100ml bleach per liter of water (1:10 dilution) yields approximately 5250ppm. Stock bottles of bleach shall be replaced no later than 12 months after the date of manufacture, while dilutions of bleach shall be prepared fresh.
   - F. Iodofors: Recommended for general use (75-150 ppm). Effective against vegetative bacteria and viruses but poor activity against spores. Brown or yellow solution is still active. Wescodyne diluted 1 to 10 is popular disinfectant for washing hands.
   - G. Glutaraldehydes: Two percent solutions exhibit good activity against vegetative bacteria, spores, and viruses. Toxic and capable of eye damage (e.g., Cidex, Sporicidin, 3M Glutarex).

6. Sterilization

   A. Steam Heat. Required approximately 15 psi pressure with a chamber temperature of at least 250 °F (121 °C). The cycle time begins when the materials being sterilized reach the predetermined temperature. Then the length of time is dependent upon the volume size of the load (usually 30-60 min.). Monitor steam sterilization effectiveness with a biological indicator (e.g., Bacillus sterothermophilus).

   B. Dry Heat. Less effective than steam, and requires more time (two to four hours) and higher temperature (320-338 °F or 160-170 °C). Monitor effectiveness with biological indicator (e.g. Bacillus subtilis).

   C. Ethylene Oxide (EtO). Lethal for all known microorganisms and best for heat-resistant organisms or heat-sensitive equipment.

      1. Temperature affects the penetration of EtO through microbial cell wall and wrapping and/or packaging materials. The activity of EtO increases approximately 2.7 times for each 18 degrees F (10 °C) rise in temperature (between ranges of 41 and 98.6 °F or 5 and 37 °C) using a concentration of 884 mg/L. Normal temperature range is 120-140 °F (49-60 °C.)
      2. A concentration of 500-1000 mg/L at 120 to 140 °F (49-60 °C) is normally recommended.
      3. Humidity recommended between 30 and 60 percent.
      4. Exposure time is determined by the factors above. Follow the manufacturer's recommendation and monitor with biological indicators (e.g., Bacillus subtilis var. niger)
      5. Precautions. Mixtures of 3-10% EtO in air can be explosive. Commercially available mixtures of EtO in freon or carbon dioxide are not explosive. Personal exposures to EtO may result in harmful physical effects. The current permissible exposure for EtO is one ppm for an eight hour time weighted average. Concentrations may exceed 1000 ppm as the sterilizer door is opened for aeration. Local exhaust at the door opening and a 15 minute wait before removing the articles to the mechanical aeration chamber will minimize exposures.

7. Antiseptics. Formulated to be used on skin or tissue-not a disinfectant (e.g., Betadine, Clinedine, Hibiclens, etc).

## S. Biological Spill Kits

It is recommended that all Biological laboratories maintain Biological Spill Kits: they are required in all BSL2-enhanced or higher laboratories. The contents of the biological spill kit include:

- Bleach or other EPA-registered disinfectant: see Section R. Non-diluted bleach shall be replaced 1 year after purchase
- Biohazard bag
- Disposable lab coat
- Disposable shoe covers
- Hand sanitizing wipes
- Nitrile gloves (4 pair)
- Mini brush and dustpan (or something to scoop spilled materials)
- Paper towels or other absorbent material
- Safety goggles
- Tong or forceps to pick up broken glass
- Spray bottle (to make fresh bleach solution)
- Rigid, leak-proof container for sharps
- “Biohazard Spill” and “Do Not Enter” signs

## T. Spill Procedures

### 1. Spills of Biological Materials

All spills or breaks involving Recombinant DNA or Synthetic Nucleic Acid Molecules and hazardous biological materials must be cleaned up using appropriate biosafety procedures, described below. If there is any doubt about what to do, call the PI, or the Biosafety Officer 1-6590, or the University’s internal emergency number: 5-2677. Identify a disinfectant that is appropriate for the material that is to be cleaned up.

A. Spills inside of a Biosafety Cabinet
   a. Stop work.
   b. If you are splashed by the material, change PPE. Always change gloves.
   c. Keep the biosafety cabinet running.
   d. Contain the spill by covering with paper towels (to avoid splashes or aerosols).
   e. Saturate spill with disinfectant. Let sit for 20 minute exposure time.

        i. In the event of a spill into the drip pan/catch basin, add an equal volume of disinfectant and wait for 20 minutes to clean up the disinfected material.

f. Wipe up spill, disposing of towels in biohazard bag.
g. Wipe spill area with disinfectant.
h. Disinfect all materials used in the biosafety cabinet by wiping the surface with disinfectant. Do not attempt to disinfect contaminated cardboard or other paper items that absorb liquid: contaminated items must be disposed of.
i. If bleach or other corrosive disinfectant used, wipe spill area and disinfected equipment with water.
j. Place all towels or absorbent materials into a designated container for biohazardous waste.
k. Remove PPE, discard disposable PPE as biohazardous waste and wash hands.
l. Run the biosafety cabinet for 10 minutes to purge the air before re-starting work.

B. Spills outside of a Biosafety Cabinet

a. Stop work.
b. If you are splashed by the material, dispose of PPE and wash hands.
c. Ensure that any other people in the vicinity are notified that a spill has occurred and that the room be evacuated. Post a “Do Not Enter” notice on the door. Notify the PI or lab supervisor.
d. If you need assistance with the spill clean-up, call OEHS (1-6590).
e. Wait 30 minutes before re-entering the room to allow aerosols to settle.
f. Assemble Spill cleanup materials and don PPE, including lab coat, eye protection and/or face shield, 2 pair of gloves, shoe covers. If the lab coat does not have cuffed sleeves, disposable sleeve covers must be worn.
g. Contain the spill by covering with paper towels (to avoid splashes or aerosols)
h. Saturate spill with disinfectant pouring from the outside in. Avoid splashing or generating aerosols. Do not use alcohol for large spills.
    i. Wipe areas around the spill that may have splatter and any reusable equipment with disinfectant.
i. Let sit for 20 minute exposure time.
j. Wipe up spill, disposing of towels in biohazard bag: if sharps may be present use tongs or a brush and pan and dispose in biohazard sharps container.
    i. Work concentrically to clean up the absorbent material. Always work from the outer edge of the spill toward the center.
k. Wipe spill area with disinfectant.
l. Place all towels or absorbent materials into a designated container for biohazardous waste.

m. Remove PPE, discard disposable PPE as biohazardous waste and wash hands.
n. Remove the “Do Not Enter” sign and inform others that it is safe to re-enter the room.
o. Once the spill has been contained, complete the “**SPILLS OR EXPOSURE EVENT REPORTING PROCEDURE”** form (Appendix D) and have the PI send to OEHS.

C. Spills Inside of a Centrifuge Contained Within a Closed Cup, Bucket, or Rotor

a. Put on lab coat, gloves, and proper eye protection prior to opening centrifuge. Open carefully to assess the damage.
b. If the spill is contained within a closed cup, bucket, or rotor, spray the exterior with disinfectant and allow at least 20 minutes of contact time. Remove the carrier to the nearest biosafety cabinet (BSC).
    *i. Note, if possible, avoid using bleach on centrifuge rotors and buckets to avoid damaging the equipment. If bleach is used, ensure all surfaces are wiped down with soap and water after disinfection. Alternatively, use an EPA-registered disinfectant, such as Cidex or Cavicide.*
c. Gather supplies needed, such as a sharps container for broken glass and bins filled with disinfectant and place into the BSC.
d. Open the centrifuge rotor or bucket inside of the BSC. Use a mechanical device (forceps, tongs, etc.) to remove broken glass and place directly into sharps container. Carefully remove any unbroken tubes and place into a bin filled with the appropriate disinfectant for at least 20 minutes. Wipe carrier/bucket with disinfectant.
e. After disinfection, carrier, bucket, or rotor shall be washed with a mild soap and water.
f. Spray the interior of the centrifuge chamber with the appropriate disinfectant, let sit for at least 20 minutes and then wipe down.
g. Dispose of all clean-up materials (except sharps) in an appropriate biohazardous waste container. Dispose of sharps in a biohazard sharps container.
h. Remove PPE, discard disposable PPE as biohazardous waste and wash hands.

If you are concerned that the spill is not contained within the rotor or bucket:

i. Ensure that any other people in the vicinity are notified that a spill has occurred and the room be evacuated. Post a “Do Not Enter” notice on the door. Notify the PI or lab supervisor.
ii. If you need assistance with the spill clean-up, call OEHS (801-581-6590)
iii. Wait 60 minutes before re-entering the room to allow aerosols to settle.
iv. Proceed with clean up as described above.

**Note**: Many centrifuge rotors may be disinfected by autoclaving. Check the manufacturer’s instructions.

D. Exposure to skin or clothing
    a. Stop work.
    b. Take off contaminated clothing and wash affected area thoroughly with soap and water, but not so hard the skin is abraded.
    c. If necessary, exit lab area and immediately take a shower. Wash thoroughly with soap and water, but not so hard the skin is abraded.
    d. Notify lab supervisor or PI, who must notify the Biosafety Officer.
    e. If exposed to BSL-2/RG2 (or above) agent, proceed directly to RedMed Clinic, Redwood Occupational Medicine Clinic, or the University of Utah Hospital Emergency Room (if after 8pm).

E. Penetrating wound
    a. Stop Work.
    b. Wash immediately with soap and water.
    c. Notify lab supervisor or PI, who must notify the Biosafety Officer.
    d. Proceed directly to Redwood Occupational Medicine Clinic, RedMed Clinic or the University of Utah Hospital emergency Room (if after 8pm).

F. Eyes, or mucous membrane exposure
    a. Stop work.
    b. Immediately flush eyes or mucous membrane with water for 10-15 minutes.
    c. Notify lab supervisor or PI, who must notify the Biosafety Officer.
    d. Proceed directly to Redwood Occupational Medicine Clinic, RedMed Clinic or the University of Utah Hospital emergency Room (if after 8pm).

G. Emergency Spills: Environmental Risk
    a. Stop work.
    b. Ensure that any other people in the vicinity are notified that a spill has occurred and that the room be evacuated. Post a “Do Not Enter” notice on the door. Notify the PI or lab supervisor.
    c. Call OEHS (801-581-6590)
    d. Take appropriate precautions to limit exposure or spread of spill to other areas.

**NOTE: Spill Procedures must be clearly posted in the BSL-2 suite**

## 2. Spills of Biohazardous and Radioactive Material

Plan ahead. Contact the Radiation Safety Office at (801) 581-6141 regarding procedures that must be followed to prevent and mitigate spills of radioactive material. Develop a list containing the name, chemical form, and annual limit on intake (ALI) for all radioisotopes used in the laboratory. A spill involving material which is both a biohazard and radioactive hazard requires recovery procedures different from those appropriate for aqueous, low energy beta radiation emitters alone. Recovery from a spill requires consideration of the types or radionuclide, pathogenicity of the microorganism or its components, the chemical composition and volume of the spill. Spills involving carbon-14 and/or tritium present no external hazard. Good aseptic techniques will prevent internal radiation exposure to these nuclides and prevent personnel contamination with either the pathogen or the radioactive material.

However, higher energy beta or gamma radiation emitters and mixtures containing volatile radioisotopes may require additional protective measures. High temperature (autoclave or dry) and gas sterilization procedures involving radioisotopes must be approved in advance through the Radiation Safety Office.

When a spill occurs:

1. Notify others in the room. Avoid inhaling airborne material and quickly evacuate the area. Close door and post with a warning sign.
2. Remove contaminated clothing turning exposed side in upon itself. Place in a biohazard bag labeled with a radioactive material sticker.
3. Thoroughly wash all exposed skin with disinfectant. Rinse for three minutes, dry and monitor for residual radioactive contamination. If radioactive contamination remains, repeat the disinfection and decontamination procedure. Do not use harsh or abrasive cleansers on skin.
4. Inform the laboratory supervisor, notify the Radiological Health Department and monitor all potentially exposed personnel for radioactive contamination. Wait at least 30 minutes before reentering the laboratory to allow dissipation of aerosols created by the spill. During this time review cleanup procedures and assemble decontamination equipment.
5. Depending on the severity and virulence of the spill, dress in protective clothing (long sleeved gown, gloves, and shoe covers). It may be advisable to wear a respirator with high efficiency particulate air (HEPA) cartridges. However, use of respirators requires knowledge of their application and appropriate fitting before beginning recovery procedures.
6. Carefully lay disinfectant-soaked towels over the spill and pour disinfectant around the spill. To minimize aerosolization, do not pour disinfectant directly onto the

spill. Use more concentrated disinfectant if the volume of material will significantly dilute the disinfectant.

7. Allow 30 minutes contact time.
8. Use forceps to place sharp objects into a sharps container. Wipe surrounding surfaces with disinfectant to cover all splash areas. Wipe flat surfaces to remove any aerosol which may have settled out on those surfaces.
9. Place all contaminated materials, including protective clothing, into a disposable plastic container lined with a heavy plastic bag labeled with radioactive materials warning tape. Do not autoclave without approval from the Radiological Health Department. If it cannot be autoclaved, add additional disinfectant to ensure decontamination of all materials.
10. Following recovery efforts thoroughly wash all exposed skin with disinfectant. Rinse for three minutes, dry and monitor for residual radioactive contamination. If radioactive contamination remains, repeat the disinfection and decontamination procedure. Do not use harsh or abrasive cleansers on skin.
11. Allow time for thorough drying of all disinfected surfaces and then monitor the spill area for residual radioactive contamination. The presence of radioactivity on surfaces warrants repeated disinfection and decontamination efforts.

## U. Shipments

### 1. Background

A. The most current regulations affecting the transportation of Dangerous Goods will go in to effect January 1, 2013. For the purposes of the Principal Investigator conducting biomedical research, all potentially infectious substances come under classification of Dangerous Goods. Carriers (e.g., FedEx and UPS) are legally bound to reject any packages that are not in compliance with these regulations.

B. The specific requirements of the Dangerous Goods Regulations are outlined in the International Air Transport Association (IATA) tariffs and the International Civil Aviation Organization (ICAO) tariff. The following represents the relevant details of the regulations.

C. All biohazard materials shipped off campus must be packed in special packaging which meets UN 6.2” packaging standards. Some of the vendors providing appropriate packaging are VWR, Uline, or NU Packaging.

### 2. Training

A. Strict government regulations must be followed when transporting hazardous materials. An infectious substance is regulated as a hazardous material under the

U.S. Department of Transportation's (DOT) Hazardous Materials Regulations (HMR; 49 CFR Parts 171-180). Shipments must arrive at their destination in good condition and present no hazard during shipment. All individuals who package and ship category B infectious materials, potentially infectious materials and/or dry ice are required by federal law to complete Shipping Category B Infectious Substances and Dry Ice training every two years.

B. A Biological specimen, Category B (previously known as Clinical specimen and Diagnostic specimen), is an infectious substance that does not cause permanent disability or life-threatening or fatal disease to humans or animals when exposure to it occurs.

C. Category A materials are infectious substances that are transported in a form that is capable of causing permanent disability or life-threatening or fatal disease to otherwise healthy humans or animals when exposure to it occurs.

D. If you wish to ship a Category A substance or are unsure about the regulations concerning shipping a diagnostic or infectious specimen, please call the Biosafety Officer at 801-581-6590. Category A shipments have to be made by OEHS.

E. To read the regulations concerning the transport of a diagnostic or infectious specimen, see:

1. U.S. Department of Transportation, Office of Hazardous Materials Safety, 49 CFR Regulations 173.134.
2. International Air Transportation Association (IATA) Dangerous Goods information Online.

**3. Category B Infectious Substances, Exempt Materials, or Dry Ice shipments**

All shipment of this type are required to have the appropriate labels with the assigned UN numbers on the outer package.

A. Number 3373 is assigned to Category B Infectious Substances.

B. Number 1845 is assigned to dry ice shipments.

C. "Exempt" is assigned to shipments that do not contain a known infectious substance.

**4. Packaging**

Shippers of infectious and potentially infectious substances must comply with these regulations and must ensure that shipments are prepared in such a manner that they arrive at their destination in good condition, and that they present no hazards to persons or animals during shipment. The packaging must include both inner packaging and outer packaging.

A. Inner Packaging Specifications:

1. The primary receptacle must be leak-proof or sift-proof and must not contain

more than 1L.

2. Sufficient absorbent material must be placed around the primary receptacle to absorb its entire contents if a leak occurs.
3. Leak-proof secondary packaging will contain the primary receptacle and absorbent material.
    a. For liquid shipments by aircraft, the primary receptacle or secondary packaging must be capable of withstanding without leakage an internal pressure producing a pressure differential of not less than 95kPa.

