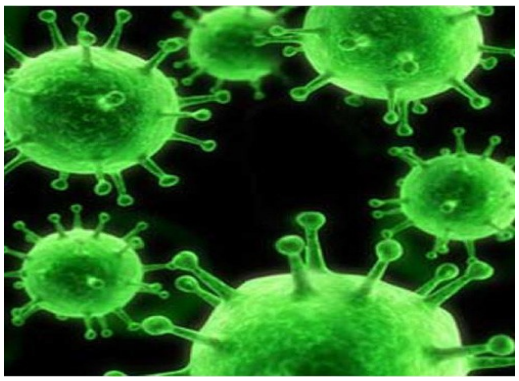
Institutional Biosafety Manual



Revision 01.2021

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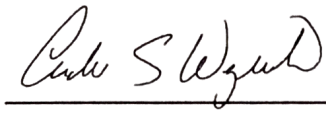
Contents

University of Utah Biosafety Manual Approved By:

Dr. Andy Weyrich, Ph.D., Vice President for Research



Signature

1/11/2021

Date

Dr. Erin Rothwell, Ph.D., Associate Vice Present for Research Integrity and Compliance

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01/11/2021

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Dr. David Gillespie, Ph.D., Chair, Institutional Biosafety Committee

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01/15/2021

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01/21/2021

Date

Fred Monette, M.S., Executive Director, EHS

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1/27/2021

Date

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 Environmental Health and Safety (EHS) 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 Associate Vice President for Research Integrity and Compliance 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 the conduct of 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 EHS, 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, work with human samples (including human cell lines), work with acute biological toxins, and other work with infectious agents, as needed. Visit the IBC website for more information and guidance.
   - The PI must complete the Lab Profile in the EHS Safety Administrative Management System (SAM), at least annually and ensure that the classes of biological materials in use in the lab are appropriately identified.

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, chemical hygiene plans, 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 EHS.
   - The PI must ensure that all visiting personnel, including those who are not employees of the University of Utah, receive all required trainings and are added to IBC registrations prior to starting work.

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 EHS 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 EHS 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 EHS 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. For any materials exported from the United States, the PI must contact Todd Nilsen, Export Control Officer (todd.nilsen@osp.utah.edu or (801) 581-8948), prior to shipment.

n. The PI must maintain an accurate inventory of all acute biological toxins. These toxins must be stored in a secured location.

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 EHS. 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 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 this 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 human 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.

a. Immediately report all exposure incidents to your supervisor and EHS using the online Hazard/Near Miss Report Form (https://oehs.utah.edu/resource-center/forms/hazard-report).
b. Inform your immediate supervisor of any unsafe practices or conditions in the work area. Reports of unsafe practices can also be reported to EHS (https://oehs.utah.edu/resource-center/forms/hazard-report). Concerns over research misconduct or misbehavior can be reported anonymously at www.ethicspoint.com.

d. Lab personnel and students must report any change in health status to the supervisor if there is a possibility it may be work related.
e. 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 EHS.
f. Lab personnel and students shall seek immediate medical attention following exposure to biohazardous agents.
g. 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 human samples.
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, tissue and cell lines, 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 develops 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 I for details of the DURC policy.

7. Environmental Health and Safety (EHS) and the University of Utah 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 for Research Integrity and Compliance on 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. Keep personal items away from the lab bench area to avoid the possibility of contaminating them. (e.g., personal cell phones, backpacks, car keys, pens, pencils, notebooks, calculators, laptop/tablet/iPad, coat, hat, etc.)
3. Do not remove potentially contaminated items such as pens, pencils, notebooks, etc. that are used at the lab bench from the lab unless they have been decontaminated with a disinfectant. Do not take them to an office, car, home, etc. to avoid the possibility of fomite transmission of biohazardous agents.
4. 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.
5. Control access to restricted biohazard areas.
6. 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 into the area.
    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. Hand Hygiene

- Cover cuts or abrasions on hands when working with biohazardous materials.
- Before entering the lab, hydrate dry, cracked skin with lotion to improve skin integrity on hands.
- Always keep hands gloved when working with biohazardous agents, making sure your wrists are not exposed.
- Never touch your face with gloved hands.
- Never put your hands in your personal pockets while gloved.

- If you need to access your lab coat pockets while working, train yourself to only access them while gloved, not when ungloved. However, if the gloves are contaminated or potentially contaminated, change the gloves prior to doing this.
- When gloved, never touch any work surface that you or others touch bare-handed. (e.g., phones, keyboard, computer mouse, pencils/pens, doorknobs, equipment handles, telephone, faucets, etc.)
- Practice effective hand washing, which involves:
    - Wet hands with clean, warm running water and apply soap. Rub hands together to lather soap, washing backs of hands and thumbs, between fingers, around nails; wash wrists up to slightly above area covered by gloves.
    - Gently scrub for 20 seconds.
    - Rinse hands under clean running water; dry using clean paper towels. Turn off the faucet with the paper towel.
    - If no soap or running water is available, use moist disposable wipes to physically remove dirt/debris from your hands, then use hand sanitizer on all hand/wrist surfaces. Wipe hands with paper towels before product dries on skin, then reapply sanitizer and let air dry. Wash with soap and water as soon as possible.
    - The CDC has a number of hand-washing Fact Sheets that can be printed and posted to remind lab members of good handwashing techniques.

C. 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" and from the CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition.

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.

There are a number of resources that can provide valuable information on the risks associated with a range of human pathogens, including the CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) and Pathogen Safety Data Sheets produced by the Public Health Agencies of Canada. These include information for many agents that are associated with laboratory acquired infections (LAIs) or are of increased public concern. Agent summary statements also identify known and suspected routes of transmission of LAIs, and, when available, information on infective dose, host range, agent stability in the environment, protective immunizations, and attenuated strains of the agent. A thorough examination of the agent hazards is necessary when the intended use of an agent does not correspond with the general conditions described in the agent summary statement or when an agent summary statement is not available. In addition, it is always helpful to seek guidance from colleagues with experience in handling the agent and from biological safety professionals.

The *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)* has incorporated an agent Risk Group (RG) classification for laboratory use that describes four general Risk Groups based on these principle characteristics and the route of transmission of the natural disease; this list is found in Appendix B of the *NIH Guidelines*. ABSA International also has a compendium of organisms and Risk Group assignments from several countries and organizations available at https://my.absa.org/Riskgroups. Agent Risk Group assignments assist with an initial estimate of the pathogen's risk; the assessment must be modified appropriately based on the unique risks faced by each laboratory for the specific work being done. **The four groups address the risk to both the laboratory worker and the community and correlate with, but do not equate to, Biosafety Levels.** In addition, the IBC has developed Fact Sheets providing information for conducting risk assessments for work with many recombinant viral vectors. These can be found here.

TABLE 1. Laboratory activities associated with exposure to infectious agents	
Routes of exposure/transmission	**Activities/practices**
Ingestion/oral	• Pipetting by mouth • Splashing infectious material • Placing contaminated material or fingers in mouth • Eating, drinking, using lipstick or lip balm
Percutaneous inoculation/nonintact skin	• Manipulating needles and syringes • Handling broken glass and other sharp objects • Bites and scratches from animals and insects • Using scalpels to cut tissue for specimen processing • Waste disposal (containers with improperly disposed sharps)
Direct contact with mucous membranes	• Splashing or spilling infectious material into eye, mouth, nose • Splashing or spilling infectious material onto intact and nonintact skin • Working on contaminated surfaces • Handling contaminated equipment (i.e., instrument maintenance) • Inappropriate use of loops, inoculating needles, or swabs containing specimens or culture material • Bites and scratches from animals and insects • Waste disposal • Manipulation of contact lenses
Inhalation of aerosols	• Manipulating needles, syringes, and sharps • Manipulating inoculation needles, loops, and pipettes • Manipulating specimens and cultures • 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: Bovine rhinotracheitis virus Bovine diarrhea virus Parainfluenza type 3 Bovine enterovirus Bovine herpesvirus 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

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.
-

Risk Communication

An effective culture of safety depends on the effective communication and reporting of risk indicators, including incidents and near misses, in a non-punitive manner. Documents communicating the fundamental elements of a safety program are an important part of this culture and form the basis of the risk assessment; this includes hazard communication to all stakeholders. Institutional leadership can engage workers at all levels by collaborating with institutional safety programs and committing to and supporting a safe working environment.

The principal investigator or the facility equivalent has the primary responsibility for communicating hazards and risks in the laboratory. Staff must have the ability to report issues, including incidents and near misses without fear of reprisal. Laboratory staff, IBCs or equivalent resource, biosafety professionals, Institutional Animal Care and Use Committees (IACUCs), and laboratory animal veterinarians also have responsibility for identifying biological risks associated with laboratory work and communicating institute-wide risk management practices.

Facilitating a Culture of Safety through Risk Assessment

The goal of your risk assessment is to address all realistic, perceivable risks to protect personnel, the community, and the environment. Research progress, changes in personnel, and changes in regulation over time drive programmatic change and demand reconsideration of all factors, as periodically necessary. Risk assessment is an ongoing process, and all personnel have a role in its success.

The challenge is to develop good habits and procedures through training and competency checks with the support of leadership. Once established, these practices will persist to further instill a culture of safety. A sound risk communication strategy is also critical for both hazard identification and successful implementation. While policies and plans are tangible assets derived from the risk assessment process, the ultimate success will be measured by whether you establish, strengthen, and sustain a culture of safety while encouraging communication about risks between management and staff to prevent accidents before they happen.

The regular review of all hazards, prioritization of risk, multidisciplinary review of priority risks, and establishment of risk mitigation measures demonstrate the institution’s commitment to a safe and secure working environment and form the cornerstone of a biosafety program. The approach to risk assessment outlined in the preceding section is not static and benefits from active participation by all relevant stakeholders. Aim for ongoing evaluation and periodic readjustments to stay aligned with

the changing needs of the institution and to protect all persons from potential exposure to biological materials in laboratories and associated facilities.

D. 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 BMBL and Pathogen Safety Data Sheets provided by the Public Health Agency 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 (except those listed as RG3, including SARS-associated Coronavirus (SARS-CoV), SARS-associated Coronavirus-2 (SARS-CoV-2 or COVID-19) or Middle East Respiratory Syndrome Coronavirus (MERS-CoV))

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 *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 below, *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 *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)
- SARS-associated coronavirus-2 (SARS-CoV-2 or COVID-19)
- 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 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*. Routine operations with dilute toxin solutions are conducted using **Biosafety Level 2 (BSL-2)** practices: see below).