B. Outer Packaging Specifications:

1. Packaging materials must be of sufficient strength to meet the design type test standards. Items must be placed in a rigid container that will not break if dropped from less than 1.2 meters.
2. Do not use bubble wrap as packing material.
3. An itemized list of contents must be enclosed between the secondary packaging and the outer packaging.
4. All packages containing infectious substances and/or dry ice must be marked durably and legibly on the outside of the package with the required labels and the name and phone number of a person responsible for the shipment.
5. Dry ice must never be shipped or placed in a sealed container.

**5. Physical Condition**

A. Substances shipped at or above ambient temperature:

1. Primary receptacle may only be of glass, metal, or plastic.
2. Must provide a positive means of ensuring a leakproof seal; e.g., heat seal, skirted stopper, metal crimp seal.
3. Screw caps must be reinforced with adhesive tape.

B. Substances shipped refrigerated or frozen:

1. Ice, wet or dry, must be placed outside the secondary packaging.
2. Interior support must be in place to secure secondary packaging in its original position after the ice has dissipated.
3. If wet ice is used; packaging must be leakproof.
4. If dry ice is used, the outer packaging must permit the release of carbon dioxide.

The primary and secondary packaging must maintain containment integrity at the temperature of the refrigerant used, as well as, the temperature and pressure ranges of air transport to which the receptacle could be subjected if refrigeration is lost.

C. Lyophilized substances

1. Primary receptacles must be either flame-sealed glass ampoules or rubber-stoppered glass vials with metal seals.

### 6. Importation and Exportation

A license may be required from the Department of Commerce to export certain biological agents. Please contact Biosafety Officer at (801)585-9325 regarding shipment of the following items:

A. Etiologic agents
B. Biological materials
C. Animals
D. Insects
E. Snails
F. Bats

### 7. Special handling Requirements

Certain etiologic agents require special handling. Most of these agents are Risk Group 3 or 4. They must be shipped by registered mail or an equivalent system which requires or provides for sending notification of receipt to the sender immediately upon delivery. Please contact Biosafety Officer at (801) 581-6590 regarding shipment of these items.

## V. Laundry

The University of Utah School of Medicine can be used to clean contaminated clothing and other articles that require laundering. Linen Services can be found in the AA120 level of the School of Medicine, 801-581-2200. Alternatively, there are laundry services that can clean contaminated lab coats, such as ALSCO, Cintas and Aramark.

The following laundering requirements must be met:

- Handle contaminated laundry as little as possible, with minimal agitation.
- Place wet contaminated laundry in leak-proof, labeled or color-coded containers before transport to the University Hospital Laundry.
- Contact outside providers for information on their transport requirements.

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This document has been developed from earlier University of Utah Biosafety Manuals and Standard Operating Procedures (SOP) documents, as well as Exposure Control Plans, Safety Manuals and SOPs developed at other Universities, including Arizona State University, Rutgers University, University of California Los Angeles, University of California Berkeley, University of California Irvine, University of Colorado, Duke University, Montana State University, University of Virginia and templates provided by the American Biological Safety Association.

# APPENDICES

## Appendix A: Guidelines for Working in a Type II Biological Safety Cabinet

Type II Biological Safety Cabinets (BSCs) are available for use in many laboratories at the University of Utah. Any work or task with a potential for splash or aerosol generation with infectious materials requires the use of a BSC or other appropriate containment device. Proper use of BSC includes:

1. Turn on the blower in the cabinet at least 10 minutes before placing infectious materials into the hood.

2. Check the certification sticker and all biosafety cabinet monitors to verify that the biosafety cabinet is working properly. Biosafety cabinets must be certified prior to use. A qualified outside contractor must certify these cabinets annually. Check the certification sticker on the front of the unit to verify your biosafety cabinet's condition. If the re-certification date has passed contact OEHS.

3. The biosafety cabinet air flow monitor must be checked to assure proper operation of the cabinet before placing any materials into it. Readings indicate relative pressure drop across the HEPA filter. Higher readings may, therefore, indicate filter clogging. Zero readings may indicate loss of filter integrity. In either of these cases, notify the Laboratory Manager or PI and OEHS. University of Utah Facilities Management do not perform maintenance on biological safety cabinets. If the BSC needs to be serviced, contact OEHS.

4. Gloves must be worn at all times.

5. Prior to beginning work, the BSC must be decontaminated. Don appropriate PPE (rear closing, fluid resistant lab coat, gloves, eye protection). Clean the inside surfaces of the BSC with appropriate EPA-registered disinfectant and follow with water (if using bleach). DO NOT put head inside the cabinet. To reach the back of the cabinet use an extension, such as a Swiffer handle.

6. Let blower run for 10 min to filter the cabinet air of any particulates.

7. DO NOT disrupt the airflow through the hood by placing ANY item on the grills or by opening the door to the corridor. Disrupting the airflow into the front grill allows contaminated air from inside the cabinet to blow into the lab or directly at the person sitting at the cabinet. It also allows non-sterile air from the room to blow into the biosafety cabinet over the experiments.

8. Organize the work surface for a clean-to-dirty work flow. Place clean pipets, flasks, and sterile media bottles at one side of the cabinet; place discard or kill pans containing disinfectant, biohazard waste containers, used flasks, spent cultures, and other wastes on the other side.

9. While working, keep all material and perform work at least 4 inches back from the front opening of the cabinet, and minimize rapid movements or activity.

10. In general, the interior of the hood shall be considered to be a contaminated zone, even though every effort is made to keep the surfaces clean, as is consistent with accepted good microbiological practice and sterile technique.

11. After manipulating infectious agents, make sure all containers are tightly closed.

12. Plastic pipettes with a cotton plug shall be used for pipetting liquids containing viral particles. The electric pipettor shall be fitted with a 0.2 µm filter to prevent aerosol-based contamination.

13. A beaker or discard pan, containing a freshly prepared 1:10 solution of commercial bleach, shall be placed inside the biosafety cabinet during the cell culture work.

14. After pipetting liquid containing viral particles, the dilute bleach solution in the beaker shall be pipetted up and down the full length of the pipette or left in the pan. Serological pipettes and tips must be placed in a in a puncture resistant sharps container or other approved receptacle.

15. After decontamination, pipette tips shall be removed from the pipettor and temporarily left in the beaker containing bleach in the biosafety cabinet.

16. At the completion of the work, all materials to be removed from the biosafety cabinet must be decontaminated.

17. At the completion of the work, the beaker containing the plastic tip pipettes shall be removed from the biosafety cabinet. Pipettes tips shall be lifted out of the beaker, the bleach solution allowed to drain back into the beaker, and the pipette tips placed in a puncture resistant sharps container or other approved receptacle. NOTE plastic pipette tips and serological pipettes are treated as sharps.

18. Small volumes of liquid waste containing viral particles shall be collected in a beaker containing undiluted bleach inside the biosafety cabinet. The final concentration of bleach shall be at least

10% of the final volume. After completing work, wait at least 30 minutes before disposing down the drain.

19. Large volumes shall be collected by vacuum aspiration into a flask containing an appropriate disinfectant, such as Wescodyne or bleach, up to 1 or 10% of the volume of the flask, respectively. **NOTE: No untreated or non-disinfected biological agent-containing material must be allowed into any drain connected to the sanitary sewer system (e.g., from a sink).**

    i. Bleach in the vacuum traps must be changed at least twice per week or when the flask is half full. Wait at least 20 minutes after finishing work to discard waste.
    **ii.** Wescodyne is more stable and can be used for up to 3 months before it is discarded. Wescodyne-treated waste must be transferred to a sealed container marked "Unwanted materials". When full or 6 months after your start date (whichever happens first), arrange pickup by OEHS through the LMS website. **NO DRAIN DISPOSAL.**
    iii. *The flask must be placed in a secondary container to prevent it from tipping over, be labeled with a biohazard sticker and the vacuum line must be protected by a hydrophobic (HEPA) filter. The vacuum filters must be replaced if clogged or if liquid makes contact with the filter. Examples include Whatman Vacu-guard and Pall Gelman Vacushield in-line disk filters. Used filters must be placed in the biohazard waste.*

20. Turn off the house vacuum when not in use.

21. Clean the inside surfaces of the BSC with an EPA-registered disinfectant after completion of work, and follow with water (if using bleach).

22. Allow the blower to run for at least 10 minutes following use.

23. If UV lights are used, the UV light is turned on between procedures (at least 30 minutes). UV lights must be turned off before work begins in the hood. **Do not look directly at UV lights as this can cause eye damage**.

    *During decontamination, a sign shall be placed stating that the biosafety cabinet is being decontaminated and shall not be used. Also, it shall be stated that the user (with contact information) shall be contacted if there are any questions or concerns.*

    *UV light is effective only for decontaminating clean, solid surfaces with which it comes in contact. It is not effective in decontaminating the cabinet air flow. UV light is not effective against bacterial spores. UV germicidal light tubes are cleaned regularly and be replaced*

*frequently (at least every 6 months for biosafety cabinets in use on a daily basis) to assure that they are emitting light at 254 nm and at an intensity appropriate for decontamination.* ***Due to concerns over the effectiveness of these lights and the risks to individuals in the room, some Institutions, such as the NIH, have banned their use in BSCs. The University of Utah strongly discourages the use of UV lights in BSCs.***

NOTE: Any use of volatile solvents, such as ethanol, shall be kept to a minimum or done elsewhere. **Dangerously high levels of volatile vapors can accumulate inside the cabinet and pose a threat of fire or explosion.**

**NOTE:** *Be very careful when using small pieces of materials in the BSC as they can be blown into the grilles and disrupt the motor operations.*

Annual certification of the BSC confirms that it will provide the user and experimental material the protection for which it is designed. The airflow, filters, and cabinet integrity are checked to ensure that the cabinet meets minimum performance standards. Certification and decontamination are arranged through OEHS and provided by an outside vendor. A sticker on the BSC will list when certification is due. If certification is past due, please contact OEHS.

BSCs intended for research with biohazardous materials must be certified:

- After they are received and installed (before use with infectious materials).
- After filter changes.
- After being moved (even a few feet).
- Annually.
- By an NSF-certified technician.

BSC decontamination (e.g., using a peroxide gas process) must be provided and needs to be done:

- Before any maintenance work requiring disassembly of the air plenum, including filter replacement.
- Prior to cabinet recertification.
- Before moving the cabinet to a new laboratory.
- Before discarding or salvaging.
- By an NSF-certified technician.

Note: all maintenance work inside of the biosafety cabinet must be performed by an NSF-certified technician. Work on the exterior of the cabinet, such as connecting vacuum or gas lines can be performed by University of Utah Facilities. Please contact OEHS (801-581-6590) prior to having any work performed on the BSC.

**Open Flames (Bunsen burners) in Biosafety Cabinets**

Open flames, including the use of Bunsen burners, are not allowed to be used in Biological Safety cabinets (BSCs) at the University of Utah. Early microbiologists had to rely on open flames to ensure sterility. However, with the advancement of modern technology, including the introduction of the BSC, the use of an open flame is almost always no longer necessary. Alternative options include:

- Use disposable sterile loops and sterile lab supplies. This eliminates the need to use open flames for sterilizing.
- Autoclave utensils and equipment prior to use. Place loops, spreaders, needles, forceps, scalpels and other tools in autoclavable plastic or wrap in autoclavable foil.
- Use a Bacti-Cinerator to sterilize loops and needles safely and conveniently while preventing infectious spatter and cross-contamination.
- The Electrical Bunsen Burner combines the efficiency of a gas burner with the safety and control of an electric heater. It is ideal for sterilizing inoculating needles and loops, and for heating small flasks, test tubes, and beakers.
- The Glass Bead Sterilizer provides a safe, effective, and convenient method for sterilizing small instruments without using flames, gases, or chemicals.
- If it is deemed absolutely necessary for the experiment being done, use a pilotless burner or touch-plate microburner (Touch-O-Matic) to provide a flame on demand.

The use of Bunsen burners inside of a BSC is not recommended because it:

- Disrupts airflow, compromising the protection of the worker and the product. The Class II BSC maintains product protection through delivery of laminar flow (air volumes traveling in a single direction at a constant speed – without turbulence) down over the work area of the cabinet. The heating of air from the Bunsen burner causes up-flow of air that mixes with the down flowing airstreams to produce turbulence and recirculation within the working area. The notion of laminar flow may be completely destroyed and any aerosols generated beneath the burner may be carried to other parts of the cabinet, jeopardizing the product and personnel working within the cabinet.
- Causes excessive heat build-up within the cabinet. As most Class II BSCs recirculate the majority of the air within the cabinet, heat from the Bunsen burner builds up over time. The excessive heat can inactivate and degrade components in media such as vitamins, amino acids and growth factors, possibly below the threshold for finicky cell lines. In

addition, the excessive heat may make it an uncomfortable environment for the worker, leading to errors and mistakes.

- May damage the HEPA filter or melt the adhesive holding the filter together, compromising the cabinet's integrity. An open flame has the capacity for melting the bonding agent that holds the HEPA filter media to its frame. This destroys the HEPA filters effectiveness, leading to loss of containment in the positive pressure plenum. ENV will charge $250 for decontamination of the cabinet, $250-$1000 for the filter, and $145 for recertification each time the HEPA filter needs to be replaced.
- Presents a potential fire or explosion within the cabinet. The cabinets are not constructed to be explosion proof. If the flame was to go out, there was a leak, or the valve was not shut off completely, flammable gas would be introduced to the cabinet at a steady rate. In the case of a Class II A2, where 70% of the air in the BSC is recirculated, concentrations of the flammable gas could reach explosive potential and pose a serious risk to not only the cabinet, but to the user and the laboratory it is occupied in. Electrical components like the fan motor, lights, or electrical outlets could ignite a flash fire with a spark in this volatile environment. Manufacturers often post their cabinets with warning labels stating that flammable materials must not be used in the cabinet.
- Inactivates manufacturer's warranties on the cabinet. Biological safety cabinet manufactures are opposed to the practice and will assume no liability in the event of fire, explosion or worker exposure due to the use of a flammable gas in their cabinet.
- Automatically voids UL approval. Underwriters Laboratories Inc. (UL) is an OSHA approved independent product safety certification organization that develops standards and test procedures for products, materials, components, assemblies, tools and equipment, chiefly dealing with product safety. The use of a Bunsen burner in the cabinet will void UL approval of that piece of equipment.
- Requires hook-up of central gas source. Some laboratories may not be fitted with gas lines and will require costly room renovations for retrofitting. Facilities will also need to install plumbing from the house lines to the cabinet. If the cabinet needs to be moved, this will incur additional costs from Facilities. In addition, gas connectors are generally not supplied with new biosafety cabinets without customer insistence and at an additional cost.

Quotes on Open Flames in BSCs:

NIH/CDC: National Institutes of Health and the Centers for Disease Control and Prevention (Appendix A of the BMBL): "Open flames are not required in the near microbe-free environment of a biological safety cabinet. On an open bench, flaming the neck of a culture vessel will create an upward air current which prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates

turbulence which disrupts the pattern of HEPA-filtered air supplied to the work surface. When deemed absolutely necessary, touchplate microburners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized. The burner must be turned off when work is completed. Small electric "furnaces" are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable or recyclable sterile loops can also be used."

WHO: World Health Organization's Laboratory Biosafety Manual: "Open flames should be avoided in the near microbe-free environment created inside the BSC. They disrupt the airflow patterns and can be dangerous when volatile, flammable substances are also used. To sterilize bacteriological loops, micro-burners or electric "furnaces" are available and are preferable to open flames."

Public Health Agency of Canada; The Laboratory Biosafety Guidelines: "The provision of natural gas to BSC's is not recommended. Open flames in the BSC create turbulence, disrupt airflow patterns and can damage the HEPA filter. When suitable alternatives (e.g., disposable sterile loops, micro-incinerators) are not possible, touch-plate micro-burners that have a pilot light to provide a flame on demand may be used."

NSF/ANSI Standard 49 – 2009 published by NSF International, Annex G; Section G.3.3.1: "Service valves allow inert gases, air, or vacuum lines to be plumbed into the BSC. Although many users connect gas to a service valve in the cabinet, this practice should be avoided if possible, because open flames in a Class II BSC disrupts the airflow, and there is the possibility of a buildup of flammable gas in BSC's that recirculate their air."