E. Biosafety Levels (BSL)

In general, the four biosafety levels correspond directly to the four risk groups of microorganisms listed in Section D. 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, 6th Edition.

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 (BSL-1) is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans and that present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open benchtops using standard microbiological practices. Special containment equipment or facility design is not generally required but may be used as determined by appropriate risk assessment. Laboratory personnel receive specific training in the procedures conducted in the laboratory and are supervised by a scientist with 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 EHS: more information can be found on the EHS 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. A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated, as necessary.
    a. The safety manual contains sufficient information to describe the biosafety and containment procedures for the organisms and biological materials in use, appropriate agent-specific decontamination methods, and the work performed.
    b. The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, and other potential emergencies. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.

3. A sign is posted at the entrance to the laboratory when infectious materials are present. Posted information includes: the laboratory's Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the laboratory.
4. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the laboratory.
5. 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. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.
    b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
6. Eating, drinking, smoking, chewing gum, handling contact lenses, and applying cosmetics are not permitted in the work area. Food must be stored in cabinets or refrigerators located OUTSIDE of the work area.
7. Do not put items (e.g., pens and pencils) in the mouth.
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 are always taken with sharp items. These include:
    a. Plasticware is substituted for glassware whenever possible.
    b. Use of needles and syringes or other sharp instruments is limited in the laboratory and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.
        i. Careful management of needles and other sharps are of primary importance.
        ii. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
        iii. If absolutely necessary to remove a needle from a syringe (e.g. to prevent lysing blood cells) or recap a need (e.g. loading syringes in one room and injecting

animals in another), a hands-free device or comparable safety procedure must be used (e.g. a needle remover on a sharps container, the use of forceps to hold the cap when recapping a needle), or placing the syringe and needle inside a puncture resistant tube.

iv. Used disposable needles and syringes are carefully placed in conveniently located puncture-resistant containers used for sharps disposal immediately after use.

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. 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. Gloves are not worn outside of the laboratory.

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

    c. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

    d. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

12. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.

13. Persons wash their hands after working with potentially hazardous materials and animals and before leaving the laboratory.

14. Long pants, or other clothing (such as scrubs), to cover exposed skin down to the shoes, must be worn.

15. Solid, closed toe and heel shoes that cover the entire foot must be worn.

16. Long hair is restrained so that it cannot contact hands, specimens, containers, or equipment.

17. An effective integrated pest management program is implemented.

18. 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 EHS.

19. The laboratory supervisor ensures that laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and that appropriate records are maintained. Personnel must receive annual updates or additional training when procedural or policy changes occur. All persons entering the facility are advised of the potential hazards, are instructed on the appropriate safeguards, and read and follow instructions on practices and procedures.
20. Personal health status may affect an individual's susceptibility to infection and ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. 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

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

1. Special containment equipment is generally not required for manipulation of agents assigned BSL-1.
2. Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory to prevent contamination of personal clothing. Before leaving the laboratory for non-laboratory area (e.g., elevators, 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.
3. 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 in laboratories shall also wear eye protection. Eye protection and face protection are disposed of with other contaminated laboratory waste or decontaminated after use.
4. In circumstances where research animals are present in the laboratory, the risk assessment considers appropriate eye, face, and respiratory protection, as well as potential animal allergens.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratories have doors for access control.
2. Laboratories have a sink for handwashing.
3. An eyewash station is readily available in the laboratory.
4. The laboratory is designed so that it can be easily cleaned.
    a. Carpets and rugs in laboratories are not appropriate.
    b. Spaces between benches, cabinets, and equipment are accessible for cleaning.
5. Laboratory furniture can support anticipated loads and uses.
    a. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
    b. Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
6. Laboratory windows that open to the exterior are fitted with screens.
7. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.

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

Biosafety Level 2 (BSL-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 unless approved by the Biosafety Officer.

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 at the discretion of the Principal Investigator/Supervisor when experiments are in progress.
2. A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated, as necessary.
    a. The manual must, at a minimum, provide a risk assessment for the organisms and biological materials used, describe the physical containment and PPE requirements, describe appropriate disinfectants, waste disposal, and describe agent-specific

decontamination methods. Personnel are advised of special hazards and are required to read instructions on practices and procedures and how to follow them.

b. The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, and other potential emergencies. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.

3. A sign incorporating the universal biohazard symbol is posted at the entrance to the laboratory when infectious materials are present. Posted information includes: the laboratory's Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the laboratory.
4. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the laboratory.
5. 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. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.
   b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
6. Eating, drinking, smoking, chewing gum, handling contact lenses, and applying cosmetics are not permitted in the work area. Food must be stored in cabinets or refrigerators located OUTSIDE of the work area.
7. Do not put items (e.g., pens and pencils) in the mouth.
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 are always taken with sharp items. These include:
   a. Plasticware is substituted for glassware whenever possible.
   b. Use of needles and syringes or other sharp instruments is limited in the laboratory and is restricted to situations where there is no alternative (e.g., parenteral injection, blood

collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.

i. Careful management of needles and other sharps are of primary importance.
ii. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
iii. If absolutely necessary to remove a needle from a syringe (e.g. to prevent lysing blood cells) or recap a need (e.g. loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g. a needle remover on a sharps container, the use of forceps to hold the cap when recapping a needle), or placing the syringe and needle inside a puncture resistant tube.
iv. Used disposable needles and syringes are carefully placed in conveniently located puncture-resistant containers used for sharps disposal immediately after use.

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. Gloves are 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-2 workers must:
    a. Gloves are not worn outside of the laboratory.
    b. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    c. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
    d. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
12. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
13. Persons wash their hands after working with potentially hazardous materials and animals and before leaving the laboratory.
14. Long pants, or other clothing (such as scrubs), to cover exposed skin down to the shoes, must be worn.
15. Solid, closed toe and heel shoes that cover the entire foot must be worn.

16. Long hair is restrained so that it cannot contact hands, specimens, containers, or equipment.
17. An effective integrated pest management program is implemented.
18. 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 EHS.
19. The laboratory supervisor ensures that laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and that appropriate records are maintained. Personnel must receive annual updates or additional training when procedural or policy changes occur. All persons entering the facility are advised of the potential hazards, are instructed on the appropriate safeguards, and read and follow instructions on practices and procedures.
20. Personal health status may affect an individual's susceptibility to infection and ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. 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 and techniques before working with BSL-2 agents.

5. 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.
    c. If it is not possible to perform a procedure within a BSC or other physical containment device, a combination of appropriate personal protective equipment and administrative controls are used, based on a risk assessment.

    Note: See Section K and Appendix A for instruction on the use of a Class II Biosafety Cabinet.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment 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.
8. A method for decontaminating all laboratory wastes must be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). If autoclaves are used, they must be tested weekly or every 40 hours (whichever is longer) with a biological indicator, such as *Bacillus stearothermophilus* (See Appendix H).
9. Incidents that result in exposures to infectious materials are immediately reported to the Principal Investigator/Supervisor and to the IBC/EHS. 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.

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

1. Protective laboratory coats, gowns, or uniforms designated for laboratory use are worn while working with hazardous materials and removed before leaving for non-laboratory areas (e.g., cafeteria, library, and administrative offices). Protective clothing is disposed of appropriately or deposited for laundering by the institution. Laboratory clothing is not taken home.
2. Eye protection and face protection (e.g., safety glasses, goggles, mask, face shield or other splatter guard) are used for manipulations or activities that may result in splashes or sprays of infectious or other hazardous materials. Eye protection and face protection are disposed of with other contaminated laboratory waste or decontaminated after use.
3. The risk assessment considers whether respiratory protection is needed for the work with hazardous materials. If needed, relevant staff are enrolled in a properly constituted respiratory protection program.
4. In circumstances where research animals are present in the laboratory, the risk assessment considers appropriate eye, face, and respiratory protection, as well as potential animal allergens.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors are self-closing and have locks in accordance with the institutional policies.
2. Laboratories have a sink for handwashing. It should be located near the exit door.
3. An eyewash station is readily available in the laboratory.
4. The laboratory is designed so that it can be easily cleaned.
    a. Carpets and rugs in laboratories are not appropriate.
    b. Spaces between benches, cabinets, and equipment are accessible for cleaning.
5. Laboratory furniture can support anticipated loads and uses.
    a. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
    b. Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
6. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they are fitted with screens.
7. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.

8. Vacuum lines in use are protected with liquid disinfectant traps and in-line HEPA filters or their equivalent. Filters are replaced, as needed, or are on a replacement schedule determined by a risk assessment.
9. There are no specific requirements for ventilation systems. However, the planning of new facilities considers mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
10. BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness. See Appendix A.
    a. BSCs are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
    b. BSCs can be connected to the laboratory exhaust system by either a canopy connection (Class IIA only) or directly exhausted to the outside through a hard connection (Class IIB, IIC, or III). Class IIA or IIC BSC exhaust can be safely recirculated back into the laboratory environment if no volatile toxic chemicals are used in the cabinet.
    c. BSCs are certified at least annually to ensure correct performance.

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 CDC 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-2 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 unless approved by the IBC. 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 the IBC and/or an 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" 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 even when working in the biosafety cabinet.
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.*
7. ***Black box warning (Samples from COVID-19 Patients):*** *SARS-CoV-2, the etiologic agent that causes COVID-19, is a Risk Group 3 agent that is readily transmitted through*

aerosolization. This research poses a risk of accidental human exposure to SARS-CoV-19 and could result in the development of COVID-19.

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 and controlled by the Principal Investigator/Supervisor. Lab doors are secured and locked at the end of the day.
2. A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated, as necessary.
    a. The manual must, at a minimum, provide a risk assessment for the organisms and biological materials used, describe the physical containment and PPE requirements, describe appropriate disinfectants, waste disposal, and describe agent-specific decontamination methods. Personnel are advised of special hazards and are required to read instructions on practices and procedures and how to follow them.
    b. The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, and other potential

emergencies. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.