**Reference:** *Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health.*

## Appendix B: Guidelines for Working with Recombinant Viral Vectors

The following provides information on the use and containment of recombinant viral vectors. Investigators must use these guidelines as part of their risk assessment when planning experiments with these vectors and preparing applications to the Institutional Biosafety Committee (IBC). Note the listed containment levels are the minimum that may be employed with these vectors: some experiments, such as the expression of toxins or oncogenes, may require higher levels of containment. The appropriateness of the containment must be considered as part of the investigator's risk assessment and will be reviewed by the IBC.

| **Lentivirus** | |
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| NIH Risk Group | RG2<br>Lentiviruses are a subset of retroviruses, which are simple, enveloped single-stranded RNA viruses. |
| Biocontainment Level | The Recombinant DNA Advisory Committee of the NIH Office of Biotechnology Activities issued a report that reviewed biosafety issues relating to lentivirus vectors. This report advised that reduced biosafety level containment was appropriate in the laboratory setting for research involving the use of advanced lentivirus vector systems that 1) separated vector and packaging functions onto multiple plasmids, 2) were produced at laboratory scale quantities, and 3) lacked expression of oncogenic transgenes. They specifically recommended that 4-plasmid systems that met specific criteria could be used at BSL-2 without the need to assay for replication competent virus (RCV).<br><br>BSL-2 enhanced:<br>*Oncogenic transgenes*<br>Lentivirus vectors that incorporate transgenes with oncogenic potential must be generated and used at BSL-2 enhanced containment regardless of whether second or third generation systems are used.<br><br>*Scale of production*<br>Lentivirus vectors made at a level of production > 100 ml volume must be generated and used at BSL-2 enhanced containment regardless of whether second or third generation systems are used. |

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| | BSL-2 enhanced<br>Second generation or 3-plasmid lentivirus systems shall be generated and used at BSL-2 enhanced. These systems generally have one packaging plasmid, which includes all the important packaging components: Gag, Pol, Rev, and Tat, an envelope plasmid and the transfer vector. In general, lentiviral transfer vectors with a wildtype 5' LTR need the 2nd generation packaging system because these vectors require TAT for activation.<br><br>The investigator may request a downgrade in biosafety level to BSL-2 following demonstration that virus preparations have no detectable RCV based on results of an accepted RCV assay as described below. A protocol modification requesting reduction in biosafety level and including data from the RCV test must be submitted to *and* approved by the IBC before any BSL-2 or ABSL-2 work can be performed.<br><br>BSL-2<br>Third generation or 4-plasmid system vectors may be generated and used at BSL-2, as may second generation lentivirus systems that use a self-inactivating vector (see below) The 4 plasmids of the third generation system include 2 packaging plasmids, an envelope plasmid, and a transfer plasmid. 3rd generation packaging system offers maximal biosafety but require the transfection of four different plasmids into the producer cells.<br>The main differences in the 3rd generation system are as follows:<br>The Tat gene has been eliminated from the packaging completely<br>Rev is expressed on a separate plasmid<br>The 5'LTR of the transfer plasmid has been modified to include a conventional promoter and the U3 region of the 3’LTR has been deleted. This is termed a self-inactivating (SIN) vector and can be packaged by both 2nd and 3rd generation packaging systems. |

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| | The potential for generation of RCV from HIV-1 based lentivirus vectors depends upon several factors, the most important of which are:<br>The number of recombination events necessary to reassemble a replication competent virus genome<br>The number of essential genes that have been deleted from the vector/packaging system.<br><br>Earlier vector systems (such as two-plasmid vector systems) may have a higher potential for generation of RCV. The IBC does not require testing for RCV when 4-plasmid (third generation) systems are used or when a SIN vector is used with a $2^{nd}$ generation packaging system (see IBC guidelines for RCV testing). |
| Infectious to Humans/Animals | Yes |
| Route of Transmission | Lentiviruses are transmitted via direct exposure to infected bodily fluids, sexual contact, sharing unclean needles. Lentiviruses may persist lifelong due to their ability to integrate into the host chromosome and ability to evade host immunity. Lentiviruses replicate, mutate and undergo selection by host immune responses. |
| Laboratory Hazards | Risks include direct contact with skin and mucous membranes of the eye, nose and mouth, parenteral inoculation, ingestion. |
| Disease | The clinical manifestation of HIV infection includes non-specific symptoms such as lymphadenopathy, anorexia, chronic diarrhea, weight loss, fever, and fatigue. Can cause severe immunologic and neurological disease in hosts. The major risks associated with lentiviral vectors are insertional mutagenesis and local inflammation. |
| Treatment/Prophylaxis | NRT inhibitors, Protease inhibitors |
| Pathogenesis | Local inflammation. Insertional mutagenesis. Can infect non-dividing cells including immune cells. Can infect non-target cells. Can persist lifelong. High mutation rates. Inappropriate expression of gene product. Rescue by other human pathogenic viruses |
| Replication Competent | Possible |
| RCV Testing | Can be performed by the investigator using a standard p24 ELISA kit providing the assay has a sensitivity of < 12.5 pg/ml. A positive control for virus infection is not required; the IBC does not want the investigator to |

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| | work with infectious HIV-1 for this assay. However, the assay must contain a positive control for the ELISA itself in the form of p24 antigen.<br><br>Virus shall be tested for RCV by serial passage of tissue culture supernatant on 293T cells for 3 passages with subsequent testing of supernatant from each passage for p24 antigen by ELISA.<br><br>Investigators who are not generating their own viruses from 2 or 3-plasmid system but are acquiring already constructed virus stocks from a commercial source that has documentation filed with the IBC of acceptable RCV testing will not be required to test for RCV. |
| Disinfection | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:<br>RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach)<br>5% Phenol<br>70% Ethanol or Isopropanol |
| Animals | ABSL-2: When animals are infected with lentiviral vectors, the Animal Biosafety Level of the project will be generally assigned to ABSL-2: the use of vectors where RCV may be generated requires ABSL-2-enhanced. Animals must be injected in a Biological Safety Cabinet.<br><br>Infected animals can excrete lentivirus, so cages and bedding are considered biohazardous for a minimum of 72 hours post-exposure (replication incompetent vectors). Take precautions to avoid creating aerosols when emptying animal waste material. Soiled cages are disinfected prior to washing.<br><br>Animal cages must be labeled with a biohazard sign.<br><br>ABSL2 to ABSL1 stepdown: When using third or later generation vectors, or RCV testing is negative, the IBC may approve ABSL stepdown. On the fourth day following infection, animals injected with replication incompetent vectors can be transferred to ABSL-1 standard conditions. The animals will be transferred to a clean cage, and the ABSL-2 cage will stay in the ABSL-2 quarantine space for appropriate waste disposal and |

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| | cleaning. Once animals have been transferred to ABSL-1, they can be handled as with other ABSL-1 animals. However, for rodents that contain any human cells or tissues, step down to BSL1 will generally not be allowed: determined by IBC.<br><br>ABSL-2 or ABSL-1 for xenografts of transduced human/animal cells. Determined by IBC. |

| Adenovirus | |
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| NIH Risk Group | RG2<br>Adenoviruses are non-enveloped icosahedral viruses containing double-stranded DNA. |
| Biocontainment Level | BSL-2.<br>1st Generation: Deletion of regions E1, E3 genes (less safe)<br><br>2nd Generation: Deletion of regions E1, E2, E3, E4 genes (more safe)<br><br>Expression of oncogenes or toxins may raise BSL containment requirements |
| Infectious to Humans/Animals | Yes |
| Route of Transmission | Wild-type adenoviruses are spread directly by oral contact and droplets. They are indirectly spread by handkerchiefs, eating utensils and other articles freshly soiled with respiratory discharge of an infected person. It is possible for a person who is infected, but asymptomatic, to shed virus for many months or years. |
| Laboratory Hazards | Inhalation of aerosolized droplets, mucous membrane contact, parenteral inoculation, or ingestion.<br><br>Adenovirus is unusually stable in the environment. Adenovirus can still be infective after having been extracted with ether and/or chloroform. |
| Disease | Apart from respiratory involvement, illnesses and presentations of adenovirus include gastroenteritis, conjunctivitis, cystitis, and rash illness. Symptoms of respiratory illness caused by adenovirus infection range from the common cold syndrome to pneumonia, croup, and bronchitis. Patients with compromised immune systems are especially susceptible to severe complications of adenovirus infection.<br><br>*Pharyngoconjunctival fever* is a specific presentation of adenovirus infection:<br>high fever that lasts 4–5 days<br>pharyngitis (sore throat) |

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| | conjunctivitis (inflamed eyes, usually without pus formation like pink eye)<br>enlargement of the lymph nodes of the neck<br>headache, malaise, and weakness<br>Incubation period of 5–9 days<br><br>Replication-defective recombinant adenoviral vectors have caused corneal and conjunctival damage. |
| Treatment/Prophylaxis | Most infections are mild and require no therapy or only symptomatic<br><br>Treatment/Prophylaxis. Because there is no virus-specific therapy, serious adenovirus illness can be managed only by treating symptoms and complications of the infection. |
| Pathogenesis | Can infect a variety of non-dividing cells. Stays episomal (does not integrate) |
| Replication Competent | Possible |
| RCV Testing | The probability of producing replication competent virus (RCV), although low, increases with each successive amplification. RCA is produced when adenoviral DNA recombines with E1-containing genomic DNA in HEK 293 cells. It is suggested to use early amplification stocks when needed to produce additional quantities of adenovirus. RCV testing is recommended for 1st generation vectors. PCR for E1 prior to use or plate on non-susceptible cell types |
| Disinfection | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:<br>RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach)<br>5% Phenol<br><br>Note: Alcohol is NOT an effective disinfectant against non-enveloped viruses such as adenovirus. |
| Animals | ABSL-2: When animals are infected with adenoviruses/adenoviral vectors, the Animal Biosafety Level of the project will be generally assigned to ABSL-2.<br><br>Animals must be injected in a Biological Safety Cabinet. Infected animals can excrete adenovirus, so |

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| | cages and bedding are considered biohazardous for a minimum of 5 days post-exposure (replication incompetent vectors). Take precautions to avoid creating aerosols when emptying animal waste material: adenovirus is excreted by animals. Soiled cages are disinfected prior to washing.<br><br>Animal cages must be labeled with a biohazard sign.<br><br>ABSL2 to ABSL1 stepdown: When using third or later generation vectors, or RCV testing is negative, the IBC may approve ABSL stepdown. After 5 days animals can be transferred to ABSL-1 standard conditions. The animals will be transferred to a clean cage, and the ABSL-2 cage will stay in the ABSL-2 quarantine space for appropriate waste disposal and cleaning. Once animals have been transferred to ABSL-1, they can be used handled as with other ABSL-1 animals.<br><br>For first generation vectors or infection of animals containing human cells or tissues, ABSL-2 containment may be required for longer periods. This will be determined by the IBC.<br><br>ABSL-2 or ABSL-1 for xenografts of transduced human/animal cells. Determined by IBC. |

| Adeno-Associated Virus | |
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| NIH Risk Group | RG1<br>AAV are non-enveloped icosahedral viruses with a single stranded DNA genome. |
| Biocontainment Level | BSL-1; unless it encodes oncogene/toxin or helper virus present (BSL-2) |
| Infectious to Humans/Animals | Yes (Humans/Primates) |
| Route of Transmission | AAV may be transmitted through direct contact with an infected individual or through indirect contact with the contaminated environment.<br>Transmission routes include respiratory, gastrointestinal and possibly sexual transmission.<br>A concern for vertical transmission from mother to fetus also exists.<br>Most adults (85-90% in the US) are seropositive for AAV and about 30% have neutralizing antibodies. |
| Laboratory Hazards | Inhalation of aerosolized droplets, mucous membrane contact, parenteral injection, or ingestion. |
| Disease | AAV is not associated with any human disease; however, there is evidence of AAV infection in the human embryo and an association of AAV with male infertility.<br>A significant correlation was found between the presence of AAV DNA in amnion fluids and premature amniorrhexis (rupture of the amnion) and premature labor.<br>Recombinant AAV vectors lose site specific integration into chromosome 19, thereby raising the theoretical concern of insertional mutagenesis. |
| Treatment/Prophylaxis | Supportive care. No specific Treatment/Prophylaxis |
| Pathogenesis | Infects multiple cell types. May be associated with insertional mutagenesis and cancer. WT AAV inserts itself on human chromosome 19 and remains latent. Can be potentially reactivated later in the presence of a helper virus and produce infection. Shown to cause insertional mutagenesis in murine cell lines.<br><br>High doses have resulted in neurotoxic effects in primates. |
| Replication Competent | Only in presence of helper virus (CMV, adenovirus, herpesvirus, vaccinia) |
| RCV Testing | If helper virus is adenovirus, test for presence of RCV after heat inactivation (56°C for 15min) |

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| Disinfection | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:<br>RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach)<br>Alkaline solutions at pH >9.<br>5% phenol.<br><br>Note: Alcohol is NOT an effective disinfectant against non-enveloped viruses, such as AAV. |
| Animals | ABSL-1: If helper virus is used follow rules for that virus. In general, ABSL-2 will be required if a helper virus used or if host animal could house helper virus: animals must be injected in a Biological Safety Cabinet. 72 hours following infection, animals can be transferred to ABSL-1 standard conditions. The animals will be transferred to a clean cage, and the ABSL-2 cage will stay in the ABSL-2 quarantine space for appropriate waste disposal and cleaning. Once animals have been transferred to ABSL-1, they can be used handled as with other ABSL-1 animals.<br><br>Special handling of bedding and cages for 48 hours post injection. Bedding disposed in biohazardous waste.<br><br>Animal cages at ABSL-1 need not be labeled with a biohazard sign. |

| Murine Retroviruses, such as Moloney Murine Leukemia Virus: (MoMuLV/MMLV) or Mouse Mammary Tumor Virus (MMTV) | |
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| NIH Risk Group | RG1 (ecotropic)<br>RG2 (Others: amphotropic or pseudotyped)<br>MMLV is a member of the gammaretroviruses and MMTV is a beta retroviruses genera. Both are enveloped, icosahedral, diploid viruses with a single-stranded, linear RNA genome. MMLV integrates into the host genome and is present in infected cells as a DNA provirus. Cell division is required for infection. |
| Biocontainment Level | BSL-1 (ecotropic)<br>BSL-2 (Others: amphotropic or pseudotyped) |
| Infectious to Humans/Animals | Possible if amphotropic or pseudotyped |
| Route of Transmission | Bloodborne |
| Laboratory Hazards | In mice, virus is transmitted via blood from infected mother to offspring; may also occur via germline infection.<br><br>In vivo infection in humans appears to require direct parenteral injection with amphotropic or pseudotyped MLV. However, contact with feces or urine from transduced animals for 72 hours post infection or with tissues and body fluids of transduced animals must be avoided. |
| Disease | Cell transformation and tumor formation |
| Treatment/Prophylaxis | None |
| Pathogenesis | Insertional mutagenesis possible, leading to cell transformation/tumor formation. Amphotropic Env gene or pseudotyped viruses can infect non-murine cells including human cells |
| Replication Competent | Yes |
| RCV Testing | Use permissive cell line (*Mus dunni*); screen by marker rescue assay (PG-4S+L-). In general no RCV testing for 3rd generation or later vector systems: determined by IBC. |
| Disinfection | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:<br>RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach)<br>5% Phenol<br>70% Ethanol or Isopropanol |
| Animals | ABSL-1: Ecotropic replication incompetent murine retroviruses |

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| | ABSL-2: Amphotrophic or pseudotyped murine retroviruses must be handled at ABSL-2 for at least 72-hours post administration. Animals must be injected in a Biological Safety Cabinet. Infected animals can excrete retrovirus, so cages and bedding are considered biohazardous for a minimum of 72 hours post-exposure (replication incompetent vectors). Take precautions to avoid creating aerosols when emptying animal waste material. Soiled cages are disinfected prior to washing.<br><br>Animal cages must be labeled with a biohazard sign.<br><br>For rodents that do not or will not contain any human cells or tissues, on the fourth day following infection, animals injected with replication incompetent vectors can be transferred to ABSL-1 standard conditions. The animals will be transferred to a clean cage, and the ABSL-2 cage will stay in the ABSL-2 quarantine space for appropriate waste disposal and cleaning. Once animals have been transferred to ABSL-1, they can be used handled as with other ABSL-1 animals.<br><br>IBC may require RCV testing for viruses to be administered at ABSL1 or studies where containment is reduced after administration.<br><br>ABSL-2 or ABSL-1 for xenografts of transduced human/animal cells. Determined by IBC. |

| Herpes Simplex Virus | |
|---|---|
| NIH Risk Group | RG2<br>Herpesviruses are enveloped, icosahedral, double-stranded linear DNA viruses. |
| Biocontainment Level | BSL-2 |
| Infectious to Humans/Animals | Yes |
| Route of Transmission | HSV-1 is typically transmitted by saliva or by the infection on hands of healthcare personnel. HSV-2 is typically transmitted through sexual contact. HSV can be transmitted by direct contact with epithelial or mucosal surfaces. |
| Laboratory Hazards | In the laboratory, HSV can be transmitted by ingestion, parenteral injection, droplet exposure of the mucous membranes (eyes, nose or mouth), and inhalation of aerosolized materials. |
| Disease | Depends on type:<br>Oral Herpes<br>Genital Warts<br>Herpes esophagitis<br>Herpes encephalitis or meningitis |
| Treatment/Prophylaxis | Antivirals may reduce shedding |
| Pathogenesis | After infection, the viruses are transported along sensory nerves to the nerve cell bodies, where they reside lifelong. Causes of recurrence may include: decreased immune function, stress, and sunlight exposure. The first episode is often more severe and may be associated with fever, muscle pains, swollen lymph nodes and headaches. Over time, episodes of active disease decrease in frequency and severity |
| Replication Competent | All versions of HSV vectors are prone to recombination. Additionally, approximately 50% - 90% of adults possess antibodies to HSV type 1; 20% - 30% of adults possess antibodies to HSV type 2. This is a concern since reactivation from latency is not well understood. Infection by HSV vectors into latently infected cells could potentially reactivate the wild-type virus, or spontaneous reactivation of a latent infection could produce an environment where replication defective vectors could replicate. |
| RCV Testing | Viral preparations used for *in vitro* studies must be tested every 6 months for replication competent |

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|---|---|
| | viruses by plaque assay. These assays must be tested at a sensitivity limit of 1 infectious unit per mL. |
| Disinfection | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:<br>RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach)<br>5% Phenol<br>70% Ethanol or Isopropanol |
| Animals | ABSL-2: Animals will be maintained at ABSL-2 for the duration of the study. Animals must be injected in a Biological Safety Cabinet. All bedding, waste and animals infected with HSV shall be treated as biohazardous. After all animals are removed from their primary enclosure immediately autoclave or treat with chemical disinfectant. After disinfection, dump the cage contents and begin cleaning the cage for re-use. All waste must be decontaminated by autoclaving or chemical disinfection prior to disposal. Animal carcasses must be placed in autoclave bags and be designated for infectious waste disposal. All necropsies must be performed in a designated room using animal BSL-2 practices and procedures.<br><br>Animal cages must be labeled with a biohazard sign. |

| Sendai Virus | |
|---|---|
| NIH Risk Group | RG2 for human paramyxoviruses. |
| Biocontainment Level | BSL-2<br>Sendai virus (SeV) causes respiratory disease in rodents and sometimes swine. There is limited evidence of zoonotic transmission to humans. However, the virus is capable of infecting human cell lines and is similar to human parainfluenza virus type 1. For these reasons, SeV work is usually classified as BSL-2.<br><br>Recombinant constructs expressing oncogenes or toxins must be handled at BSL-2 enhanced |
| Infectious to Humans/Animals | Mice |
| Route of Transmission | SeV is responsible for a highly transmissible respiratory tract infection in mice, hamsters, guinea pigs, rats, and occasionally pigs, with infection passing through both air and direct contact routes. |
| Laboratory Hazards | No reported cases of laboratory acquired disease but inhalation of aerosolized droplets, mucous membrane contact, parenteral inoculation, or ingestion are possible routes of infection. |
| Disease | Respiratory disease. Infections of mice are usually associated with a high mortality rate although latent infections can occur. |
| Treatment/Prophylaxis | Antivirals may reduce shedding |
| Pathogenesis | The respiratory infection of Sendai virus in mice is acute. Virus may first be detected in the lungs 48 to 72 hours following exposure. As the virus replicates in the respiratory tract of an infected mouse, the concentration of the virus grows most quickly during the third day of infection. After that, the growth of the virus is slower but consistent. Typically, the peak concentration of the virus is on the sixth or seventh day, and rapid decline follows that by the ninth day. A fairly vigorous immune response mounted against the virus is the cause of this decline. |
| Replication Competent | Yes |
| RCV Testing | No |
| Disinfection | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following: |

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| | RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach)<br>5% Phenol<br>70% Ethanol or Isopropanol |
| Animals | ABSL-3: Animal cages must be labeled with a biohazard sign. Note there are currently no ABSL-3 suites at the University of Utah. |

| Vaccinia Virus | |
|---|---|
| NIH Risk Group | RG2<br>The poxviruses are the largest known DNA viruses and are distinguished from other viruses by their ability to replicate entirely in the cytoplasm of infected cells. Vaccinia is an enveloped double-stranded DNA virus that is highly stable and can cause severe infections in immunocompromised persons, persons with certain underlying skin conditions, or pregnant women. |
| Biocontainment Level | The biocontainment level of the vector is based on CDC criteria for the parental virus strain. Containment level is established by the IBC.<br><br>BSL-1<br>Vectors derived from highly attenuated strains including MVA (Ankara strain) TROVAC (fowlpox) and ALVAC (canarypox) strains that do not replicate in human cells, and NYVAC (derived from the Copenhagen strain) that replicates poorly in human cells: if these strains are used in work areas where NO other orthopoxviruses are manipulated.<br><br>BSL-2<br>Non-attenuated vaccinia strains, such as NYCBOH (the strain used in the vaccinia vaccine), Western Reserve (WR), Copenhagen, Temple of Heaven, Lister or Cowpox. |
| Infectious to Humans/Animals | Yes |
| Route of Transmission | Vaccinia virus may be transmitted via surface contact with contaminated object(s) and subsequently spread to mucus membranes (eyes, nose, and mouth) and/or to open sores on skin. |
| Laboratory Hazards | Accidental needlestick is a mode of transmission within research laboratories. Accidental ingestion of viral contaminated materials and inhalation are other routs of transmission. If working with infectious animal models, then bite wounds could transmit vaccinia virus infection. |
| Disease | Infection of the skin can cause a localized lesion that then scabs over and heals in about 10-14 days. |
| Treatment/Prophylaxis | Vaccination is not recommended for persons who work only with replication-deficient poxvirus strains (e.g., MVA, NYVAC, TROVAC, and ALVAC). |

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| | The CDC recommends vaccination every 10 years for laboratory workers in the United States who have any contact with replication-competent vaccinia viruses and recombinant viruses developed from replication-competent vaccinia viruses. However, individuals who are pregnant; breastfeeding; have skin conditions such as eczema or atopic dermatitis; those with heart disease; or those with altered immune systems, are at increased risk from the vaccine, and must not be vaccinated and not work with the virus.<br><br>The vaccination can be accompanied by fever, rash, lymphadenopathy, fatigue, myalgia and headaches. Serious complications such as ocular vaccinia, myopericarditis, eczema vaccinatum (a papular, vesicular and pustular rash that is very infectious), progressive vaccinia (progressive necrosis at the vaccination site), postvaccinial CNS disease (headache, lethargy, seizures and coma), fetus malformations and abortion (very rare) sometimes occur after vaccination. Complications are more serious in immunosuppressed individuals and the smallpox vaccine usually causes one death for every million doses.<br><br>https://www.cdc.gov/mmwr/volumes/65/wr/mm6510a2.htm |
| Replication Competent | Yes |
| RCV Testing | NA |
| Disinfection | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:<br>RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach)<br>70% Ethanol or Isopropanol |
| Animals | ABSL-2: Animals must be injected in a Biological Safety Cabinet. Animals will be maintained at ABSL-2 for the duration of the study. All bedding, waste and animals infected with vaccinia shall be treated as biohazardous. After all animals are removed from their primary enclosure immediately autoclave or treat with chemical disinfectant. After disinfection, dump the cage contents and begin cleaning the cage for re-use. All waste must be decontaminated by autoclaving or chemical disinfection prior to disposal. Animal carcasses must be placed in autoclave bags and be designated for infectious waste disposal. All necropsies must be performed in a designated room using animal BSL-2 practices and procedures. |

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| | Animal cages must be labeled with a biohazard sign. |

| **Rabies Virus** | |
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| NIH Risk Group | RG2<br>Rabies virus is a member of the Rhabdoviridae family and is a common zoonotic infection from bats and other wild mammals. Rabies is an enveloped, single-stranded, negative sense RNA virus.<br><br>Replication-deficient rabies vectors can be useful tools for investigation into neuronal trafficking or targeted expression in neurons. SADΔG-mCherry/EnvASADΔG is an example of a modified rabies virus. This modified version of the rabies virus forces neurons it infects to produce a red fluorescent protein called mCherry. mCherry makes the infected cells glow red so they are visible under a microscope. The benefit is the ability to trace a neural circuit on the cellular level as only connected/attached neurons are affected. Initial deletion: This modification deletes a gene which encodes the rabies virus envelope B19- glycoprotein (RG) and which is required for the production of competent or infectious viral particles from the virus genome in transduced cells. As a result, the mutant virus cannot spread to any other surrounding cells from the originally infected cells. If the B19-glycoprotein is over-expressed as a transgene in a defined group of infected cells, the virus can trans-synaptically transport to adjacent cells only (single-step) and never go beyond.<br><br>The tropism of the viral vector may also be changed so that it cannot infect any mammalian cells except those that express a genetically-specified neuronal population transgene that encodes the envelope receptor. Examples of this include EnvA, VSV-g, avian sarcoma leucosis virus glycoprotein, or HIV env. EnvA pseudotyped virus can only infect cells expressing the complementary receptor TVA. Since mammalian neurons do not express TVA, the injected virus cannot infect wild-type human neurons. If the virus is able to infect a TVA-positive neuron (for example, in transgenic mice), it can replicate and strongly label the first-order (initially infected) neurons, but since its |

| | |
|---|---|
| | genome lacks the B19 glycoprotein, it cannot infect other neurons by itself. In short, the risk for infection is specified by transgene expression and retrograde transport is limited to a single synapse. Thus the resultant virus becomes a “mono-synaptic” transneuronal tracer and significantly reduces the biohazardous risk because the virus has no potential to infect or trans-synaptically transport to any mammalian cells, including human and mice.<br><br>Since the rabies virus is a negative-strand RNA virus, it does not integrate into the cell genome and has no chance to produce a G protein RNA template. Therefore, there is essentially no risk to generate replication competent rabies virus with this vector. |
| Biocontainment Level | BSL-2 |
| Infectious to Humans/Animals | Yes |
| Route of Transmission | Percutaneous injury, such as animal bites. Potential non-bite modes of transmission include contamination of a pre-existing wound, contact of mucous membrane or respiratory tract with the saliva of an infected animal, exposure to aerosolised rabies virus in the laboratory (or from bats), or via organ transplantation from an infected donor, or inhalation of droplets |
| Laboratory Hazards | Accidental needlestick is a mode of transmission within research laboratories. Accidental ingestion of viral contaminated materials and inhalation are other routs of transmission. If working with infectious animal models, then bite wounds could transmit rabies virus infection. |
| Disease | Rabies virus can cause an acute infection, marked by progressive encephalomyelitis, and is usually fatal. The initial symptoms of rabies resemble those of other systemic viral infections, including fever, headache, malaise, and upper respiratory and gastrointestinal tract disorders. This prodromal phase typically lasts about 4 days, but can last as long as 10 days before specific symptoms develop. |
| Treatment/Prophylaxis | Consultation is available to determine if vaccination is appropriate for personnel working with recombinant rabies vectors. Vaccination is not needed for working with SAD B19 vaccine strain. |

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| | Post-exposure rabies prophylaxis with vaccines together with the administration of rabies immunoglobulin (RIG) is highly effective but is a medical urgency. There is no established treatment for wild-type rabies once symptoms have begun, but supportive therapy may include intubation, sedation, mechanical ventilation, fluid and electrolyte management, and nutrition. |
| Replication Competent | Usually no but depends on pseudotyping and expression of envelope protein |
| RCV Testing | No effective methods for RCV testing |
| Disinfection | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:<br>RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach)<br>5% Phenol<br>70% Ethanol or Isopropanol |
| Animals | ABSL-2. |

| Epstein Barr Virus | |
|---|---|
| NIH Risk Group | RG2<br>Epstein-Barr virus, frequently referred to as EBV, is a member of the herpesvirus family and one of the most common human viruses. EBV are enveloped, icosahedral viruses with a double stranded linear DNA genome. |
| Biocontainment Level | BSL-2 |
| Infectious to Humans/Animals | Yes |
| Route of Transmission | Ingestion, accidental parenteral injection, droplet exposure of the mucous membranes, inhalation of concentrated aerosolized materials. |
| Laboratory Hazards | Accidental needlestick is a mode of transmission within research laboratories. Accidental ingestion of viral contaminated materials and inhalation are other routs of transmission. Note that cell lines are often immortalized by transformation with EBV. |
| Disease | The virus is found worldwide, and most people become infected with EBV sometime during their lives, most commonly causing infectious mononucleosis - acute viral syndrome with fever, sore throat, splenomegaly and lymphadenopathy. A few carriers of this virus may develop Burkitt's lymphoma or nasopharyngeal carcinoma. EBV is a transforming virus and is often used to produce immortalized cell lines and cause lymphoma in various animal models. |
| Treatment/Prophylaxis | No specific treatment |
| Replication Competent | Usually no but there is the potential for recombination with a latent viral infection. |
| Disinfection | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:<br>RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach)<br>5% Phenol<br>70% Ethanol or Isopropanol |
| Animals | ABSL-2: Animals must be injected in a Biological Safety Cabinet. Animals will be maintained at ABSL-2 for the duration of the study. All bedding, waste and animals infected with EBV shall be treated as biohazardous. After all animals are removed from their primary enclosure immediately autoclave or treat with chemical disinfectant. After disinfection, dump the cage contents and begin cleaning the cage for re-use. |

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| | All waste must be decontaminated by autoclaving or chemical disinfection prior to disposal. Animal carcasses must be placed in autoclave bags and be designated for infectious waste disposal. All necropsies must be performed in a designated room using animal BSL-2 practices and procedures.<br><br>Animal cages must be labeled with a biohazard sign. |

| Baculovirus | |
|---|---|
| NIH Risk Group | NA<br>Baculoviruses are non-mammalian enveloped, circular DNA viruses that infect insects. |
| Biocontainment Level | BSL1<br><br>Containment levels may be raised per IBC review if the vector is amphotropic and can infect human cells or can achieve expression of an oncogene or biological toxin in mammalian cells. |
| Infectious to Humans/Animals | Generally, non-genetically modified wild type baculoviruses are not capable of replicating in vertebrate cells |
| Route of Transmission | NA |
| Laboratory Hazards | Direct contact, droplet exposure of the mucous membrane, direct injection<br><br>Since they are not capable of replicating in vertebrate cells they do not pose any inherent hazards to laboratory workers. However, more recent studies with the use of mammalian specific promoters have achieved expression of foreign genes in a wide variety of mammalian cell lines and primary cell cultures. |
| Disinfection | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:<br>RECOMMENDED: Sodium hypochlorite (1.0%: use 1:5 dilution of fresh bleach)<br>70% Ethanol or Isopropanol |
| Animals | ABSL1 |

## Appendix C: Risk Assessments for Biological Agents

Complete for each of the agents/viral vectors in the laboratory

| **Date:** | |
|---|---|
| **PI Name:** | |
| **Name of Agent:** | |
| **Mode(s) of Transmission of Parental Agent (e.g. for recombinant lentivirus vectors describe modes of transmission of HIV):** | |
| **Signs and Symptoms of Exposure/Infection with this Agent (e.g. for recombinant lentivirus vectors describe signs and symptoms of exposure/infection with HIV):** | |

| Assessment of Additional Risks | | |
|---|---|---|
| | Yes | No |
| Is this agent replication-defective? | | |
| Could the agent integrate into the genome? | | |
| Does the agent encode a transgene that could lead to an increased risk of oncogenesis?<br>e.g. Does the transgene encode an oncogene?<br>Could the transgene inactivate a tumor suppressor? | | |
| Has the agent been modified to alter drug resistance?<br>If yes, explain: | | |
| Is the agent propagated in cell lines? | | |
| a. If yes, are the cell lines human or primate? | | |
| b. If yes, are there any hazards associated with the cells? | | |

| Vaccination and Treatment Options | | |
|---|---|---|
| | Yes | No |
| Is there a Vaccine available to protect against this agent? | | |
| If yes, have personnel been offered vaccination? | | |
| Are there Post Exposure Prophylaxis options for this agent?<br><br>If yes, describe: | | |

## Appendix D: Spills or Exposure Event Reporting Procedure

Any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses must be reported to the IBC so that a report can be sent to the NIH Office of Science Policy (OSP) within 30 days. Certain types of accidents must be reported on a more expedited basis. Spills or accidents in BL2 laboratories resulting in an overt exposure must be immediately reported to the IBC and OSP. Spills or accidents occurring in high containment (BL3 or BL4) laboratories resulting in an overt or potential exposure must be immediately reported to OSP.

Any spill or accident involving recombinant or synthetic DNA research of the nature described in the previous paragraph or that otherwise leads to personal injury or illness or to a breach of containment must be reported to the IBC and OSP. These kinds of events might include skin punctures with needles containing recombinant or synthetic DNA, the escape or improper disposition of a transgenic animal, or spills of high-risk recombinant materials occurring outside of a biosafety cabinet. Failure to adhere to the containment and biosafety practices articulated in the *NIH Guidelines* must also be reported to IBC and OSP.