3. A sign incorporating the universal biohazard symbol is posted at the entrance to the laboratory when infectious materials are present. Posted information includes: the laboratory's Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the laboratory.
4. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the laboratory.
5. 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. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.
    b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
6. Eating, drinking, smoking, chewing gum, handling contact lenses, and applying cosmetics are not permitted in the work area. Food must be stored in cabinets or refrigerators located OUTSIDE of the work area.
7. Do not put items (e.g., pens and pencils) in the mouth.
8. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
9. Sharps are not allowed unless approved by the IBC. If they are approved, 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: see the precautions described under BSL-2 Standard Microbiological Practices for examples.
10. All procedures are performed carefully to minimize the creation of aerosols.
11. Gloves are 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-3 workers must:
    a. Gloves are not worn outside of the laboratory.

b. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
c. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
d. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

12. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
13. Persons wash their hands after working with potentially hazardous materials and animals and before leaving the laboratory.
14. Long pants, or other clothing (such as scrubs), to cover exposed skin down to the shoes, must be worn.
15. Solid, closed toe and heel shoes that cover the entire foot must be worn.
16. Long hair is restrained so that it cannot contact hands, specimens, containers, or equipment.
17. An effective integrated pest management program is implemented.
18. 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 EHS.
19. The laboratory supervisor ensures that laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and that appropriate records are maintained. Personnel must receive annual updates or additional training when procedural or policy changes occur. All persons entering the facility are advised of the potential hazards, are instructed on the appropriate safeguards, and read and follow instructions on practices and procedures.
20. Personal health status may affect an individual's susceptibility to infection and ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having these conditions shall be encouraged to self-identify to the Occupational Medicine Clinic for appropriate counseling and guidance.

B. Special Practices

1. All persons entering the laboratory are advised of the potential hazards and meet specific entry/exit requirements in accordance with institutional policies. Only persons whose presence in the facility or laboratory areas is required for scientific or support purposes are authorized to enter.
2. All persons who enter operational laboratory areas are provided information on signs and symptoms of disease and receive occupational medical services including medical evaluation, surveillance, and treatment, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
3. The laboratory supervisor is responsible for ensuring that laboratory personnel demonstrate proficiency in standard microbiological practices and techniques for working with agents requiring BSL-3 containment.
4. A system is established for reporting and documenting near misses, laboratory accidents, exposures, unanticipated absences due to potential Laboratory-associated infection, and for the medical surveillance of potential laboratory-associated illnesses.
5. Incidents that result in exposure to infectious materials are immediately evaluated per institutional policy. All such incidents are reported to the laboratory supervisor, institutional management, and appropriate safety, compliance, and security personnel according to institutional policy. Appropriate records are maintained.
6. Biological materials that require BSL-3 containment are placed in a durable leak-proof sealed primary container and then enclosed in a non-breakable, sealed secondary container prior to removal from the laboratory. Once removed, the primary container is opened within a BSC in BSL-3 containment unless a validated inactivation method is used. See Appendix J. The inactivation method is documented in-house with viability testing data to support the method.
7. All procedures involving the manipulation of infectious materials are conducted within a BSC or other physical containment device, when possible. No work with open vessels is conducted on the bench. If it is not possible to perform a procedure within a BSC or other physical containment device, a combination of personal protective equipment and other administrative and/or engineering controls, such as centrifuge safety cups or sealed rotors, are used, based on a risk assessment. Loading and unloading of the rotors and centrifuge safety cups take place in the BSC or another containment device.
8. Laboratory equipment must be routinely decontaminated, as well as after spills, splashes, or other potential contamination, and before repair, maintenance, or removal from the laboratory.

a. Equipment or material that might be damaged by high temperatures or steam is decontaminated using an effective and verified method, such as a gaseous or vapor method.

9. A method for decontaminating all laboratory waste is available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, or other validated decontamination method). If autoclaves are used, they must be tested weekly or every 40 hours (whichever is longer) with a biological indicator, such as *Bacillus stearothermophilus* (See Appendix H).

10. Decontamination of the entire laboratory is considered when there has been gross contamination of the space, significant changes in laboratory usage, major renovations, or maintenance shutdowns. Selection of the appropriate materials and methods used to decontaminate the laboratory is based on a risk assessment.

11. Decontamination processes are verified on a routine basis.

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

1. Laboratory workers wear protective clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.

2. Based on work being performed, additional PPE may be required.

   a. Eye protection and face protection (e.g., safety glasses, goggles, mask, face shield or other splash guard) are used for manipulations or activities that may result in splashes or sprays of infectious or other hazardous materials. Eye protection and face protection are disposed of with other contaminated laboratory waste or decontaminated after use.

   b. Two pairs of gloves are worn, when appropriate.

   c. Respiratory protection is considered. Staff wearing respiratory protection are enrolled in a properly constituted respiratory protection program.

   d. Shoe covers are considered.

3. In circumstances where research animals are present in the laboratory, the risk assessment considers appropriate eye, face, and respiratory protection, as well as potential animal allergens.

D. Laboratory Facilities (Secondary Barriers)

1. The laboratory is separated from areas that are open to unrestricted traffic flow within the building.

a. Laboratory access is restricted. Laboratory doors are lockable in accordance with institutional policies. Access to the laboratory is through two consecutive self-closing doors. A clothing change room and/or an anteroom may be included in the passageway between the two self-closing doors.

2. Laboratories have a sink for handwashing. The sink is hands-free or automatically operated and should be located near the exit door.
   a. If a laboratory suite is segregated into different zones, a sink is also available for handwashing in each zone.
3. An eyewash station is readily available in the laboratory.
4. The laboratory is designed, constructed, and maintained to facilitate cleaning, decontamination, and housekeeping.
   a. Carpets and rugs are not permitted.
   b. Spaces between benches, cabinets, and equipment are accessible for cleaning.
   c. Seams, floors, walls, and ceiling surfaces are sealed. Spaces around doors and ventilation openings are capable of being sealed to facilitate space decontamination.
   d. Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Flooring is seamless, sealed, or poured with integral cove bases.
   e. Walls and ceilings are constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
5. Laboratory furniture can support anticipated loads and uses.
   a. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
   b. Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant.
6. All windows in the laboratory are sealed.
7. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
8. Vacuum lines in use are protected with liquid disinfectant traps and in-line HEPA filters or their equivalent. Filters are replaced, as needed, or are on a replacement schedule determined by a risk assessment. Vacuum lines not protected as described are capped. The placement of an additional HEPA filter immediately prior to a central vacuum pump is considered.
9. A ducted mechanical air ventilation system is required. This system provides sustained directional airflow by drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas. The laboratory is designed such that under failure conditions the airflow will not be reversed at the containment barrier.

a. A visual monitoring device that confirms directional airflow is provided at the laboratory entry. Audible alarms to notify personnel of airflow disruption are considered.
b. The laboratory exhaust air is not re-circulated to any other area in the building.
c. The laboratory exhaust air is dispersed away from occupied areas and from building air intake locations or the exhaust air is HEPA filtered.

10. BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness. See Appendix A.
    a. a. BSCs are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
    b. b. BSCs can be connected to the laboratory exhaust system by either a canopy connection (Class IIA only) or directly exhausted to the outside through a hard connection (Class IIB, IIC, or III). Class IIA or IIC BSC exhaust can be safely recirculated back into the laboratory environment if no volatile toxic chemicals are used in the cabinet.
    c. c. BSCs are certified at least annually to ensure correct performance.
    d. d. Class III BSCs are provided supply air in such a manner that prevents positive pressurization of the cabinet or the room.
11. Equipment that may produce infectious aerosols is used within primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters are tested annually and replaced as needed.
12. Facility is constructed to allow decontamination of the entire laboratory when there has been gross contamination of the space, significant changes in usage, major renovations, or maintenance shutdowns. Selection of the appropriate materials and methods used to decontaminate the laboratory is based on the risk assessment.
    a. Facility design consideration is given to means of decontaminating large pieces of equipment before removal from the laboratory.
13. Enhanced environmental and personal protection may be necessary based on risk assessment and applicable local, state, or federal regulations. These laboratory enhancements may include 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; containment of other piped services; or advanced access control devices, such as biometrics.

14. When present, HEPA filter housings have gas-tight isolation dampers, decontamination ports, and/or bag-in/bag-out (with appropriate decontamination procedures) capability. All HEPA filters are located as near as practicable to the laboratory to minimize the length of potentially contaminated ductwork. The HEPA filter housings allow for leak testing of each filter and assembly. The filters and housings are certified at least annually.
15. The BSL-3 facility design, operational parameters, and procedures are verified and documented prior to operation. Facilities are tested annually or after significant modification to ensure operational parameters are met. Verification criteria are modified as necessary by operational experience.
16. Appropriate communication systems are provided between the laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and emergency access or egress are developed and implemented.

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

Biosafety Level 4 (BSL-4) is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening diseases that are frequently fatal, agents for which there are no vaccines or treatments, or work with a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment are handled at this level until sufficient data are obtained to re-designate the level. Laboratory staff receive specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors are competent in handling agents and procedures requiring BSL-4 containment. The laboratory supervisor controls access to the laboratory in accordance with institutional policies.

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 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 biosafety, biosecurity, and emergencies within the animal facility.
2. Access to the animal room is limited. Only those persons required for experimental, husbandry, or support purposes are authorized to enter the facility.
3. Each institution ensures that worker safety and health concerns are addressed as part of the animal protocol review process. Consideration is given to specific biohazards unique to the animal species and protocol in use. Prior to beginning a study, animal protocols are reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) as well as the Institutional Biosafety Committee (IBC), as appropriate.
4. The supervisor ensures that animal care, facility, and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization). Personnel receive annual updates and additional training when equipment, procedures, or policies change. Records are maintained for all hazard evaluations, training sessions, and staff attendance. All persons, including facility equipment personnel, service workers, and visitors, are advised of the potential hazards (e.g., naturally acquired or research pathogens, allergens); are instructed on the appropriate safeguards; and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.
5. Personal health status may affect an individual's susceptibility to infection or ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and

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

6. Appropriate occupational medical services are in place, as determined by risk assessment.
    a. An animal allergy prevention program is part of the medical surveillance.
    b. Personnel using respirators for animal allergy prevention are enrolled in an appropriately constituted respiratory protection program.
7. A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated, as necessary.
    a. The safety manual contains sufficient information to describe the biosafety and containment procedures for the experimental animals, organisms, and biological materials in use, appropriate agent-specific decontamination methods, and the work performed.
    b. The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, escape of animals within the animal facility, and other potential emergencies. A plan for the disposition of animals during emergency situations is included. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.
8. A sign is posted at the entrance to the animal room when infectious agents are present. Posted information includes: the room's Animal Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the animal room. Agent information is posted in accordance with the institutional policy.
9. Long hair is restrained so that it cannot contact hands, animals, specimens, containers, or equipment.
10. Gloves are worn to protect hands from exposure to hazardous materials and when handling animals.
    a. Glove selection is based on an appropriate risk assessment.
    b. Consider the need for bite and/or scratch-resistant gloves.
    c. Gloves worn inside the animal facility are not worn outside the animal facility.
    d. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    e. Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated animal facility waste.

11. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
12. Persons wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or manipulated.
13. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in animal areas.
14. Mouth pipetting is prohibited. Mechanical pipetting devices are used.
15. 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 are always taken with sharp items. These include:
    a. Plasticware is substituted for glassware whenever possible.
    b. Use of needles and syringes or other sharp instruments is limited in the laboratory and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.
        i. Careful management of needles and other sharps are of primary importance.
        ii. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
        iii. If absolutely necessary to remove a needle from a syringe (e.g. to prevent lysing blood cells) or recap a need (e.g. loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g. a needle remover on a sharps container, the use of forceps to hold the cap when recapping a needle), or placing the syringe and needle inside a puncture resistant tube.
        iv. Used disposable needles and syringes are carefully placed in conveniently located puncture-resistant containers used for sharps disposal immediately after use.
    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.
16. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
17. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly

trained and equipped to work with infectious material. A spill procedure is developed and posted within the animal facility.

18. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:
    a. Materials to be decontaminated outside of the immediate animal room are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.
    b. Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.
19. An effective integrated pest management program is required.
20. Animals and plants not associated with the work being performed are not permitted in the areas where infectious materials and/or animals are housed or manipulated.

B. Special Practices

None required.

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

1. Specialized devices or equipment for restraint or containment may be required as determined by appropriate risk assessment.
2. Laboratory coats, gowns, or uniforms are the minimum recommended to prevent contamination of personal clothing. Protective outer clothing is not worn outside areas where infectious materials and/or animals are housed or manipulated. Gowns and uniforms are not worn outside the animal facility.
3. Eye protection and face protection (e.g., safety glasses, goggles, mask, face shield, or other splatter guard) are used for manipulations or activities that may result in splashes or sprays of infectious or other hazardous materials. Eye protection and face protection are disposed of with other contaminated facility waste or decontaminated after use.
4. Persons having contact with NHPs assess the risk of mucous membrane exposure and wear protective equipment (e.g., face shield, surgical mask, goggles), as appropriate.
5. Additional PPE is considered for persons working with large animals.

D. Animal Facilities (Secondary Barriers)

1. ABSL-1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Consider placing animal areas away from exterior walls of buildings to minimize the impact from the outside environment temperatures.
    a. External facility doors are self-closing and self-locking.
    b. Access to the animal facility is restricted.
    c. 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 never propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
2. The animal facility has a sink for handwashing.
    a. Emergency eyewash and shower are readily available, easily accessible, and appropriately maintained.
    b. Sink traps are filled with water and/or appropriate disinfectant to prevent the migration of vermin and gases.
    c. If open floor drains are provided, the traps are filled with water and/or appropriate disinfectant or sealed to prevent the migration of vermin and gases.
3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (e.g., walls, floors, ceilings) are water-resistant.
    a. Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Floors with drains are sloped toward drains to facilitate cleaning.
    b. It is recommended that penetrations in floors, walls, and ceilings be sealed, including openings around ducts, doors, doorframes, outlets, and switch plates to facilitate pest control and proper cleaning.
    c. Internal facility fixtures, such as light features, air ducts, and utility pipes, are designed and installed to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
    d. External windows are not recommended; if present, they are resistant to breakage. Where possible, windows are sealed. If the animal facility has windows that open, they are fitted with fly screens.
    e. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
4. Furniture can support anticipated loads and uses.
    a. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

b. Chairs used in animal areas are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant and sealed to prevent harboring of insects/vermin.

c. Equipment and furnishings are carefully evaluated to minimize exposure of personnel to pinch points and sharp edges and corners.

5. Ventilation is provided in accordance with the *Guide for the Care and Use of Laboratory Animals*.
   a. Ventilation system design considers the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
6. Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washers have a final rinse temperature of at least 180°F. If manual cage washing is utilized, ensure that appropriate disinfectants are selected.

7. Animal Biosafety Level 2

Animal Biosafety Level 2 (ABSL-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 posing a moderate hazard to personnel and the environment. It also addresses hazards from ingestion and from percutaneous and mucous membrane exposure.

ABSL-2 requires that, in addition to the requirements for ABSL-1, a BSC or other physical containment equipment is used when procedures involve the manipulation of infectious materials or where aerosols or splashes may be created.

Appropriate PPE is worn to reduce exposure to infectious agents, animals, and contaminated equipment. An appropriate occupational health program is in place, as determined by risk assessment.

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 biosafety, biosecurity, and emergencies within the animal facility.
2. Access to the animal room is limited. Only those persons required for experimental, husbandry, or support purposes are authorized to enter the facility.

3. Each institution ensures that worker safety and health concerns are addressed as part of the animal protocol review process. Consideration is given to specific biohazards unique to the animal species and protocol in use. Prior to beginning a study, animal protocols are reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) as well as the Institutional Biosafety Committee (IBC), as appropriate.
4. The supervisor ensures that animal care, facility, and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization). Personnel receive annual updates and additional training when equipment, procedures, or policies change. Records are maintained for all hazard evaluations, training sessions, and staff attendance. All persons, including facility equipment personnel, service workers, and visitors, are advised of the potential hazards (e.g., naturally acquired or research pathogens, allergens); are instructed on the appropriate safeguards; and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.
5. Personal health status may affect an individual's susceptibility to infection or ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance. Facility supervisors ensure that medical staff are informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care, and manipulations.
6. Appropriate occupational medical services are in place, as determined by risk assessment.
    a. An animal allergy prevention program is part of the medical surveillance.
    b. Personnel using respirators for animal allergy prevention are enrolled in an appropriately constituted respiratory protection program.
7. A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated, as necessary.
    a. The safety manual contains sufficient information to describe the biosafety and containment procedures for the experimental animals, organisms, and biological

materials in use, appropriate agent-specific decontamination methods, and the work performed.

b. The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, escape of animals within the animal facility, and other potential emergencies. A plan for the disposition of animals during emergency situations is included. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.

8. A sign is posted at the entrance to the animal room when infectious agents are present. Posted information includes: the room's Animal Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the animal room. Agent information is posted in accordance with the institutional policy.
9. Long hair is restrained so that it cannot contact hands, animals, specimens, containers, or equipment.
10. Gloves are worn to protect hands from exposure to hazardous materials and when handling animals.
    a. Glove selection is based on an appropriate risk assessment.
    b. Consider the need for bite and/or scratch-resistant gloves.
    c. Gloves worn inside the animal facility are not worn outside the animal facility.
    d. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    e. Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated animal facility waste.
11. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
12. Persons wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or manipulated.
13. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in animal areas.
14. Mouth pipetting is prohibited. Mechanical pipetting devices are used.
15. 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 are always taken with sharp items. These include:

a. Plasticware is substituted for glassware whenever possible.
b. Use of needles and syringes or other sharp instruments is limited in the laboratory and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.
    i. Careful management of needles and other sharps are of primary importance.
    ii. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
    iii. If absolutely necessary to remove a needle from a syringe (e.g. to prevent lysing blood cells) or recap a need (e.g. loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g. a needle remover on a sharps container, the use of forceps to hold the cap when recapping a needle), or placing the syringe and needle inside a puncture resistant tube.
    iv. Used disposable needles and syringes are carefully placed in conveniently located puncture-resistant containers used for sharps disposal immediately after use.
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.

16. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
17. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the animal facility.
18. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:

    a. Materials to be decontaminated outside of the immediate animal room are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.
    b. Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.

19. An effective integrated pest management program is required.
20. Animals and plants not associated with the work being performed are not permitted in the areas where infectious materials and/or animals are housed or manipulated.

C. Special Practices

1. Animal care staff are provided information on signs and symptoms of disease, receive occupational medical services including medical evaluation, surveillance, and treatment, as appropriate, and are offered available immunizations for agents handled or potentially present in the facility.
2. All procedures involving the manipulation of infectious materials that may generate an aerosol are conducted within a BSC or other physical containment device, when possible. If it is not possible to perform a procedure within a BSC or other physical containment device, a combination of appropriate personal protective equipment, administrative and/or engineering controls (e.g., downdraft table) are used, based on a risk assessment.
    a. Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint, chemical restraint) are used whenever possible.
    b. Equipment, cages, and racks are handled in a manner that minimizes contamination of other areas. Cages are decontaminated prior to washing.
3. Develop and implement an appropriate decontamination program in compliance with applicable institutional, local, and state requirements.
    a. Equipment is decontaminated before repair, maintenance, or removal from the animal facility. A method for decontaminating routine husbandry equipment and sensitive electronic or medical equipment is identified and implemented.
    b. Decontamination of an entire animal room is considered when there has been gross contamination of the space, significant changes in usage, and for major renovations or maintenance shutdowns. Selection of the appropriate materials and methods used to decontaminate the animal room is based on the risk assessment.
    c. Decontamination processes are verified on a routine basis.
4. Incidents that may result in exposure to infectious materials are immediately evaluated per institutional policies. All such incidents are reported to the animal facility supervisor and any other personnel designated by the institution. Appropriate records are maintained

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

1. Properly maintained BSCs and 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 the necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals. A risk assessment dictates the type of other physical containment devices used when BSCs may not be suitable.

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 micro-isolator lids or other equivalent primary containment systems for larger animals.
b. If used, actively ventilated caging systems are designed to contain microorganisms. Exhaust plenums for these systems are sealed. Safety mechanisms are in place to prevent the cage and exhaust plenums from becoming positively pressurized if the exhaust fan fails. The system is also alarmed to indicate operational malfunctions. Exhaust HEPA filters and filter housings are certified annually.

2. Protective clothing, such as gowns, uniforms, scrubs, or laboratory coats, and other PPE are worn while in the areas where infectious materials and/or animals are housed or manipulated.

   a. Scrubs and uniforms are removed before leaving the animal facility.
   b. Reusable clothing is appropriately contained and decontaminated before being laundered. Animal facility and protective clothing is never taken home.
   c. Disposable PPE and other contaminated waste are appropriately contained and decontaminated prior to disposal.

3. Eye protection and face protection (e.g., safety glasses, goggles, mask, 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 when the animal or microorganisms is handled outside the BSC or another containment device. Eye protection and face protection are disposed of with other contaminated facility waste or decontaminated after use.
4. Persons having contact with NHPs assess the risk of mucous membrane exposure and wear protective equipment (e.g., face shield, surgical mask, goggles), as appropriate.
5. Additional PPE is considered for persons working with large animals.
6. Based on the pathogen and work performed, respiratory protection may be considered for staff enrolled in a properly constituted respiratory protection program.

D. Animal Facilities (Secondary Barriers)

1. ABSL-2 facilities should be separated from the general traffic patterns of the building and restricted, as appropriate. Consider placing animal areas away from exterior walls of buildings to minimize the impact from the outside environment temperatures.

a. External facility doors are self-closing and self-locking.

b. Access to the animal facility is restricted.

c. 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 are never to be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.

2. A handwashing sink is located at the exit of the areas where infectious materials and/or animals are housed or manipulated. Additional sinks for handwashing are located in other appropriate locations within the facility. If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink is also available for handwashing at the exit from each segregated area.

   a. Emergency eyewash and shower are readily available, easily accessible, and appropriately maintained.

   b. Sink traps are filled with water and/or appropriate disinfectant to prevent the migration of vermin and gases.

   c. If open floor drains are provided, the traps are filled with water and/or appropriate disinfectant or sealed to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (e.g., walls, floors, and ceilings) are water-resistant.

   a. Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Floors with drains are sloped toward drains to facilitate cleaning.

   b. Penetrations in floors, walls, and ceiling surfaces are sealed, including openings around ducts, doors, doorframes, outlets, and switch plates to facilitate pest control and proper cleaning.

   c. Internal facility fixtures, such as light features, air ducts, and utility pipes, are designed and installed to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.

   d. External windows are not recommended; if present, they are sealed and resistant to breakage.

   e. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.

4. Furniture is minimized and can support anticipated loads and uses.

   a. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

b. Chairs used in animal areas are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant and sealed to prevent harboring of insects/vermin.

a. Equipment and furnishings are carefully evaluated to minimize exposure of personnel to pinch points and sharp edges and corners.

5. Ventilation is provided in accordance with the *Guide for the Care and Use of Laboratory Animals*.

   a. Ventilation system design considers the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

   b. The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways.

   c. A ducted exhaust air ventilation system is provided.

   d. Exhaust air is discharged to the outside without being recirculated to other rooms.

6. Mechanical cage washers have a final rinse temperature of at least 180°F. The cage wash area is 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.

7. BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness.

   a. BSCs are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, windows that can be opened, heavily traveled areas, and other possible airflow disruptions.

   b. BSCs can be connected to the animal facility exhaust system by either a canopy connection (Class IIA only) or directly exhausted to the outside through a hard connection (Class IIB, IIC, or III). Class IIA or IIC BSC exhaust can be safely recirculated back into the animal facility environment if no volatile toxic chemicals are used in the cabinet.

   c. BSCs are certified at least annually to ensure correct performance.

8. Vacuum lines in use are protected with liquid disinfectant traps and in-line HEPA filters or their equivalent. Filters are replaced, as needed, or on a replacement schedule determined by a risk assessment.

9. An autoclave is present in the animal facility to facilitate decontamination of infectious materials and waste. A validated alternative process (e.g., alkaline digestion, incineration) may be used for decontamination and disposal of carcasses.

8. ABSL-1+ (ABSL-2 to ABSL-1 Stepdown)

Inoculating non-permissive mice or rats with some replication deficient viral vectors are eligible for containment at ABSL-1+, also known as "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 6th 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: screening for other viruses may be required depending on the cell type. 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

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 in addition to the requirements for ABSL-2, all procedures are conducted in BSCs or by use of other physical containment equipment. Inward airflow at the containment boundary is maintained. Handwashing sinks are capable of hands-free operation.

Appropriate PPE is worn to reduce exposure to infectious agents, animals, and contaminated equipment.

The following standard and special safety practices, safety equipment, and facility specifications are necessary for ABSL-3.

A. Standard Microbiological Practices

1. The animal facility director establishes and enforces policies, procedures, and protocols for biosafety, biosecurity, and emergencies within the animal facility.
2. Access to the animal room is limited. Only those persons required for experimental, husbandry, or support purposes are authorized to enter the facility.
3. Each institution ensures that worker safety and health concerns are addressed as part of the animal protocol review process. Consideration is given to specific biohazards unique to the animal species and protocol in use. Prior to beginning a study, animal protocols are reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) as well as the Institutional Biosafety Committee (IBC), as appropriate.
4. The supervisor ensures that animal care, facility, and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization). Personnel receive annual updates and additional training when equipment, procedures, or policies change. Records are maintained for all hazard evaluations, training sessions, and staff attendance. All persons, including facility equipment personnel, service workers, and visitors, are advised of the potential hazards (e.g., naturally acquired or research pathogens, allergens); are instructed on the appropriate safeguards; and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.
5. Personal health status may affect an individual's susceptibility to infection or ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance. Facility supervisors ensure that medical staff are informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care, and manipulations.
6. Appropriate occupational medical services are in place, as determined by risk assessment.
    a. An animal allergy prevention program is part of the medical surveillance.
    b. Personnel using respirators for animal allergy prevention are enrolled in an appropriately constituted respiratory protection program.

7. A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated, as necessary.
    a. The safety manual contains sufficient information to describe the biosafety and containment procedures for the experimental animals, organisms, and biological materials in use, appropriate agent-specific decontamination methods, and the work performed.
    b. The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, escape of animals within the animal facility, and other potential emergencies. A plan for the disposition of animals during emergency situations is included. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.
8. A sign is posted at the entrance to the animal room when infectious agents are present. Posted information includes: the room's Animal Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the animal room. Agent information is posted in accordance with the institutional policy.
9. Long hair is restrained so that it cannot contact hands, animals, specimens, containers, or equipment.
10. Gloves are worn to protect hands from exposure to hazardous materials and when handling animals.
    a. Glove selection is based on an appropriate risk assessment.
    b. Consider the need for bite and/or scratch-resistant gloves.
    c. Gloves worn inside the animal facility are not worn outside the animal facility.
    d. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    e. Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated animal facility waste.
11. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
12. Persons wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or manipulated.
13. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in animal areas.

14. Mouth pipetting is prohibited. Mechanical pipetting devices are used.
15. Sharps are not allowed unless approved by the IBC. If they are approved, 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: see the precautions described under Animal BSL-2 Standard Microbiological Practices for examples.
16. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
17. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the animal facility.
18. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:
    a. Materials to be decontaminated outside of the immediate animal room are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.
    b. Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.
19. An effective integrated pest management program is required.
20. Animals and plants not associated with the work being performed are not permitted in the areas where infectious materials and/or animals are housed or manipulated.

B. Special Practices

1. Animal care staff are provided information on signs and symptoms of disease, receive occupational medical services including medical evaluation, surveillance, and treatment, as appropriate, and are offered available immunizations for agents handled or potentially present in the facility.
2. A system is established for reporting and documenting near misses, animal facility accidents, exposures, unanticipated absences due to potential Laboratory-associated infection, and for the medical surveillance of potential laboratory-associated illnesses.

3. Incidents that result in exposure to infectious materials are immediately evaluated per institutional policy. All such incidents are reported to the animal facility director, facility supervisor, institutional management, and appropriate facility safety, compliance, and security personnel according to institutional policy. Appropriate records are maintained.
4. Only necessary equipment and supplies are recommended to be taken inside the animal facility.
5. All procedures involving the manipulation of infectious materials that may generate an aerosol are conducted within a BSC or other physical containment device, when possible. If it is not possible to perform a procedure within a BSC or other physical containment device, a combination of appropriate personal protective equipment, administrative and/or engineering controls (e.g., downdraft table) are used, based on a risk assessment.
    a. Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint, chemical restraint) are used whenever possible.
    b. Equipment, cages, and racks are handled in a manner that minimizes contamination of other areas.
6. Biological materials that are to remain in a viable state during removal from the animal facility are placed in a durable leak-proof sealed primary container and then enclosed in a non-breakable, sealed secondary container prior to removal from the facility by authorized personnel. Once removed, the primary container is opened within a BSC in BSL-3 or ABSL-3 containment unless a validated inactivated method is used. See Appendix J. The inactivation method is documented in-house with viability testing data to support the method.
7. Develop and implement an appropriate decontamination program in compliance with applicable institutional, local, and state requirements.
    a. Equipment is decontaminated before repair, maintenance, or removal from the animal facility. A method for decontaminating routine husbandry equipment and sensitive electronic or medical equipment is identified and implemented.
    b. Decontamination of an entire animal room is considered when there has been gross contamination of the space, significant changes in usage, and for major renovations or maintenance shutdowns. Selection of the appropriate materials and methods used to decontaminate the animal room is based on the risk assessment.
    c. Decontamination processes are verified on a routine basis.

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

1. Properly maintained BSCs and other physical containment devices or equipment are used for manipulations of infectious materials and animals as determined by risk assessment.

a. The risk of infectious aerosols from infected animals or their bedding can be reduced if animals are housed in containment caging systems, such as solid wall and bottom cages covered with micro-isolator lids, open cages placed in inward flow ventilated enclosures, HEPA filter isolators and caging systems, or other equivalent primary containment systems.
    i. Actively ventilated caging systems are designed to prevent the escape of microorganisms from the cage. Exhaust plenums for these systems are sealed to prevent the escape of microorganisms if the ventilation system becomes static, and the exhaust is HEPA-filtered. Safety mechanisms are in place to prevent the cage and exhaust plenums from becoming positive to the surrounding area should the exhaust fan fail. The system is alarmed to indicate operational malfunctions.
b. When animals cannot be housed in ventilated containment cages/units, certain features of the animal room act as the primary barriers. The procedures in place include how workers are protected from agents shed by the animals (e.g., PPE enhancements) as well as how the environment is protected from such agents through the use of biocontainment enhancements such as some combination of boot or PPE change or surface decontamination at the door, a personal shower at the room level, and/or other procedures.