In addition, exposure to Infectious Agents or Other Potentially Infected Material must be reported to the IBC.

Minor spills of low-risk agents not involving a breach of containment that were properly cleaned and decontaminated generally do not need to be reported. If the investigator, or other institutional staff are uncertain whether the nature or severity of the incident warrants reporting, contact the Biosafety Officer who can assist in making this determination, with guidance from OSP, if necessary.

**Please complete the form below and submit to the Biosafety Officer (801-581-6590).**

**SPILL OR EXPOSURE EVENT REPORT**

If a spill or exposure to Recombinant DNA or Synthetic Nucleic Acid Molecules, Infectious Agents Biological Toxins or Other Potentially Infected Material occurs in your laboratory, please complete the following information and provide to the Biosafety Officer at biosafety@ehs.utah.edu. This will serve as a record of the event and be used for NIH OSP reporting, if necessary.

| **Incident Investigation Report** | | | |
|---|---|---|---|
| **Instructions: The supervisor of the employee is requested to complete this form thoroughly within 24 hours after the event, although some investigations may take longer. If you have questions contact OEHS at 1-6590** | | | |
| Employee Name: | | Date of Incident: | |
| Employee Job Title: | | Date Reported: | |
| Employee Dept.: | | Investigation Date: | |
| Supervisor Name: | | Building: | |
| Supervisor Job Title: | | Room #: | |
| Injury Type (strain, cut, etc.): | | Body Part: | |
| | | | |
| **Preliminary Root Cause Analysis For Consideration (check all that apply)** | | | |
| **Contributing Actions** | | **Contributing Conditions** | |
| Use of safety devices | Recapped needle | Housekeeping | Exposure |
| Use of PPE | Material Handling | Condition of surface | Noise |
| Equipment | Use of tools | Ergonomic issue | Chemicals |
| Appropriate equipment use | Warning method | Guards/barriers | Fire/explosion hazard |
| Procedural issues | Type of clothing | Tools/equipment | Radiation |
| Speed of | Authorization | Tools/Equipment not | Sharp object |
| Lifting technique | Awareness | Lighting/Temp/Ventilation | Inclement weather |
| Operator skill | Lost balance | Work area | Training |
| **Other:** | | | |
| **Root Cause Narrative: Based on your analysis, please describe what caused this incident. (If more in-depth analysis is needed, use the 5-Why process below).** | | | |
| **5-Why Root Cause Analysis** | | | |
| By repeatedly asking the question "Why" (five is a good rule of thumb), you can peel away the layers of symptoms which can lead to the root cause of a problem. Example: Someone slipped and fell. (the problem)<br>1.Why? - The floor was wet. (first why)<br>2.Why? - The weather was bad and people tracked snow into the building. (second why)<br>3. Why? - The floor tile was not slip-resistant and did not absorb moisture. (third why)<br>4.Why? - The floor mats that are normally put out during bad weather were not put down. (fourth why)<br>5.Why? - The person that puts out floor mats during bad weather was absent that day and no one assumed | | | |

| Why 1: |
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| Why 2: |
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| Why 3: |
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| Why 4: |
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| Why 5: |
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**Possible Corrective Actions For Consideration (check those items that will help prevent recurrence)**

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|---|---|---|---|
| Isolate & guard the hazard | Procedure change | Gloves | Hard hat |
| Automate a manual process | Safety training | Respirator | Face shield |
| Design out/remove hazard | Add signs/warning labels | Safety glasses | Cut/Puncture resistant clothing |
| Ventilation | Improve housekeeping | Safety shoes | |
| | New/different tools/equip | Hearing protection | Lab Coat |

| Other: |
|---|
| |

| **Incident Description:** Please provide a detailed description of the incident. If possible, have the employee re-create the incident; including who, what, when, where, and why. If more space is needed use blank pages for additional description. Attach photos, if appropriate. | |
|---|---|
| | |
| Agent (Recombinant Lentivirus, recombinant adenovirus, etc); | |
| Gene encoded by vector: | |
| Known Oncogene or predicted to increase risk of oncogenesis (Y or N): | |
| Amount exposed (pfu, µl, etc: if known): | |
| Did the affected individuals need medical treatment and, if so, where were they treated? | |
| Narrative of post exposure/spill response (please describe disinfectant agents, washing of exposed sites, PPE worn during clean up, etc.) and whether the employee visited an Occupational Medicine clinic | |

| For work involving recombinant or synthetic nucleic acids: | |
|---|---|
| Is the work NIH funded? | |
| If yes, please provide the grant number, NIH funding institute or center, NIH program officer contact information (name, email etc): | |

If the incident involves a sharps injury, complete a sharps injury log:

# Sharps Injury Log

**Laboratory Name:________________** **Year 2_____**

| Date | Case/ Report # | Type of Device (e.g. syringe, suture needle) | Brand Name of Device | Work Area where injury occurred (e.g. Geriatrics, Lab) | Brief description of how incident occurred (i.e., procedure being done, action being performed (disposal, injection, etc.), body part injured) |
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29 CRF 1910.1030, OSHA's Bloodborne Pathogens Standard, in paragraph (h)(5), requires an employer to establish and maintain a Sharps Injury Log for recording all percutaneous injuries in a facility occurring from contaminated sharps. The purpose of the Log is to aid in the evaluations of devices being used in healthcare and other facilities and to identify problem devices or procedures requiring additional attention or review. This log must be kept in addition to the injury and illness log required by 29 CFR 1904. The Sharps Injury Log must include all sharps injuries occurring in a calendar year. The Log must be retained for five years following the end of the year to which it relates. The Log must be kept in a manner that preserves the confidentiality of the affected employee.

# Appendix E: Chemical Disinfectants

In the laboratory setting, chemical disinfection is the most common method employed to decontaminate surfaces and disinfect waste liquids. In most laboratories, dilutions of household bleach is the preferred method but there are many alternatives that may be considered and could be more appropriate for some agents or situations. There are numerous commercially available products that have been approved by the Environmental Protection Agency (EPA). Selected EPA Registered Sterilizers, Tuberculocides, and Antimicrobial Products Against Certain Human Public Health Bacteria and Viruses can be found at https://www.epa.gov/pesticide-registration/selected-epa-registered-disinfectants. Most EPA-registered disinfectants have a 10-minute label claim. **However, OEHS Biosafety recommends a 15-20 minute contact time for disinfection/decontamination.**

Prior to using a chemical disinfectant always consult the manufacturer's instructions to determine the efficacy of the disinfectant against the biohazards in your lab and be sure to allow for sufficient contact time. In addition, consult the Safety Data Sheet for information regarding hazards, the appropriate protective equipment for handling the disinfectant and disposal of disinfected treated materials. Federal law requires all applicable label instructions on EPA-registered products to be followed (e.g., use-dilution, shelf life, storage, material compatibility, safe use, and disposal). Do not attempt to use a chemical disinfectant for a purpose for which it was not designed.

When choosing a disinfectant consider the following:

- The microorganisms present
- The item to be disinfected or surface(s)
- Corrosivity or hazards associated with the chemicals in the disinfectant
- Ease of use

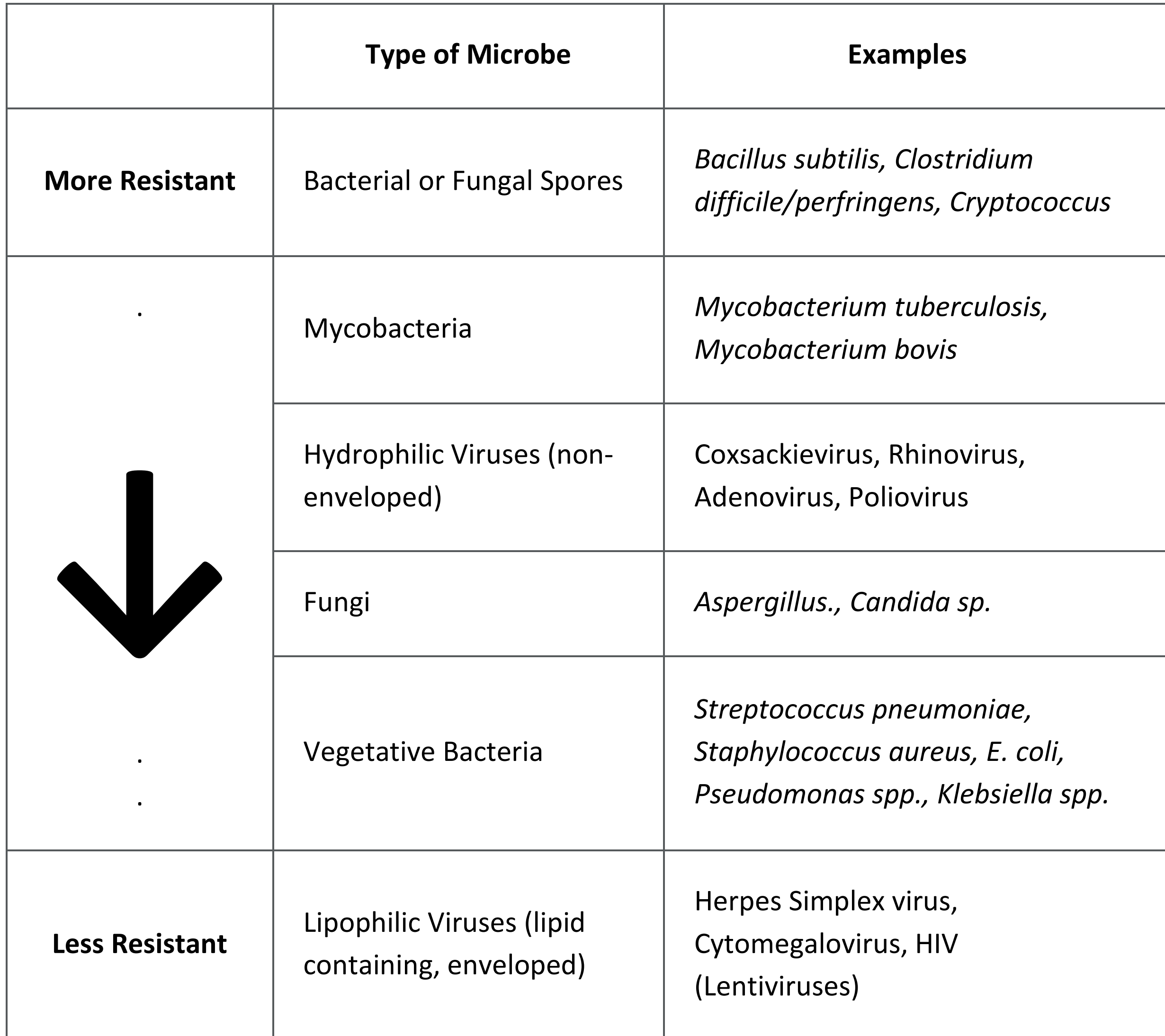
The OSHA Bloodborne Pathogen standard CFR 1910.1030 requires an EPA-registered disinfectant effective against HIV-1 and Hepatitis B virus. Therefore, diluted ethanol or isopropanol may not be used to disinfect materials and surface contaminated by human or non-human primate blood or other potentially infectious material (OPIM), as defined in the standard. However, alcohol-based disinfectants, such as Cavicide are registered by the EPA as virucidal and tuberculocidal.

## 1) Organism Sensitivity and Resistant Organisms

The innate characteristics of microorganisms often determine its sensitivity to chemical disinfection (**Table 9**). Some agents such as *Cryptosporidium*, *Clostridium difficile*, *Bacillus* spores and prions are very resistant to the usual disinfectants. OEHS Biosafety is available to assist you in determining the appropriate disinfectant and provides guidance on use of appropriate disinfection techniques and

materials for researchers.

**Table 9. Sensitivity of Microorganisms to Chemical Disinfectants**



| | Type of Microbe | Examples |
|---|---|---|
| **More Resistant** | Bacterial or Fungal Spores | *Bacillus subtilis, Clostridium difficile/perfringens, Cryptococcus* |
| . | Mycobacteria | *Mycobacterium tuberculosis, Mycobacterium bovis* |
| ↓ | Hydrophilic Viruses (non-enveloped) | Coxsackievirus, Rhinovirus, Adenovirus, Poliovirus |
| | Fungi | *Aspergillus., Candida sp.* |
| . . | Vegetative Bacteria | *Streptococcus pneumoniae, Staphylococcus aureus, E. coli, Pseudomonas spp., Klebsiella spp.* |
| **Less Resistant** | Lipophilic Viruses (lipid containing, enveloped) | Herpes Simplex virus, Cytomegalovirus, HIV (Lentiviruses) |

**2) Chemical Disinfectant Groups (Table 10)**

a. Halogen-Based Biocides: (Chlorine Compounds and Iodophores)

i. Chlorine Compounds (e.g., Household Bleach)

Chlorine compounds are good disinfectants on clean surfaces, but are quickly inactivated by organic matter, thus, reducing their biocidal activity. They have a broad spectrum of

antimicrobial activity and are inexpensive and fast acting. Hypochlorites, the most widely used of the chlorine disinfectants, are available in liquid (e.g., Sodium hypochlorite), household bleach and solid (e.g., calcium hypochlorite, sodium dichloroisocyanurate) forms. Household bleach has an available chlorine content of 5.25%, or 52,500 ppm. For most purposes, **a 1:10 dilution of bleach** (approximately 0.5% or 5,000 ppm sodium hypochlorite) is recommended in the laboratory. Because of its oxidizing power, diluted bleach loses potency quickly and **must be made fresh** and used within the same day it is prepared. **Bleach must be diluted with cold water in order to prevent breakdown of the disinfectant.** The free available chlorine levels of hypochlorite solutions in both opened and closed polyethylene containers are reduced to 40% to 50% of the original concentration over a period of one month at room temperature. Bleach must be stored between 50 and 70°F. Undiluted household bleach has a shelf life of six months to one year from the date of manufacture, after which it degrades at a rate of 20% each year until totally degraded to salt and water, and a **1:10 bleach solution has a shelf life of 24 hours**.

There are two potential occupational exposure hazards when using hypochlorite solutions. The first is the production of the carcinogen bis-chloromethyl ether when hypochlorite solutions come in contact with formaldehyde. The second is the rapid production of chlorine gas when hypochlorite solutions are mixed with an acid. Care must also be exercised in using chlorine–based disinfectants, which can corrode or damage metal, rubber, and other susceptible surfaces. Bleached articles must never be autoclaved without reducing the bleach with sodium thiosulfate or sodium bisulfate.

Chloramine T, which is prepared from sodium hypochlorite and p-toluenesulfonamide, is a more stable, odorless, less corrosive form of chlorine but has decreased biocidal activity in comparison to bleach.

ii. Iodophors (e.g. Wescodyne)

Iodophors are used both as antiseptics and disinfectants, typically at a concentration of 25-1600 ppm of titratable iodine: for Wescodyne the recommended final concentration is 75 to 150ppm. Wescodyne, Betadyne, Povidone-Iodine and other iodophors are commercially available Iodine-based disinfectants, which give good control when the manufacturer's instructions for formulation and application are followed. **Iodophors must be diluted in cold water in order to prevent breakdown of the disinfectant.**

An iodophor is a combination of iodine and a solubilizing agent or carrier; the resulting complex provides a sustained-release reservoir of iodine and releases small amounts of free iodine in aqueous solution. Antiseptic iodophors are not suitable for use as hard-surface disinfectants

because they contain significantly less free iodine than do those formulated as disinfectants.

b. Alcohols (ethanol and isopropanol)

Alcohols work through the disruption of cellular membranes, solubilization of lipids, and denaturation of proteins by acting directly on S-H functional groups. Ethyl and isopropyl alcohols are the two most widely used alcohols for their biocidal activity. These alcohols are effective against lipid-containing viruses and a broad spectrum of bacterial species, but ineffective against spore-forming bacteria and many non-enveloped viruses. They evaporate rapidly, which makes extended contact times difficult to achieve unless the items are immersed.

The optimum bactericidal concentration for ethanol and isopropanol is in the range of 70% to 85% by volume. Their cidal activity drops sharply when diluted below 50% concentration. Absolute alcohol is also not very effective. They are used to clean sensitive equipment and are generally regarded as being non-corrosive.

Due to the evaporative nature of the solution, aqueous alcohol is not recommended as the primary disinfectant of spills, especially in areas with significant airflow, such as a Biosafety cabinet. For surface decontamination, a spray, wipe, spray approach is recommended to achieve the desired contact time.