2. Special consideration is given to the potential for cross-contamination when open caging is used.
3. Personnel within the animal facility wear protective clothing, such as uniforms or scrubs.
    a. Disposable PPE such as non-woven, olefin cover-all suits, or wrap-around or solid-front gowns are worn over this clothing before entering areas where infectious materials and/or animals are housed or manipulated. Front-button, laboratory coats are unsuitable.
    b. Reusable clothing is appropriately contained and decontaminated before being laundered. Animal facility and protective clothing is never taken home.
    c. Disposable PPE is removed when leaving the areas where infectious materials and/or animals are housed or manipulated. Scrubs and uniforms are removed before leaving the animal facility.
    d. Disposable PPE and other contaminated waste are appropriately contained and decontaminated prior to disposal.
4. All personnel entering areas where infectious materials and/or animals are housed or manipulated wear appropriate head covering, eye, face, and respiratory protection. To prevent cross-contamination, boots, shoe covers, or other protective footwear are used where indicated and disposed of or decontaminated after use.
5. Head covering, eye protection, and face protection are disposed of with other contaminated animal facility waste or decontaminated after use.

6. Procedures may require wearing two pairs of gloves (i.e., double-glove). Change outer gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
7. Additional PPE is considered for persons working with large animals.

D. Animal Facilities (Secondary Barriers)

1. ABSL-2 facilities should be separated from the general traffic patterns of the building and restricted, as appropriate. Consider placing animal areas away from exterior walls of buildings to minimize the impact from the outside environment temperatures.
    a. External facility doors are self-closing and self-locking.
    b. Access to the animal facility is restricted.
    c. 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 are never to be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
    d. Entry into the containment area is via a double-door entry, which constitutes an anteroom/airlock and a change room. Exit showers may be considered based on risk assessment. An additional double-door anteroom or double-doored autoclave may be provided for movement of supplies and wastes into and out of the facility.
2. A handwashing sink is located at the exit of the areas where infectious materials and/or animals are housed or manipulated. Additional sinks for handwashing are located in other appropriate locations within the facility. If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink is also available for handwashing at the exit from each segregated area.
    a. The sink is hands-free or automatically operated.
    b. Emergency eyewash and shower are readily available, easily accessible, and appropriately maintained.
    c. Sink traps are filled with water and/or appropriate disinfectant to prevent the migration of vermin and gases.
    d. Floor drains are maintained and filled with water and/or appropriate disinfectant or sealed to prevent the migration of vermin and gases.
3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (e.g., walls, floors, and ceilings) are water-resistant.
    a. Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Floors with drains are sloped toward drains to facilitate cleaning.

b. Penetrations in floors, walls, and ceiling surfaces are sealed, including openings around ducts, doors, doorframes, outlets, and switch plates to facilitate pest control and proper cleaning. Walls, floors, and ceilings form a sanitizable and sealed surface.

c. Internal facility fixtures, such as light features, air ducts, and utility pipes, are designed and installed to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.

d. External windows are not recommended; if present, they are sealed and resistant to breakage.

e. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.

4. Furniture is minimized and can support anticipated loads and uses.
   a. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
   b. Chairs used in animal areas are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant and sealed to prevent harboring of insects/vermin.
   c. Equipment and furnishings are carefully evaluated to minimize exposure of personnel to pinch points and sharp edges and corners.
5. Ventilation is provided in accordance with the *Guide for the Care and Use of Laboratory Animals*.

   a. Ventilation system design considers the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

   b. The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways. A visual monitoring device, which confirms directional airflow, is provided at the animal room entrance.

   c. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms. This system creates directional airflow, which draws air into the animal room from “clean” areas and toward “contaminated” areas.

   d. The exhaust air is dispersed away from occupied areas and from building air intake locations or the exhaust air is HEPA-filtered.

   e. The ABSL-3 animal facility is designed such that under failure conditions the airflow will not be reversed at the containment barrier. Alarms are considered to notify personnel of ventilation and HVAC system failure.

6. Cages are decontaminated prior to removal from the containment barrier and prior to washing in a mechanical cage washer. The cage wash area is 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.

7. BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness.
    a. BSCs are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, windows that can be opened, heavily traveled areas, and other possible airflow disruptions.
    b. BSCs can be connected to the animal facility exhaust system by either a canopy connection (Class IIA only) or directly exhausted to the outside through a hard connection (Class IIB, IIC, or III). Class IIA or IIC BSC exhaust can be safely recirculated back into the animal facility environment if no volatile toxic chemicals are used in the cabinet.
    c. BSCs are certified at least annually to ensure correct performance.
8. Equipment that may produce infectious aerosols is contained in primary barrier devices that exhaust air through HEPA filtration, or other equivalent technology, before being discharged into the animal facility. These HEPA filters are tested annually and replaced as needed.
9. All vacuum lines are protected with HEPA filters, or their equivalent, or are capped. Vacuum lines in use are protected with liquid disinfectant traps and in-line HEPA filters or their equivalent. Filters are replaced, as needed, or are on a replacement schedule determined by a risk assessment. The placement of an additional HEPA filter immediately prior to a central vacuum pump is considered.
10. An autoclave is available within the containment barrier. The autoclave is utilized to decontaminate infectious materials and waste before moving these materials to the other areas of the facility. If not within the containment barrier, special practices are developed for the transport of infectious materials to designated alternate locations for decontamination. A validated alternative process (e.g., alkaline digestion, incineration) may be used for decontamination and disposal of carcasses.
11. The ABSL-3 facility design, operational parameters, and procedures are verified and documented prior to operation. Facilities are tested annually or after significant modification to ensure operational parameters are met. Verification criteria are modified as necessary by operational experience.
12. Enhanced environmental and personal protection may be necessary based on risk assessment and applicable local, state, or federal regulations. These enhancements may include 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 animal room isolation; final HEPA filtration of the animal room exhaust air; animal room effluent

decontamination; containment of other piped services; or advanced access control devices, such as biometrics.

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 2019 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**
Arthropods free of specific pathogens	Indigenous/ no change in local fauna	Exotic/ inviable or transient only	Exotic with establishment potential or transgenic	n/a	n/a
Infection status	Uninfected or infected with RG1 agent		Up to RG2/BSL-2	Up to RG3/BSL-3	RG4/BSL-4
Practices	ACL-1 Standard Arthropod-Handling Practices		ACL-2 and BSL-2 limited access, training, signage containment and disposal	ACL-3 and BSL-3 restricted access, training, appropriate PPE, signage containment, disposal, and record keeping[a]	ACL-4 and BSL-4 isolation, training, appropriate PPE, signage containment, disposal, and record keeping[a]
Primary Barriers	Species-appropriate containers		Appropriate PPE, Escape-Proof Containers	Appropriate PPE, Escape-proof arthropod containers, pesticide available for emergency use[a]	Appropriate PPE, Escape-proof arthropod containers, pesticide available for emergency use[a]

Arthropod containment level:	1	2	3	4
Secondary Barriers		BSL-2 facilities, breeding sites, and harborage minimized	BSL-3 facilities, biological safety cabinets, other physical containment devices, pest control[a]	BSL-4 and facility-specific procedures and equipment for arthropod handling while wearing positive pressure containment suit[a]
General guidelines for best laboratory containment practices are shown for vector species of arthropod that are uninfected or infected according to biosafety and ACLs. Indigenous species are those species whose current range includes the research location. All others are considered exotic. For uninfected arthropods, containment guidelines take into account the consequences of accidental escape from a laboratory, in which the arthropod would be (1) inviable as a result of exposure to unfavorable conditions; (2) transient because conditions vary such that the arthropod would die during typical year climate cycle; or (3) has potential for establishment because escaped arthropods could reasonably be expected to persist through a typical climatic year. Arthropod containment specifics for each BSL should always be reviewed in the context of a laboratory-, vector-, and pathogen-specific risk assessment that is based on consultation between the investigator and the appropriate institutional oversight committee(s) and according to the constraints of the infrastructure available. [a]Additional restrictions apply for work with arthropods in association with Select Agents.				

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. Injection of male mosquitoes with dengue virus or homogenizing them afterward to assay for viral replication might expose personnel to infection. Even if the actual infection status of each individual male mosquito inoculated with dengue virus is not known, the standard of practice with diagnostic materials that may contain an infectious agent is to manipulate them at BSL-2. 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.

Another practical example of the utility of the ACL guidelines relates to the use of exotic arthropods. Biological introductions are to be prevented by all reasonable means, and handling exotic arthropods at ACL-2 or higher greatly reduces that risk. At any rate, even before the formal ACL guidelines, USDA import and possession permits would stipulate the equivalent of the ACL-2 recommendations for use of most exotic arthropods. It is impossible to prescribe universal levels of containment for a particular

species since the risks associated with accidental release from a laboratory are determined by several factors, for example, the climate at the facility and history of transmission in that location. For example, the accidental release of an uninfected anthropophilic tropical vector species, during the winter in Wisconsin, should be considered 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 bridge vector of an established zoonotic pathogen to humans. Thus, one of a number of possible approaches to reduce the risk of a release might be to perform relatively high-risk experiments during the winter when any escaped arthropods would quickly be killed by adverse environmental conditions; or a rainforest species could be used in a laboratory located in a xeric environment. IBCs might even use such biological considerations to "downgrade" a particular protocol from ACL-3 to ACL-2, providing that experiments are performed during a particular period or in a particular site. On the contrary, the possibility of zoonotic transmission or promoting a risk to domestic animal production means that we have to consider in risk analysis those pathogens that are predominantly an animal health issue; USDA Guidelines may need to be considered when assigning a containment level to a particular vector species.

All IBC protocols must outline an emergency 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-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. For example, personnel in mosquito laboratories should immediately eliminate any standing water.

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 or placing in biohazardous waste bags (no transgenic arthropods may be disposed of down the drain).

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. *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, including creation and breeding of transgenic arthropods.

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, in conjunction with long pants and closed, solid shoes. 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, if possible, 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. Arthropod Containment Level 2 (ACL-2)

ACL-2 should be practiced if working with exotic and indigenous arthropods infected with BSL-2 agents associated with animal and/or human disease, or that are reasonably suspected of being infected with such agents (diagnostic samples). The PI must perform a risk assessment when deciding whether arthropods are reasonably suspected of being infected with a pathogen. For example, live mosquitoes collected during the course of a disease outbreak and maintained in the laboratory would present more of a risk to laboratory personnel than those that are cold-immobilized or killed before sorting and identifying them for standard surveillance purposes. Uninfected genetically modified arthropod vectors also fall under this level provided the modification has no or only negative effects on viability, survivorship, host range, or vector capacity. ACL-2 builds on the practices, procedures, containment equipment, and facility requirements of ACL-1. It is more stringent in physical containment, disposal, and facility design requirements. Moreover, access is more restricted than ACL-1. The decision to propagate infected exotic arthropods under ACL-2 conditions in active transmission areas or in cases in which establishment is a possibility typically requires that measures that otherwise would only be recommended or preferred must be instituted as policy.

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 by laboratorians, custodians, and service persons are unlikely. This may be achieved by locating arthropods in dedicated rooms, closets, incubators located out of the traffic flow, or similar measures. Nonflying

arthropods, such as ticks, are typically held in primary containers (vials) that are placed within an environmentally controlled container such as a desiccator or plastic food container; often, this in turn is held within an environmental chamber. Although a dedicated space is recommended for long-term storage of ticks, appropriate risk assessment by the local IBC, informed by the PI or other experts, may allow for the housing of ticks in noninsectary settings.

2. *Supply Storage*. The area is designed and maintained to enhance detection of escaped arthropods. Equipment and supplies not required for operation of the insectary should not be located in the insectary. All supplies for insect maintenance that must be kept within the insectary are located in a designated area and not on open shelves. It is recommended that a closed storage room, cabinets with tight-fitting doors or drawers, be used. Doors and drawers are opened only for access. Insect diet should be kept in sealed containers.

3. *Primary Container Cleaning and Disinfestation*. In addition to cleaning cages and culture containers to prevent arthropod escape as in ACL-1, containers are disinfected chemically and/or autoclaved if used for infected material, according to an IBC-approved protocol and/or laboratory standard operating procedures. To reduce the risk of mixing up uncontaminated with potentially contaminated waste by having different methods for disposal, a laboratory may want to consider routine autoclaving, incineration, or other appropriate decontamination of all primary containers.

4. *Primary Container Construction*. Cages used to hold arthropods are shatter-proof and screened with mesh of a size to prevent escape. Containers are preferably autoclavable or disposable. Openings designed to prevent escape during removal and introduction of arthropods are recommended.

5. *Disposal of Arthropods*. All life stages of arthropods must be killed before disposal by freezing or other suitable methods. Infected arthropods should be autoclaved, or decontaminated with chemical disinfectants, such as freshly prepared 1:10 dilution of bleach or 70% ethanol based on an agent-specific risk assessment. The lack of an autoclave or means of incineration should be evaluated by local risk assessment and appropriate substitutes sought. Unless incinerated, material must be disposed of in biohazardous waste bags.

6. *Primary Container Identification and Labeling*. As per ACL-1.

7. *Prevention of Accidental Dispersal on Persons or via Sewer*. Before leaving the insectary and after handling cultures and infected arthropods, personnel wash their hands. Care

should be taken to not disperse viable life stages into the drainage system. No infected material is disposed through the sewer unless it is decontaminated. Physical barriers (overlapping sheets and screens) or air curtains are recommended as appropriate; personal protective equipment that is reused (laboratory coats, gowns) should be checked for infestation before exiting the insectary.

8. *Pest Exclusion Program*. As per ACL-1.

9. *Escaped Arthropod Monitoring* Investigators assess whether escapes are occurring by instituting an effective arthropod trapping program to monitor the escape prevention program. Oviposition traps, ground-level flea traps, oil-filled channels surrounding tick colonies, light traps for mosquitoes, and so on, are recommended. Particularly in the case when exotic arthropods are used, exterior monitoring is recommended. Records of exterior captures are maintained. Any evidence of escape should trigger a review of practices and procedures before resuming work.

10. *Source and Harborage Reduction*. Harborage and breeding areas are eliminated. Furniture and racks are minimized and can be easily moved to permit cleaning and location of escaped arthropods. Equipment in which water is stored or might accumulate (*e.g.*, humidifiers) is screened to prevent arthropod access, or contains chemicals to prevent arthropod survival.

11. *Laboratory Sharps*. Disposable sharps should be discarded in puncture-proof biohazardous waste containers. Forceps, dissecting probes, and other sharps that are reused should be frequently disinfected by chemical disinfection, autoclaving, or flame sterilization.

12. *Routine Decontamination*. Equipment and work surfaces in the insectary are routinely decontaminated with an effective chemical disinfectant.

13. *Notification and Signage*. Persons entering the area must be made aware of the presence of BSL-2 agents in arthropod vectors. If infected material is present, a BSL-2 biohazard warning sign is posted on the entrance to the insectary, listing all species handled within and is updated whenever new species are introduced or pathogenic infectious agents are present. The hazard warning sign identifies the arthropod species, agent(s) known or suspected to be present, PPE requirements, lists the name and telephone number of the responsible person(s) and emergency contacts, and indicates any special requirements for entering the insectary (*e.g.*, the need for immunizations or respirators).

14. *Procedure Design*. All procedures are carefully designed and performed to prevent arthropod escape.

15. *Safety Manual*. A site-specific safety manual is prepared, approved by the IBC, and adopted. The manual contains emergency procedures, standard operating procedures, waste disposal, and other information necessary to inform personnel of the methods for safe maintenance and operation of the insectary. The manual must be updated and reviewed by all personnel at least annually.

16. *Training*. Laboratory personnel are advised of special hazards and are required to follow instructions on practices and procedures contained in the safety manual. Adherence to established safety procedures and policies is made a condition of employment and is part of the annual performance review, if applicable, of every employee. Personnel receive annual updates and additional training as necessary for procedural or policy changes. Records of all training are maintained.

17. *Medical Surveillance*. At the minimum, all personnel should be educated by the PI about the risks associated with the specific tasks and experiments, as well as the signs and symptoms of any illness caused by the agent(s) under study. Specialty immunizations or a serum surveillance system may be required by the IBC as part of the registration process. In general, persons who may be at increased risk of acquiring infection, or for whom infection may be unusually hazardous (*e.g*., immunocompromised), are not allowed in the insectary unless special personal protection procedures are in place to eliminate extra risk.

18. *Access Restrictions*. Routine access is limited to trained persons and accompanied guests. Service persons are made aware of the hazards present and the consequences of arthropod release and contact with agents that may be present.

19. *Special Arthropod handling containers and areas*. Infected arthropods are prevented from release into the laboratory area. A dedicated area for handling infected material is recommended. This is preferably a separate cubicle, walk-in incubator, or screen room. Additional physical barriers (*e.g*., glove box, biosafety cabinet) or procedures (incapacitated arthropods, *e.g*., removing a wing from a mosquito) may be required depending on the local risk assessment.

20. *Safe Transport in the laboratory*. All infectious and potentially infectious samples are collected, labeled, transported, and processed in a manner that contains and prevents transmission of the agent(s). Transfer of arthropods between manipulation and holding areas is in non-breakable secure containers.

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, including creation and breeding of transgenic arthropods. In addition, all work at BSL-2 requires IBC approval prior to starting work.

2. *Housing of Non-Arthropod Animals*. Other animals are not accessible to the arthropods. Animals used as hosts or blood sources generally are not housed with arthropods. If present, they are adequately protected from access by escaped arthropods.

3. *Blood Source*. As per ACL-1. To prevent inadvertent contamination of the clean colony, sources of infection, such as a tube of infected blood, should not be stored in the same refrigerator as a tube of uninfected blood for maintaining uninfected colonies by membrane feeding. Colony arthropods should be maintained in an ACL-1 area and transported to an ACL-2 area for infection; there should be no transport of living arthropods from ACL-2 to ACL-1 without specific local risk assessment.

4. *Escaped Arthropod Handling*. Loose arthropods must be killed and disposed, or recaptured and returned to the container from which they escaped. Infected arthropods must not be killed with bare hands and must be manipulated using filtered mechanical or vacuum aspirators or other appropriate means (*e.g*., forceps, paintbrushes, gloved hands).

5. *Accidental Release Reporting*. A release procedure is developed and posted. This includes contacts and immediate mitigating actions. Accidents that result in release of infected arthropods from primary containment vessels or that result in overt exposure to infectious material must be reported immediately to the insectary director (PI) who is responsible for ensuring that appropriate and documented action is taken to mitigate the release. The room where the incident occurred is closed off, a warning sign indicating the location, number, and type of material released is prominently posted, and other laboratory personnel are informed until the source is eliminated. Follow-up medical evaluation, surveillance, and treatment are provided as directed by institutional policy and local risk assessment, and written records are maintained.

6. *Movement of Equipment*. All equipment must be appropriately decontaminated and disinfested before transfer between rooms within the insectary, and before removal from the insectary.

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

Personal protective equipment should be evaluated as part of the local risk assessment. It should be noted that very few infected arthropods are directly infectious by handling; virtually all require exoskeleton disruption or the act of feeding to be hazardous although there are exceptions (body lice excrete feces that contain *Rickettsia prowazekii* or *Bartonella quintana*; newly fed mosquitoes may diurese infectious virus). Clothing (primary as well as safety) should conform to institutional policy and to the risk assessment by the IBC. As an example, entering a room containing an environmental chamber holding plastic food containers with tick vials would not require personal protective equipment (PPE) beyond standard lab PPE. The use of latex or nitrile gloves, although highly recommended, may not be required as a result of risk assessment by the local IBC or equivalent; manipulation of arthropods within a glove box fitted with Hypalon gloves, for example, would not necessarily require additional gloves.

1. *Personal Protective Equipment*. Laboratory coats, gowns, and/or uniforms are worn at all times in the insectary when handling blood and vertebrate animals, in conjunction with long pants and closed, solid shoes. Gloves are worn when handling host animals or blood used to feed the arthropods. Appropriate face/eye and respiratory protection is worn by all personnel entering the insectary, if recommended by the local risk assessment.

2. *Arthropod-Specific Personal Protective Equipment*. Other equipment may be required as determined by the local risk assessment. Homogenization of infected arthropods, for example, may require an appropriate respiratory protective device if the procedure is not performed within a biosafety cabinet or glove box.

D. Laboratory Facilities (Secondary Barriers)

An insectary may simply be a room with a door that may be closed tightly; it may or may not have environmental controls. Dedicated spaces to be used as insectaries are highly recommended, but resources may not exist to permit such arrangements. The use of infected arthropods may be permitted after risk assessment by the local IBC even in the absence of a dedicated space. Ticks, for example, may be safely manipulated within general BSL-2 laboratory settings that are otherwise not considered to be insectaries.