EPA-registered alcohol based disinfectants, such as Cavicide, are appropriate for surface decontamination.

c. Aldehydes: (Formaldehyde, Paraformaldehyde, Glutaraldehyde, Ortho-Phthalaldehyde)

i. Glutaraldehyde:

Glutaraldehyde is a colorless liquid and has the sharp, pungent odor typical of all aldehydes, with an odor threshold of 0.04 parts per million (ppm). It is capable of sterilizing equipment, though to effect sterilization often requires many hours of exposure. Two percent solutions of glutaraldehyde exhibit very good activity against vegetative bacteria, spores and viruses. It is ten times more effective than formaldehyde and less toxic. However, it must be limited and controlled because of its toxic properties and hazards. It is important to avoid skin contact with glutaraldehyde as it has been documented to cause skin sensitization. Glutaraldehyde is also an inhalation hazard. The NIOSH ceiling threshold limit value is 0.2 ppm.

Cidex, a commercially prepared glutaraldehyde disinfectant is used routinely for cold surface

sterilization of clinical instruments. Glutaraldehyde disinfectants must always be used in accordance with the manufacturer's directions.

Due to its exposure hazards, US healthcare associations advocate the use of glutaraldehyde alternatives such as o-phthalaldehyde, hydrogen peroxide and peracetic acid.

ii. Ortho-phthalaldehyde

Ortho-phthalaldehyde (OPA) has been accepted as a better, safer alternative to glutaraldehyde in most US healthcare facilities. Cidex OPA by Advanced Sterilization Products (a Johnson & Johnson company) was cleared by the US FDA as a high-level disinfectant and emerged as a suitable replacement of glutaraldehyde for the disinfection of endoscopes.

OPA has excellent microbiocidal activity and superior mycobactericidal activity compared with glutaraldehyde, and has potent bactericidal and sporicidal activity. Like glutaraldehyde, it interacts with amino acids, proteins and microorganisms.

OPA has many advantages over glutaraldehyde, such as improved stability at varying pH ranges, lower inhalation exposure risk and a wider range of material compatibility, although it costs almost three times more; but, considering the cost of the sophisticated ventilation systems needed to minimise the respiratory hazards of using glutaraldehyde, OPA is more economical.

iii. Formaldehyde:

Fomaldehyde and its polymerized solid paraformaldehyde have broad-spectrum biocidal activity and are both effective for surface and space decontamination. As a liquid (5% concentration), formaldehyde is an effective liquid decontaminant. Its biocidal action is through alkylation of carboxyl, hydroxyl and sulfhydryl groups on proteins and the ring nitrogen atoms of purine bases. Formaldehyde's drawbacks are reduction in efficacy at refrigeration temperature, its pungent, irritating odor, and several safety concerns. Formaldehyde is presently considered to be a carcinogen or a cancer-suspect agent according to several regulatory agencies. The OSHA 8-hour time-weighted exposure limit is 0.75 ppm.

d. Quaternary Ammonium Compounds: (Zephirin, CDQ, A-3)

Quaternary ammonium compounds are generally odorless, colorless, nonirritating, and deodorizing. They also have some detergent action, and they are good disinfectants. However, some quaternary

ammonium compounds activity is reduced in the presence of some soaps or soap residues, detergents, acids and heavy organic matter loads. They are generally ineffective against viruses, spores and *Mycobacterium tuberculosis*. Basically these compounds are not suitable for any type of terminal disinfection. They are typically diluted to 0.1 to 2%.

The mode of action of these compounds is through inactivation of energy producing enzymes, denaturation of essential cell proteins, and disruption of the cell membrane. Many of these compounds are better used in water baths, incubators, and other applications where halide or phenolic residues are not desired.

e. Phenolics: (O-phenophenoate-base Compounds)

Phenolics are phenol (carbolic acid) derivatives and typically used at 1- 5% dilutions. These biocides act through membrane damage and are effective against enveloped viruses, rickettsiae, fungi and vegetative bacteria. They also retain more activity in the presence of organic material than other disinfectants. Cresols, hexachlorophene, alkyl- and chloro derivatives and diphenyls are more active than phenol itself. Available commercial products include Lysol, Pine-Sol, Amphyl, O-Syl, Tergisyl, Vesphene, and LpH se.

**Table 10. Summary and Comparison of Liquid Disinfectants**

| Class | Recommended Use | How They Work | Advantages | Disadvantages | Comments & Hazards | Examples |
|---|---|---|---|---|---|---|
| **Chlorine Compounds** | Spills of human body fluids<br><br>Good against:<br>Vegetative Bacteria<br>Fungi<br>Enveloped Viruses<br>Non-enveloped Viruses<br><br>Good at >1000ppm Sodium Hypochlorite:<br>Spores<br><br>Good with extended contact time:<br>Mycobacteria | Free available chlorine combines with contents within microorganism, reaction byproducts cause its death<br><br>Need 500 to 5000 ppm<br><br>Produce chemical combination with cell substances<br><br>Depends upon release of hypochlorous acid | Kills hardy viruses (e.g. hepatitis)<br><br>Kills a wide range of organisms<br><br>Inexpensive<br><br>Penetrates well<br><br>Relatively quick microbial kill<br><br>May be used on food prep surfaces | Corrodes metals, such as stainless, aluminum<br><br>Organics may reduce activity<br><br>Increase in alkalinity decreases bactericidal property<br><br>Unpleasant taste and odor<br><br>Tuberculocidal, with extended contact time | Follow spill procedure and dilution instructions<br><br>Make fresh solutions before use<br><br>Eye, skin and respiratory irritant<br><br>Corrosive<br><br>Toxic | Bleach solutions (sodium hypochlorite)<br><br>Clorox<br><br>Cyosan<br><br>Purex |

| Class | Recommended Use | How They Work | Advantages | Disadvantages | Comments & Hazards | Examples |
|---|---|---|---|---|---|---|
| **Iodophors (Iodine with carrier)** | Disinfecting some semicritical medical equipment<br><br>Very Good:<br>Fungi<br>Viruses<br>Bacteria<br>Some Spores<br><br>Good with extended contact time:<br>Mycobacteria | Free iodine enters microorganism and binds with cellular components<br><br>Carrier helps penetrate soil/fat<br><br>Probably by disorder of protein synthesis due to hindrance and/or blocking of hydrogen bonding | Kills broad range of organisms<br><br>Highly reactive<br><br>Low tissue toxicity<br><br>Kills immediately rather than by prolonged period of stasis<br><br>Not affected by hard water<br><br>May be used on food prep surfaces | May stain plastics or corrode metal<br><br>May stain skin/laundry<br><br>Stains most materials<br><br>Odor<br><br>Some organic and inorganic substances neutralize effect<br><br>Tuberculocidal, with extended contact time<br><br>Sporicidal: Some | Dilution critical<br><br>Follow directions!<br><br>Use only EPA registered hard surface iodophor disinfectants<br><br>Don't confuse skin antiseptic iodophors for disinfectants<br><br>Skin and eye irritant<br><br>Corrosive<br><br>Toxic | Wescodyne<br><br>Bactergent<br><br>Hy-Sine<br><br>Ioprep<br><br>Providone (iodine/betadine) |
| **Alcohols** | Good Against:<br><br>Vegetative Bacteria<br>Fungi<br>Enveloped Viruses | Changes protein structure of microorganism<br><br>Presence of water assists with killing action | Fairly inexpensive | < 50% or >90% Solution not very effective<br><br>Not active when organic matter present<br><br>Not active against certain types of viruses<br><br>Evaporates quickly<br><br>Contact time may not be sufficient for killing<br><br>Alcohol solutions not EPA-registered disinfectants | Flammable<br><br>Eye Irritant<br><br>Toxic | 70% Ethanol<br><br>Cavicide |
| **Glutaraldehyde** | Good Against:<br><br>Vegetative Bacteria<br>Fungi | Coagulates cellular proteins | Non-staining, relatively noncorrosive<br><br>Useable as a | Not stable in solution<br><br>Has to be in alkaline solution | Eye, skin and respiratory irritant<br><br>Sensitizer | Cidex<br><br>Calgocide 14<br><br>Vespore |

| Class | Recommended Use | How They Work | Advantages | Disadvantages | Comments & Hazards | Examples |
|---|---|---|---|---|---|---|
| | Mycobacteria<br>Viruses<br>Spores | | sterilant on plastics, rubber, lenses, stainless steel and other items that can't be autoclaved | Inactivated by organic material | Toxic | |
| **Quaternary Ammonium compounds (QUATS)** | Ordinary housekeeping (e.g. floors, furniture, walls)<br><br>Good Against:<br><br>Vegetative Bacteria<br>Enveloped Viruses<br>Fungi | Affects proteins and cell membrane of microorganism<br><br>Releases nitrogen and phosphorous from cells | Contains a detergent to help loosen soil<br><br>Rapid action<br><br>Colorless, odorless<br><br>Non-toxic, less corrosive<br><br>Highly stable<br><br>May be used on food prep surfaces | Does not eliminate spores, TB bacteria, some viruses<br><br>Effectiveness influenced by hard water<br><br>Layer of soap interferes with action | Select from EPA list of hospital disinfectants<br><br>Skin and eye irritant<br><br>Toxic | Coverage 258<br><br>End-Bac<br><br>Hi Tor<br><br>Bacdown |
| **Phenolic Compounds** | Good Against:<br><br>Vegetative Bacteria<br>Fungi<br>Enveloped Viruses<br>Some non-enveloped Viruses<br>Mycobacteria | Gross protoplasmic poison<br><br>Disrupts cell walls<br><br>Precipitates cell proteins<br><br>Low concentrations inactivate essential enzyme systems | Nonspecific concerning bactericidal and fungicidal action<br><br>When boiling water would cause rusting, the presence of phenolic substances produces an anti-rusting effect | Unpleasant odor<br><br>Some areas have disposal restrictions<br><br>Effectiveness reduced by alkaline pH, natural soap or organic material<br><br>Not Sporicidal | Skin and eye irritant<br><br>Sensitizer<br><br>Corrosive<br><br>Toxic | Hil-Phene<br><br>Amphyl<br><br>LpH se<br><br>Metar<br><br>Vesphene<br><br>Decon-Cycle |

### 3). Disposal

All liquid waste treated with chemical disinfectants must be disposed of as hazardous waste and collected for disposal by OEHS, which can be arranged through the Lab Management System. The only exception is that waste treated with bleach may be poured down the drain, with running water.

## Appendix F: Biological Toxin SOP

If you are working with acute biological toxins, the laboratory must describe the procedures in a Chemical Hygiene Plan/Standard Operating procedure. This must be provided to the IBC as part of the IBC registration. The template below may be customized for this purpose.

*[Customize text in parentheses and brackets to specific procedures and equipment in your laboratory. Please refer to the Biosafety in Microbiological and Biomedical Laboratories, Appendix I: Guidelines for work with Toxin of Biological Origin for more information, https://www.cdc.gov/biosafety/publications/bmbl5/bmbl5_appendixi.pdf#x2013.]*

| Standard Operating Procedures for *[Toxin]* | |
|---|---|
| #1 Chemicals/Hazards | *[Obtain specific toxin hazard information from MSDS/SDS.]*<br>CAS number: *[XXX]*<br>Routes of exposure: *[XXX]*<br>How exposure might occur: *[XXX]*<br>Target organs: *[XXX]*<br>Signs/symptoms of exposure: *[XXX]* |
| #2 Prior to Work | Hazardous chemical and specific SOP training will be provided to personnel working with toxin and any other personnel authorized or required to be in the laboratory during toxin work.<br>Appropriate inactivation method(s) for [*toxin*] will be determined and supplies for inactivation and spill cleanup of *[toxin]* will be readily available.<br>*[List vaccinations or antitoxins required or recommended for toxin]*. If vaccinations or antitoxins are required, contact Occupational and Environmental Health and Safety at 801-581-6590. |
| #3 Environmental/ Ventilation Controls | Work with *[toxin]* will be performed in a *[chemical fume hood/Biological Safety cabinet (BSC)]. List the type of BSC to be used (e.g. Class II, Type A2).*<br>In-line HEPA filters will be used on vacuum lines.<br>Safety centrifuge cups or sealed rotors will be used if centrifuging materials containing *[toxin]*, and the outside surfaces will be routinely decontaminated after each use. |
| #4 Personal Protective Equipment (PPE) | The following PPE will be worn when working with *[toxin]*: *[Customize list]*<br>• Laboratory coat or gown with long cuffed sleeves<br>• Disposable lab coat<br>• Disposable sleeves<br>• Safety glasses with side shields or chemical safety goggles<br>• Face protection such as a face shield if splash/spatter possible<br>• Gloves *[type]* that are impervious to *[toxin]* and diluent<br>• Respiratory protection *[if aerosol hazard is present] If respirators are used the worker must be enrolled in the Respiratory protection program; contact OEHS for information (http://d2vxd53ymoe6ju.cloudfront.net/wp-* |

| | |
|---|---|
| | *content/uploads/sites/4/20160922155338/Resp-Prot-Program-rev.2016.pdf).*<br><br>Gloves must be changed immediately if contaminated, torn, or punctured. |
| #5 Special Handling Procedures & Storage Requirements | **HANDLING**<br>Prep<br>• Sign will be posted on the room door when toxin is in use stating: "Toxins in Use -- Authorized Personnel Only."<br>• All preparation of *[toxin]* will be performed over plastic-backed absorbent pads in a *[fume hood/BSC]*. Pads will be disposed of immediately upon contamination and after completion of tasks.<br>• Describe how toxin will be prepared*: [Example: Vials of [toxin] will be purchased in pre-weighed powder form and then reconstituted in a [fume hood/biological safety cabinet (BSC)]. Weighing the [toxin] is not necessary as reconstitution will occur in the purchased vial and then aliquoted into vials with caps.]*<br><br>Use<br>• Only needle locking (Luer-Lock type) syringes or disposable syringe units will be used for injection or aspiration of *[toxin]*.<br>• A sharps container will be in the immediate vicinity for safe sharps disposal.<br>• Containers will be decontaminated before they are removed from *[fume hood/BSC]*.<br>• The *[fume hood/BSC]* will be decontaminated upon completion of tasks with *[decontaminant and concentration]* for *[contact time]*.<br>• All potentially contaminated disposable items will be placed in a hazardous waste bag and decontaminated before disposal.<br>• Hands will be washed upon completion of tasks.<br><br>**STORAGE**<br>• *[Toxin]* will be stored in locked *[freezer/refrigerator/cabinet/box/other]* in *[secure location room #]*.<br><br>**TRANSPORT**<br>• *[Toxin]* will be transported in labeled and sealed non-breakable secondary containers. |

| #6 Spill and Accident Procedures<br>*[Specific cleaning, decontamination agents (and contact times)/equipment and waste disposal procedures must be determined.]* | All spills will be cleaned by properly protected and trained personnel only. Wash hands thoroughly after completing any spill clean-up. If you are not trained or comfortable cleaning up a spill, call OEHS for assistance at 801-581-6590. If it is an emergency (risk of exposure to others such as an on-going toxin release), call 911.<br><br>**Liquid spills:**<br>Personnel cleaning up a liquid spill will wear a lab coat/gown with cuffed sleeves (or disposable sleeves), goggles, and two pairs of nitrile gloves. Cover spill with absorbent paper towels and apply *[inactivating agent + concentration]*, starting at the perimeter and working towards the center, allowing *[XX min]* contact time to deactivate *[toxin]*. Clean the spill area with *[inactivating agent]*, then soap and water. The decontaminated spill waste will be double bagged and disposed of in the biohazard waste container.<br><br>**Powder spills inside of [*fume hood/BSC]*:**<br>Personnel cleaning up a powder spill will wear a lab coat/gown with cuffed sleeves (or disposable sleeves), goggles, and two pairs of nitrile gloves. Gently cover powder spill with dampened absorbent paper towels to avoid raising dust. Apply *[inactivating agent + concentration]*, starting at the perimeter and working towards the center, allowing *[XX min]* contact time to deactivate *[toxin]*. Clean the spill area with *[inactivating agent]*, then soap and water. The decontaminated spill waste will be double bagged and disposed of in the biohazard waste container.<br><br>**Powder spills outside of a *[fume hood/BSC]*:**<br>Remove all personnel from the room and restrict access; do not attempt to clean up the spill unless personnel are authorized to use a respirator. If personnel are not cleared to use a respirator, report the spill by notifying OEHS (at 801-581-6590). Tell them that a spill has occurred, and you need OEHS to assist with the spill cleanup.<br><br>Be prepared to provide the following information:<br>• Name and phone number of knowledgeable person that can be contacted: *[emergency contact name and phone number]*<br>• *[Toxin name]*, concentration and amount spilled, liquid or solid spill<br>• Number of injured, if any<br>• Location of spill<br>This information can also be used in reporting to the Emergency Department after potential exposure.<br><br>Personnel cleaning up a powder spill will wear a lab coat/gown with cuffed sleeves [*or disposable sleeves*], goggles, two pairs of nitrile gloves and a respirator. Gently cover powder spill with dampened absorbent paper |
| --- | --- |

| | |
|---|---|
| | towels to avoid raising dust. Apply *[inactivating agent + concentration]*, starting at the perimeter and working towards the center, allowing *[XX min]* contact time to deactivate *[toxin]*. Clean the spill area with *[inactivating agent]*, then soap and water. The decontaminated spill waste will be double bagged and disposed of in the biohazard waste container.<br><br>For questions on spill cleanup, contact OEHS at 801-581-6590 for guidance.<br><br>Wash hands thoroughly after completing any spill clean-up. |
| **EXPOSURE PROCEDURES**<br>**In Case of Emergency** | **1. Provide First Aid Immediately**<br>• For sharps injury (needlestick or subcutaneous exposure), scrub exposed area thoroughly for 15 minutes using warm water and soap.<br>• For skin exposure, wash the area with soap and water. For large exposures, use the nearest safety shower for 15 minutes. Stay under the shower and remove clothing. Use a clean lab coat or spare clothing for cover-up.<br>• For eye exposure, use the eye wash for 15 minutes while holding eyelids open.<br>• For inhalation, move out of contaminated area. Get medical help.<br><br>**2. Get Help**<br>• Call 911 or go to nearest Emergency Department (ED). Give details of exposure, i.e. agent, dose, route of exposure, time since exposure. Bring to the ED the MSDS/SDS and this SOP.<br>• Notify your supervisor as soon as possible for assistance.<br>• Secure area before leaving.<br><br>**3. Report Incident to Occupational and Environmental Health & Safety**<br>• If serious accident, hospitalization or fatality, notify OEHS immediately after providing first aid and/or getting help.<br>    o Call at 801-581-6590. |

| #7 Waste Disposal and Cleaning | Any waste *[toxin]* will be decontaminated or autoclaved as appropriate before disposal or given to OEHS for disposal whenever possible.<br><br>Work space surfaces must be wiped down after completion of tasks with *[inactivating agent + concentration]* during the length of the experiment. Absorbent pads will be replaced after completion of tasks or immediately if contaminated. Used and potentially contaminated absorbent pads, PPE, etc. will be placed in a hazardous waste bag and autoclaved.<br><br>If in-lab inactivation is not possible for *[toxin]* waste, it must be managed as hazardous chemical waste. Be aware that some form of treatment in the lab may be required before it can be managed as chemical waste. Contact OEHS at 801-581-6590 for disposal instructions. For chemical waste pick up complete a request through the lab management system (http://oehs.utah.edu/topics/lab-management-system). |
|---|---|
| #8 Special Precautions for Use of *[Toxin]* in Animals *(This section must be completed if working with toxin in animals)*<br>*Identify where animals will be injected, housed and any special precautions/warnings for animal handlers.* | Use of toxins in animals will be documented and approved by IACUC.<br>*[Give detailed procedures for safely completing tasks, containment, decontamination information, and any special disposal requirements.]*<br>*[Animals will be anesthetized or placed into a restraining apparatus before procedures using [toxin] are performed. Once the animal has been properly fitted into the restraining apparatus, the syringe will be loaded just prior to injection.]*<br>After procedures are complete, the restraining apparatus and surrounding work station will be decontaminated *[inactivating agent + concentration]*. All reusable lab equipment will be autoclaved.<br>*[Give any special disposal requirements]* |
| #9 Approval Required | The protocol must be approved by the Institutional Biosafety Committee prior to commencement. All staff working with *[toxin]* must be trained on this SOP prior to starting work. They must also be trained on the *[toxin]* MSDS/SDS, and it must be readily available in the laboratory. All training must be documented and maintained by the PI. |
| #10 Decontamination | All surfaces will be decontaminated with *[inactivating agent + concentration]* after removing the plastic-backed pads. All reusable lab equipment will be autoclaved. Note that some disinfecting agents may not deactivate *[toxin]*. |
| #11 Designated Area | All work with [*toxin*] must be done in a designated laboratory, work space and *[fume hood/BSC]*. Signage must be placed on door to room when *[toxin]* is used. This work will be conducted in *[Room #]* |

## Appendix G: University of Utah Biosafety Guidelines for Teaching Laboratories

The American Society for Microbiology (ASM) Education Board published Guidelines for Teaching Laboratories in 2012.[1] The ASM publication was influenced by the lack of clear safety guidelines for microbiology teaching labs and a multistate outbreak of *Salmonella typhimuirium* originating in teaching and clinical laboratories in 2011.[2] Unfortunately, similar incidents occurred in 2014 and 2017, thus reinforcing the need for these guidelines.[3,4] The ASM guidelines include recommendations for working at Biosafety Level (BSL) 1 and BSL2. A major finding of the epidemiological investigation of the outbreak was deficiencies in biosafety awareness and proper training of staff and students. The University of Utah Department of Occupational and Environmental Health and Safety (OEHS) has compiled guidelines, based on the ASM recommendations, with input from the University of Utah Institutional Biosafety Committee (IBC) and from the Rutgers University Guidelines, in order to ensure our teaching labs are safe for students and to prevent pathogen exposure to persons and the environment.

This document contains biosafety requirements for teaching laboratories operating at BSL1 and BSL2. This document supplements the detailed resources described elsewhere in the University of Utah Biosafety Manual. Not all teaching laboratories are equipped to safely operate at BSL2. Any and all use of Risk Group 2 (RG2) or higher organisms must be preapproved by the University of Utah IBC: an IBC protocol must be submitted through the BioRAFT system, which can be accessed here. Please contact the biosafety group in OEHS at 801-581-6590 or biosafety@oehs.utah.edu with any questions or clarifications.

*Subculturing "unknown" samples and teaching about differential and selective media:*

The procedures needed to identify unknown microorganisms can be performed safely, and with little to no risk to the students. Students are permitted to culture organisms from soil, water, food materials, and the air. Subculturing from the initial culture plate is permitted for the above samples, but IBC review and approval must be obtained if differential media used in the experiment could select for the growth of organisms listed at RG2 or higher. If the samples will be used to only count and understand the types of organisms in a particular environment, and no subculturing performed, then IBC approval will not be required. If the laboratory will include subculturing and isolation from environments such as water fountains, door handles or other areas that could harbor pathogens, review and approval by the IBC must be obtained. Additionally, samples must never be cultured from the students themselves without approval from the IBC, and possibly the Institutional Review Board, as there is the potential to grow microorganisms that require BSL2, or even BSL3 containment.

It is recommended that testing of unknowns be performed from a mixture of known microorganisms (to the instructor), or from a culture where the contents are known to the instructor, instead of from the

environment.

For recommendations on surrogate microorganisms, please contact the OEHS biosafety office at biosafety@oehs.utah.edu.

**Minors Working in Biological Labs at the University of Utah**

All minors and their parent/legal guardian must sign the “Minor Participant Informed Consent Document” prepared by University of Utah Risk Management. Minors are not permitted to handle any biological agents, recombinant or synthetic nucleic acids, research animals, highly hazardous chemicals, or dangerous machinery without prior approval from OEHS. Many classes, activities, and events require a liability waiver. University of Utah events or activities which are planned, organized, controlled or supervised by University of Utah employees or authorized volunteers for minors must contact Risk Management to complete the Minor Participant Informed Consent & Parenting/Guardian Consent to Treatment, Waiver and Release for University of Utah Event or Activity form, https://riskmanagement.utah.edu/intranet/contracts/liability-field-trip-waiver.php.

## 1. Biosafety Level One

Biosafety Level One (BSL1) includes microorganisms that are not known to cause human disease, and that can be handled safely on bench tops. The use of BSL1 is the most appropriate for most teaching laboratories.

**BSL1 Requirements**

**Laboratory Facility Requirements:**

- Non-porous floor, bench tops, chairs and stools*
  - Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
  - Laboratory furniture is sturdy with surfaces for easy cleaning and decontamination. No cloth chairs. Spaces between benches, cabinet and equipment are accessible for cleaning.
- Sink for hand-washing
- Eyewash station
- Lockable door to the laboratory
- Proper pest control practices
- If the laboratory has windows that open, they are fitted with fly screens.
- *Recommended:* Separate storage area for personal belongings
- *Recommended:* Access to a working and validated autoclave

**It is understood that some current facilities may not be able to meet these requirements due to the original design of the laboratory space. Any facility renovation or new construction would need to address these requirements.*

**Stock Culture Requirements:**

- Stock cultures must be from authorized, commercial or reputable sources. As indicated above, subculturing microbes isolated from the environment, clinical samples or other unknown locations is discouraged as BSL2 classified microbes may be isolated. Subculturing from the environment must be reviewed and approved by the IBC.
  - Examples of Recommended Microbes for work at BSL1

| Microorganism | ATCC number |
|---|---|
| *Acinetobacter baylyi* | 33304 |
| *Alcaligenes faecalis* | 8750 |
| *Aspergillus niger* | 16888 |
| *Bacillus stearothermophilus* | 7953 |
| *Bacillus subtilis* | 23857 |
| *Citrobacter freundii* | 8090 |
| *Clostridium sporogenes* | 3584 |
| *Enterobacter aerogenes* | 13048 |
| *Enterococcus casseliflavus* | 700327 |
| *Enterococcus raffinosus* | 49427 |
| *Escherichia coli* B | 11303 |
| *Escherichia coli K12* | 10798 |
| *Geobacillus stearothermophilus* | 12980 |
| *Halobacterium salinarum* | 33170 |
| *Lactobacillus acidophilus* | 4356 |
| *Micrococcus luteus* | 4698 |
| *Neurospora crassa* | 44318 |
| *Penicillium chrysogenum* | 10106 |
| *Providencia alcalifaciens* | 9886 |
| *Pseudomonas fluorescens* | 13525 |
| *Pseudomonas putida* | 12633 |
| *Rhizopus stolonifer* | 14037 |
| *Saccharomyces cerevisiae* | 9763 |
| *Serratia liquefacens* | 27592 |
| *Serratia marcescens* Bizio | 13880 |
| *Staphylococcus epidermidis* | 14990 |
| *Staphylococcus saprophyticus* | 15305 |

- Laboratory instructor must maintain documentation for all stock organisms, sources and handling of stock cultures.
- Obtain fresh stock cultures of microorganisms on a regular basis (at least annually) to be certain of the source culture, minimize spontaneous mutations and to reduce contamination.
- Protocols that can be performed easily at BSL1: anaerobic growth, Gram stain, capsule stain, endospore stain, flagella stain, carbohydrate fermentation, casein hydrolase, catalase and oxidase test, bacterial enumeration, eosin methylene blue plate, gelatin hydrolysis, hanging drop, indole methyl red Vogues-Proskauer and Citrate (IMViC), Kirby-Bauer, Luria broth, litmus milk, 4-methylumbelliferyl-β-D-glucuronide *Escherichia coli* broth medium (*E. coli* MUG), MacConkey Agar, mannitol, nitrate reduction, spread, pour and quadrant streak plate, starch hydrolysis, transformation assay, urease, triple sugar iron, use of lambda bacteriophage, bacterial transformation, plasmid DNA isolation, restriction endonuclease digestion, polymerase chain reaction (PCR) and gel electrophoresis.

**Personal Protective Equipment Requirements:**

- Safety goggles or safety glasses (with side shields) must be worn when handling liquid cultures, spread plating, or when performing procedures that may create a splash. If glasses are shared among students, they must be sanitized with an appropriate disinfectant after use.
- Laboratory coats must be worn. These can be disposable or made of cloth. Disposable coats may be reused but must be replaced on any sign of contamination, damage or degradation. Lab coats must be stored within the laboratory and must be assigned to individual students, not shared. Lab coats must be laundered by an approved laundry facility. Do not take lab clothing home to launder.
- Long pants/ long skirts (ankle length), or other clothing (such as scrubs) to cover exposed skin must be worn.
- Closed toe and heel shoes that cover the entire foot must be worn.
- Gloves must be worn when the student has fresh cuts or abrasions on the hands, or any time when cultures are handled, when staining microbes and when handling hazardous chemicals. Hands must be washed immediately after handling microbial cultures and anytime accidental contact occurs with the skin. Hand cleansing must be performed with soap and water, or, if none is available, with ethanol based hand sanitizer. Soap and water must be used as soon as possible if hand sanitizer is used.

**Laboratory Work Practices:**

- Wash hands after entering and before leaving the laboratory.
- Long hair must be tied back.
- Long, dangling jewelry is not permitted in the laboratory.
- Lab benches must be disinfected upon entering the laboratory and at the end of the laboratory session. Any materials that are spilled must be immediately cleaned-up. Disinfectants used must be effective against microbes used in the laboratory. OEHS can be consulted for disinfectant recommendations.
- Teach, practice and enforce the proper wearing and use of personal protective equipment.
- Food, water bottles, gum, and drinks of any kind are prohibited in the laboratory.
- Do not touch your face, apply cosmetics, adjust contact lenses, bite nails, or chew on pens/ pencils in the laboratory.
- All personal items must be stowed in a clean area while in the laboratory. The use of cell phones, tablets and other personal electronic devices is prohibited.
- Mouth pipetting is prohibited.

- All containers must be labeled clearly.
- The laboratory door must remain closed at all times when the lab is in session. The laboratory instructor must approve all persons entering.
- Minimize use of sharps. Needles and scalpels are to be used according to institutional guidelines: do not re-cap needles. Most sharps shall be discarded in sharps containers that are closable, puncture-resistant, leakproof on sides and bottoms. However, non-contaminated pipets and pipet tips may be disposed of in broken glass receptacles.
- Contaminated sharps, including coverslips, slides, glass and plastic pipets and pipet tips, and Pasteur pipets, are discarded immediately or as soon as possible in biohazard sharps containers that are closable, puncture-resistant, leakproof on sides and bottoms, and labeled or color-coded appropriately.
- Test tube racks or other secondary containers must be used to move cultures in the laboratory.
- Stocks and other cultures must be stored in a leak-proof container when work is complete. A sealed, leak-proof container, labeled with a biohazard symbol, must be used to transport stocks and cultures from one room to another.
- Cultures must be disinfected/inactivated prior to disposal, either by chemical disinfection or autoclaving.
- Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container labeled with a biohazard symbol, which is closed before being removed from the laboratory. Hazardous waste can be picked up by OEHS, arranged through the OEHS Lab Management System (http://oehs.utah.edu/topics/lab-management-system).
- Broken glass must be handled using a dustpan and broom or forceps/tongs, not picked up by students or laboratory personnel with their hands. Broken glass must be disposed of in a broken glass box, unless it is contaminated and mustbe disposed of in a biohazard sharps container. If contaminated, broom will need to be disposed or sterilized.
- All spills or injuries must be immediately reported to the laboratory instructor. When contaminated material is spilled, inform the laboratory assistant immediately. Proper procedure require the instructor and student to secure area, deny entry to non-authorized people. The instructor must assume everything spilled is infectious, wear personal protective equipment (lab coat, eye protection, shoe covers and 2 pairs of gloves), cover spill with paper towels, prepare fresh disinfectant (e.g., 1:10 dilution of bleach) and pour slowly onto spill from outside to in, leave for>20 min, use tongs to pick up objects and place in sharps containers, place other waste in biohazard waste containers, remove PPE and wash hands. Spills or injuries must then be documented with OEHS, who can be reached at 801-581-6590.
- Should an exposure occur, immediately wash the affected areas with soap and water, or if exposure to eyes or mucous membranes occurred, immediately flush affected area with water

for 10-15 minutes. Go directly to the Student Health Center, Madsen Clinic, 555 South Foothill Boulevard, for medical evaluation and follow-up. For life threatening injury or illness call emergency medical services by calling 911. Complete and submit the Incident/Accident Report form to Risk Management within 24 hours of the incident. The form can be downloaded from the Risk Management website, https://riskmanagement.utah.edu/intranet/insurance/incident-accident-info.php.

- Advise immune-compromised students and students living with or caring for an immune-compromised person to consult physicians to determine the appropriate level of laboratory participation. (Students shall not be asked to reveal if they are immuno-compromised. A general announcement shall be made that students with a reduced immune status should consult with Student Health Services. A note from Student Health Services is sufficient to excuse a student from laboratory work.)
- *Recommended:* Supply pens and pencils for students, and keep separate from personal items.
- *Recommended:* Keep note taking and discussions separate from work with laboratory materials.
- *Recommended:* Use micro-incinerators or glass bead sterilizers rather than Bunsen burners.

**Training Practices:**

- Faculty and teaching assistants must complete University of Utah laboratory safety, bloodborne pathogens and biosafety trainings, as applicable.
- Instructors and/ or teaching assistants must review basic biosafety and microbiological practice with students on the first day of lab. The requirements listed above must be included in this training session. Training session must be documented with a sign-in sheet maintained by the instructor.
- Students and instructors are required to handle microorganisms safely and in conjunction with requirements outlined in the University of Utah Biosafety Manual.
- Inform students of safety precautions applicable to each exercise before the procedure is performed.

**Documentation**:

- Safety Data Sheets (SDS) must be available in the laboratory for all chemicals.
- Require students to sign safety agreements indicating that they have been informed about the safety requirements and the hazardous nature of the microbes and materials that they will handle throughout the semester. The laboratory instructor must maintain student signed agreements in the laboratory.
- Maintain and post caution signs on lab doors (complete with biohazard symbol). These are obtained from OEHS. https://oehs.utah.edu/resource-center/forms/hazard-warning-signage-

questionnaire.

- Instructors must provide a detailed list of microorganisms that will be handled in the laboratory to students. This list can be included in the syllabus, laboratory manual, or online at the course website.
- Emergency phone numbers and information must be posted in the laboratory.

## 2. Biosafety Level Two

Biosafety Level Two (BSL2) laboratories are suitable for working with microbes posing a moderate risk to the individual and a low community risk for infection. There are many microorganisms handled at BSL2 that can cause disease in humans via ingestion or inoculation. The guidelines for BSL2 laboratories build upon those for BSL1 facilities, and typically include additional engineering controls to protect students, such as biological safety cabinets, centrifuge safety cups and safety needle devices.

### BSL2 Requirements

**Laboratory Facility Requirements:**

- Non-porous floor, bench tops, chairs and stools*
  - Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
  - Laboratory furniture is sturdy with surfaces for easy cleaning and decontamination. No cloth chairs. Spaces between benches, cabinet and equipment are accessible for cleaning.
- Sink for hand-washing
- Eyewash station
- Lockable door to the laboratory
- Proper pest control practices
- If the laboratory has windows that open, they are fitted with fly screens.
- Separate storage area for personal belongings*
- Working and validated autoclave
- Biohazard signage where cultures are used and stored (e.g. incubators), on the door to the room and on containers used to transport cultures. Contact the OEHS Biosafety team at 1-6590 to request a BSL-2 Warning sign.
- *Recommended:* Biological Safety Cabinet. Please see requirements below. All biological safety cabinets must be certified by an approved vendor annually (contact OEHS at 801-581-6590). Biological safety cabinets (Class I or II) or other appropriate personal protective or physical containment devices are used whenever:

a. Procedures with a high potential for creating infectious aerosols are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, and harvesting infected tissues from animals or eggs.

b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed heads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

**It is understood that some current facilities may not be able to meet these requirements due to the original design of the laboratory space. Any facility renovation or new construction would need to address these requirements.*

**Stock Culture Requirements:**

- Stocks must be from authorized, commercial or reputable sources. Do not subculture microbes isolated from the environment, clinical samples or other unknown locations because they may be microbes that require BSL2 practices and facilities. Samples must never be obtained from clinical sites unless a full description of strain antibiotic susceptibility and resistance is provided, and the IBC has approved the use of these strains for the laboratory.
- Strains resistant to clinically relevant antibiotics shall not be handled in teaching laboratories.
- Maintain documentation for all stock organisms, sources and handling of stock cultures.
- Obtain fresh stock cultures of microorganisms on a regular basis to be certain of the source culture, minimize spontaneous mutations and to reduce contamination.
- Store stocks in a secure (locked) area.
- Substitute surrogates for common BSL2 pathogens

Examples of Common Microbes used at BSL2

| Microorganism | ATCC Number |
|---|---|
| *Klebsiella oxytoca* | 13182 |
| *Proteus mirabilis* | 25933,7002 |
| *Proteus vulgaris* | 29905 |
| *Salmonella enterica* | 700720 |
| *Staphylococcus aureus* | 12600 |

- When choosing a test organism, many instructors want to choose organisms that are clinically relevant, i.e. pathogens. There are six microorganisms that are considered major threats, not because they cause the most devastating illnesses but because they comprise the majority of antibiotic-resistant infections observed in health care settings. These are referred to as ESKAPE

pathogens and include *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa*, and species of Enterobacter (ESKAPE).

- ESKAPE pathogens should be replaced with "Safe Relatives". Requests to use ESAKPE pathogens rather than the safer alternatives will need to be justified to the IBC, who may require additional safeguards.

    - ESKAPE pathogen > Safe Relative
      *Enterococcus faecium* > *Enterococcus raffinosus* or *Enterococcus casseliflavus*
      *Staphylococcus aureus* > *Staphylococcus epidermidis*
      *Klebsiella pneumonia* > *Escherichia coli*
      *Acinetobacter baumannii* > *Acinetobacter baylyi*
      *Pseudomonas aeruginosa* > *Pseudomonas putida*
      *Enterobacter species* > *Enterobacter aerogenes*

**Personal Protective Equipment Requirements:**

- Safety goggles or safety glasses must be worn when handling liquid cultures, spread plating, or when performing procedures that may create a splash.
- Closed toe and heel shoes that cover the entire foot must be worn.
- Long pants/ long skirts (ankle length), or other clothing (such as scrubs) to cover exposed skin must be worn.
- Laboratory coats must be worn. These can be disposable or made of cloth. Disposable coats may be reused but must be replaced on any sign of contamination, damage or degradation. Lab coats must be stored within the laboratory and must be assigned to individual students, not shared. Lab coats must be laundered by an approved laundry facility. Do not take lab clothing home to launder.
- Gloves must be worn when handling cultures, when staining microbes and when handling hazardous chemicals. Hands must be washed immediately after handling microbial cultures and anytime accidental contact occurs with the skin. Hand cleansing must be performed with soap and water, and if none is available with ethanol based hand sanitizer. Soap and water must be used as soon as possible if hand sanitizer is used.

**Laboratory Work Practices:**

- Instructors/Supervisor limits access to the laboratory. In general, persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in

the laboratory or animal rooms. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

- Instructors/Supervisor establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet any specific entry requirements (e.g., immunization) may enter the laboratory
- When the infectious agent(s) in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign, incorporating the universal biohazard symbol, is posted on the access door to the laboratory work area. The hazard warning sign identifies the infectious agent, lists the name and telephone number of the Instructor/Supervisor or other responsible person(s) for entering the laboratory.
- An insect and rodent control program is in effect.
- Wash hands after entering and before leaving the laboratory.
- Long hair must be tied back.
- Long, dangling jewelry is not permitted in the laboratory.
- Teach, practice and enforce the proper wearing, use, donning and doffing of personal protective equipment
- Lab benches must be disinfected upon entering the laboratory and at the end of the laboratory session. Additionally, if any materials are spilled, they will be immediately cleaned-up.
    - ➢ Disinfectants used must be effective against microbes used in the laboratory. OEHS can be consulted for disinfectant recommendations.
- Food, water bottles, gum, and drinks of any kind are prohibited in the laboratory.
- Do not touch your face, apply cosmetics, adjust contact lenses, bite nails, or chew on pens/ pencils in the laboratory.
- All personal items must be stowed while in the laboratory. The use of cell phones is prohibited.
- Mouth pipetting is prohibited.
- All containers must be labeled clearly.
- The laboratory door must remain closed at all times when the lab is in session.
- Minimize use of sharps. Needles and scalpels are to be used according to institutional guidelines: do not re-cap needles. Most sharps shall be discarded in sharps containers that are closable, puncture-resistant, leakproof on sides and bottoms. However, non-contaminated pipets and pipet tips may be disposed of in broken glass receptacles.
- Contaminated sharps, including coverslips, slides, glass and plastic pipets and pipet tips, and Pasteur pipets, are discarded immediately or as soon as possible in biohazard sharps containers that are closable, puncture-resistant, leakproof on sides and bottoms, and labeled or color-coded appropriately.

- Test tube racks or other secondary containers must be used to move cultures in the laboratory.
- Stocks and other cultures must be stored in a leak-proof container when work is complete. A sealed, leak-proof container, labeled with a biohazard symbol, must be used to transport stocks and cultures from one room to another.
- Students must be taught proper technique to minimize production of aerosols. For example: when pipetting, place tip on side of tube and allow liquid to run down the side of the tube, and when flaming a loop to transfer culture, have a sterile agar plate used as a "sizzle" plate so students do not touch a culture with a really hot loop.
- All procedures that generate aerosols: centrifuging, grinding, blending, shaking, mixing, sonicating, etc., must be performed inside a biological safety cabinet or using appropriate engineering controls (centrifuge safety cups). Biological safety cabinets must also be used when opening a container that can become depressurized when opened, and could release aerosols of the stock culture, and students must be trained in the proper use of biological safety cabinets.
- All waste and cultures are appropriately labeled and must be disinfected/inactivated prior to disposal, either by chemical disinfection or autoclaving.
- Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leakproof container labeled with the biohazard symbol, which is closed before being removed from the laboratory. Hazardous waste can be picked up by OEHS, arranged through the OEHS Lab Management System (http://oehs.utah.edu/topics/lab-management-system).
- Broken glass must be handled using a dustpan and broom or forceps/tongs, not picked up by students or laboratory personnel with their hands. Broken glass must be disposed of in a broken glass box, unless it is contaminated and must be disposed of in a biohazard sharps container. If contaminated, broom will need to be disposed or sterilized.
- All spills or injuries must be immediately reported to the laboratory instructor. When contaminated material is spilled, inform the laboratory assistant immediately. Proper procedure require the instructor and student to secure area, deny entry to non-authorized people. The instructor must assume everything spilled is infectious, wear personal protective equipment (lab coat, eye protection, shoe covers and 2 pairs of gloves), cover spill with paper towels, prepare fresh disinfectant (e.g., 1:10 dilution of bleach) and pour slowly onto spill from outside to in, leave for>20 min, use tongs to pick up objects and place in sharps containers, place other waste in biohazard waste containers, remove PPE and wash hands. Spills or injuries must then be documented with OEHS, who can be reached at 801-581-6590.
- Should an exposure occur, immediately wash the affected areas with soap and water, or if exposure to eyes or mucous membranes occurred, immediately flush affected area with water for 10-15 minutes. Go directly to the Student Health Center, Madsen Clinic, 555 South Foothill Boulevard, for medical evaluation and follow-up. For life threatening injury or illness call

emergency medical services by calling 911. Clinic addresses and maps are at the end of this document and must be incorporated into training documents. Complete and submit the Incident/Accident Report form to Risk Management within 24 hours of the incident. The form can be downloaded from the Risk Management website, https://riskmanagement.utah.edu/intranet/insurance/incident-accident-info.php.

- Advise immune-compromised students and students living with or caring for an immune-compromised person to consult physicians to determine the appropriate level of laboratory participation. (Students must not be asked to reveal if they are immuno-compromised. A general announcement shall be made that students with a reduced immune status should consult with student health services. A note from Student Health Services is sufficient to excuse a student from laboratory work.)
- Supply pens and pencils for students, and keep separate from personal items.
- Keep note taking and discussions separate from work with laboratory materials. Note taking can be performed on a pull out desk shelf, if available, but must be taken away from the work area. If this is not available, lecture must be performed before any materials are brought to the bench areas.
- Use micro-incinerators rather than Bunsen burners. Bunsen burners are not permitted in biological safety cabinets. Micro-incinerators can also be used to heat fix bacterial smears on microscope slides and flaming the end of a test tube by passing these items over the entrance to the micro-incinerator.

**Training Practices:**

- Teaching assistants must complete OEHS laboratory safety, bloodborne pathogen and BSL2 biosafety trainings.
- Instructors and/ or teaching assistants must review basic biosafety and microbiological practice with students on the first day of lab. The requirements listed above must be included in this training session. Training session must be documented with a sign in sheet maintained by the instructor.
- Require students and instructors to handle microorganisms safely and in conjunction with requirements outlined in the University of Utah Biosafety Manual.
- Inform students of safety precautions applicable to each exercise before the procedure is performed.
- Require students to demonstrate proficiency in standard aseptic technique and BSL1 practices before allowing them to work at BSL2.

**Documentation:**

- A biosafety manual is prepared and adopted. Students are advised of special hazards and are

required to read instructions on practices and procedures and how to follow them

- Safety Data Sheets (SDS) sheets must be available in the laboratory for all chemicals.
- If available, Pathogen Safety Data Sheets (PSDSs) (previously titled Material Safety Data Sheets for infectious substances) are technical documents that describe the hazardous properties of a human pathogen and recommendations for work involving these agents in a laboratory setting. These documents have been produced by the Public Health Agency of Canada (the Agency) as educational and informational resources for laboratory personnel working with these infectious substances and can be accessed at http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php.
- Spills and Post-Exposure Procedures must be available in the laboratory.
- Require students to sign safety agreements indicating that they have been informed about the safety requirements and the hazardous nature of the microbes and materials that they will handle throughout the semester. Maintain student signed agreements at the institution.
- Prepare, maintain and post caution signs to the laboratory, complete with biohazard symbol.
- Instructors must provide a detailed list of microorganisms that will be handled in the laboratory to students. This list can be included in the syllabus, laboratory manual, or online at the course website.
- Register all work at BSL2 with the Institutional Biosafety Committee.
- Maintain an inventory of the quantity and location of all RG2 agents, in line with ASM recommedations.[5] Create a record of RG2 agents to include the following: (1) identification (name and species of agent), (2) quantity (e.g., approximate number of vials for each agent), (3) location (building, room and cold storage unit ID), (4) name of person familiar with that agent, (5) date entry created, and (6) other related information, such as source, and variant/strain.
- Follow all requirements for BSL2 as outlined in the University of Utah Biosafety Manual.
- Emergency numbers and information must be posted in the laboratory.

**References:**


1. ASM teaching guidelines: http://www.asm.org/index.php/education-2/22-education/8308-new-version- available-for-comment-guidelines-for-best-biosafety-practices-in-teaching-laboratories
2. CDC report regarding 2011 *Salmonella typhimurium* outbreak: http://www.cdc.gov/salmonella/2011/lab-exposure-1-17-2012.html
3. CDC report regarding 2014 *Salmonella typhimurium* outbreak: http://www.cdc.gov/salmonella/typhimurium-labs-06-14/index.html
4. CDC report regarding 2017 *Salmonella typhimurium* outbreak: https://www.cdc.gov/salmonella/typhimurium-07-17/index.html
5. ASM Statement: What is in your laboratory freezer?

http://www.asm.org/index.php/public-policy/99-policy/policy/93059-freezer-8-14

## Appendix H: Dual Use Research of Concern

The policy applies to work with 15 agents and toxins which are subject to the select agent regulations (42 CFR Part 73, 7 CFR Part 331, and 9 CFR Part 121), which set forth the requirements for possession, use, and transfer of select agents and toxins, and have the potential to pose a severe threat to human, animal, or plant health, or to animal or plant products. Research that uses one or more of the agents or toxins listed below, and produces, aims to produce, or can be reasonably anticipated to produce one or more of the effects listed below, will be evaluated for DURC potential.

**A.** **Agents**:

(i) Avian influenza virus (highly pathogenic)
(ii) *Bacillus anthracis*
(iii) Botulinum neurotoxin: For the purposes of this Policy, there are no exempt quantities of botulinum neurotoxin. Research involving any quantity of botulinum neurotoxin must be evaluated for DURC potential.
(iv) *Burkholderia mallei*
(v) *Burkholderia pseudomallei*
(vi) Ebola virus
(vii) Foot-and-mouth disease virus
(viii) *Francisella tularensis*
(ix) Marburg virus
(x) Reconstructed 1918 Influenza virus
(xi) Rinderpest virus
(xii) Toxin-producing strains of *Clostridium botulinum*
(xiii) Variola major virus
(xiv) Variola minor virus
(xv) *Yersinia pestis*

**B.** **Categories of experiments:**

(i) Enhances the harmful consequences of the agent or toxin
(ii) Disrupts immunity or the effectiveness of an immunization against the agent or toxin without clinical and/or agricultural justification
(iii) Confers to the agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies
(iv) Increases the stability, transmissibility, or the ability to disseminate the agent or toxin
(v) Alters the host range or tropism of the agent or toxin

(vi) Enhances the susceptibility of a host population to the agent or toxin

(vii) Generates or reconstitutes an eradicated or extinct agent or toxin listed above

## Appendix I: Contact Information and OEHS Guidance

Occupational and Environmental Health and Safety Mainline: 801-581-6590

E Mail: Biosafety@oehs.utah.edu

OEHS Website: https://oehs.utah.edu/

OEHS Topics: https://oehs.utah.edu/topics

OEHS Resources: https://oehs.utah.edu/resources

OEHS Forms: https://oehs.utah.edu/resource-center/forms

OEHS Biosafety FAQs: https://oehs.utah.edu/topics/biosafety-faq