1. *Location of Insectary*. The insectary is separated from areas that are open to unrestricted personnel traffic within the building. It is recommended that this be accomplished by at least two self-closing doors that prevent passage of the arthropods. Increased levels of physical isolation are recommended, for example, separate buildings, wings, and suites. However, the lack of a dedicated insectary should not imply that infected arthropods may

not be manipulated; site-specific risk assessments may provide mitigating alternative arrangements. For example, nonflying infected arthropods such as ticks or fleas may be safely manipulated in a dedicated area within a BSL-2 laboratory using a moat system (pan within a pan of water) and accounting for all specimens.

2. *Insectary Doors*. Recommended entrance to the insectary is via a double-door vestibule that prevents flying and crawling arthropod escape. For example, the two contiguous doors must not be opened simultaneously. Internal doors may open outward or be sliding, and are kept closed when arthropods are present. Self-closing doors are highly recommended. Additional barriers (*e.g.*, screened partitions, hanging curtains) may be required by the local risk assessment. Alternative arrangements may be specified by local risk assessment in the absence of a dedicated insectary.

3. *Insectary Windows*. Windows are not recommended, but if present cannot be opened and are well sealed. Windows should be resistant to breakage (*e.g.*, double paned or wire reinforced).

4. *Vacuum Systems*. If a central vacuum system is installed, each service outlet is fitted with suitable barriers/filters to prevent arthropod escape. Filters are installed to permit decontamination and servicing. Other vacuum devices are appropriately filtered to prevent transfer and exhausting of arthropods.

5. *Interior Surfaces*. The insectary is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior walls are preferably light colored so that a loose arthropod can be easily located, recaptured, or killed. Gloss finishes, ideally resistant to chemical disinfectants and fumigants, are recommended. Light-colored floors are also highly recommended, smooth and uncovered. Ceilings are as low as possible to simplify detection and capture of flying insects. Inability to conform to these recommendations may be mitigated by other physical or procedural methods as indicated by the local risk assessment. A static glove box with a light-colored interior, for example, may be used to manipulate infected arthropods where the color of walls and floors cannot be easily changed.

6. *Floor Drains*. Floor drains are modified to prevent accidental release of arthropods and agents. If present, traps must be filled with an appropriate chemical treatment to prevent survival of all arthropod stages (*e.g.*, mosquito larvae).

7. *Plumbing and Electrical Fixtures*. Internal facility appurtenances (*e.g.*, light fixtures, pipes, ducting) are minimal since these provide hiding places for loose arthropods. Penetrations

of walls, floors, and ceilings are minimal and sealed/caulked. Ideally, light fixtures are flush with the ceiling, sealed, and accessed from above.

8. *Heating, ventilations and Air Conditioning (HVAC)*. Ventilation is appropriate for arthropod maintenance, but does not compromise containment of the agent or arthropod. Examples include the following: exhaust air is discharged to the outside without being recirculated to other rooms; appropriate filter/barriers are installed to prevent escape of arthropods; the direction of airflow in the insectary is inward; a progressively negative pressure gradient is maintained as distance from the main entrance increases; fans located in the vestibule and internal corridor can be used to help prevent escape of flying arthropods; and hanging or air curtains are located in vestibules and doorways. Local risk assessments may provide site- and task-specific alternatives to these recommendations, for example, the use of a static glove box in which infected arthropods are manipulated may provide adequate security if directional airflow is not possible.

9. *Sterilization Equipment*. An autoclave is available, conveniently located in rooms containing arthropods within the insectary building. If an autoclave is not available, an appropriate decontamination system or set of practices and procedures may be recommended by the local risk assessment.

10. *Sink*. The facility has a hand-washing sink with warm water and with suitable plumbing to prevent arthropod escape

11. *Illumination*. Illumination is appropriate for arthropod maintenance, and does not compromise arthropod containment, impede vision, or adversely influence the safety of procedures within the insectary. Lighted (or dark) openings that attract escaped arthropods are avoided.

12. *Facility Compliance Monitoring*. The facility should be evaluated annually for compliance to ACL-2 by the University of Utah Biosafety Officer. The PI or insectary director inspects the facility at least annually to ensure that alterations and maintenance have not compromised the containment characteristics. Adequacy of the practices and facility in view of changes in research protocols, agents, or arthropods is considered.

15. 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 facilities or laboratories, 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.

The principal purpose of plant containment is to avoid the unintentional transmission of a recombinant or synthetic nucleic acid molecule-containing plant genome, including nuclear or organelle hereditary material or release of recombinant or synthetic nucleic acid molecule-derived organisms associated with plants. Specific regulations for research involving modified whole plants can be found in Sections III-D-5 and III-E-2 of the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*. Appendix L 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. All non-exempt work, as defined in the NIH Guidelines, must be registered with and approved by the University of Utah IBC.

The containment principles are based on the recognition that the organisms that are used pose no health threat to humans or higher animals (unless deliberately modified for that purpose), and that the containment conditions minimize the possibility of an unanticipated deleterious effect on organisms and ecosystems outside of the experimental facility, e.g., the inadvertent spread of a serious pathogen from a greenhouse to a local agricultural crop or the unintentional introduction and establishment of an organism in a new ecosystem.

BL1-P through BL4-P are designed to provide differential levels of biosafety for plants in the absence or presence of other experimental organisms that contain recombinant or synthetic nucleic acid molecules. These biosafety levels, in conjunction with biological containment conditions described in Appendix L-III of the NIH Guidelines, *Biological Containment Practices*, provide flexible approaches to ensure the safe conduct of research.

A. Regulations, Permitting Agencies, and Oversight

1. 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) United States Department of Agriculture (USDA)/Animal and Plant Health Inspection Service (APHIS)

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 in greenhouses. 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. Laboratory-based Containment

For experiments in which plants are grown at the BL1 (BSL-1) or BL-2 (BSL-2) laboratory settings, containment practices shall be followed as described above. These containment practices include the use of plant tissue culture rooms, growth chambers within laboratory facilities, or experiments performed on open benches. Additional biological containment practices will be added by the IBC, as necessary (see Appendix L-III), if botanical reproductive structures are produced that have the potential of being released.

D. Greenhouse Containment

Note, the University of Utah does not currently have greenhouse containment facilities to conduct BL2-P, BL3-P or BL4-P designated research.

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. See Appendix L-II for specific BL1-P requirements.

BL2-P

BL2-P applies to experiments with transgenic plants and plant associated organisms that have the potential for rapid and widespread dissemination, and the capability of interbreeding with weeds or related species. However, these materials are not likely to have a serious detrimental impact on natural ecosystems.

Work involving other organisms that require a containment level of BL2-P or lower may be conducted concurrently in a greenhouse bay as long as all work is conducted using BL2-P practices. See Appendix L-II for specific BL2-P requirements.

D.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.

D.2. Access

Only approved students, faculty, staff or other critical personnel may enter the University of Utah's greenhouse facility must obey facility rules (a single entrance to the facility is security card protected). Entry to greenhouse bays with ongoing experiments requiring BL1-P or BL2-P containment is restricted as specified in the greenhouse rules. All support staff and external contractors must be approved by the Greenhouse or Facility Manager to enter BL1-P or BL2-P active greenhouses, and by the PI for students or research staff deemed to critically require access to support experiments in progress: for entry into the Greenhouse, 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 or BL2-P active greenhouses are knowledgeable of special containment strategies, any required personal protective equipment, entry/exit procedures, and requirements for removal/disposal of recombinant biological materials.

D.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 should be maintained in the respective

greenhouse bay by the PI. Record logs of all in-process experiments in the greenhouse facility should be kept by the greenhouse manager (IBC registrations or a simple list of ongoing experiments are suitable records for BL1-P).

Persons working in BL2-P greenhouse bays must read and follow the University of Utah Greenhouse Manual and applicable SOPs. . A copy of the SOPs for experiments being performed in any given greenhouse bay should be maintained in the respective greenhouse bay by the PI. Record logs of all in-process BL2-P experiments must be kept by the PI. These records should include a detailed inventory of all experimental plants, microorganisms, arthropods, or small animals that are brought into or removed from the greenhouse facility or growth chambers, including the date, what was taken, where it was taken, name of person moving material, and how the container was sanitized.

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).

D.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.

BL2-P greenhouse bays are required to be composed of an impervious material (e.g. concrete). Screens are required in BL2-P greenhouse bays to exclude small arthropods and birds.

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.

*If it is suspected that structural damage has resulted in a loss in containment of transgenic plants or plant-associated organisms at BL2-P, the Biosafety Officer and IBC must be notified since this is reportable to the NIH Office of Science Policy, USDA APHIS, and/or other designated authorities.

D.5. Signs and Labeling

In BL2-P greenhouse bays and growth chambers a "Caution-Experiment in Progress" sign must be posted at the entrance to the individual greenhouse bay or growth chamber. The sign will indicate the following information: plant species and novel trait; microorganisms used; precautionary information (including if the organisms used have a recognized potential for causing detrimental impacts on the environment); and a responsible individual with a 24-hour emergency contact number. If there is a risk to human health, the universal biohazard symbol will be present on the sign, along with relevant safety information. Though not required for BL1-P, similar signage is highly encouraged.

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.

D.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, particularly at BL2-P;
- Sanitize equipment in BL2-P containment greenhouse bays at the end of the project); and
- Prohibit smoking throughout the Greenhouse Facility.

D.7. Pest Control

The NIH Guidelines specify that a weed and pest control program must be in place for all levels of greenhouse containment. Additional precautions need to be taken in BL2-P containment if macroorganisms, such as flying arthropods and nematodes, are released in the greenhouse bay since they are pollen vectors. In the Greenhouse Facility, PIs assigned to the bays are responsible for pest management. Plants must be regularly inspected for signs of insect infestation.

D.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.

D.9. Biological Containment Techniques

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

D.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.

BL2-P plant materials (including soil and pots) must be autoclaved using validated parameters prior to disposal, or as indicated by the permit. Appendix L of the NIH Guidelines states that BL2-P materials should be autoclaved. If an autoclave is not available, other suitable methods of inactivation may be used (e.g. chemicals, steam carts). However, the efficacy of these alternatives must be validated and documented. After plant materials are inactivated, they may be disposed of in the regular trash. If plant materials contain transgenic materials that may harm humans, a biohazard symbol must be present on the outside of the plastic bag prior to sterilization. After treating the plant materials using validated parameters, the biohazard symbol must be covered (i.e. place in non-see-through trash bag) prior to final disposal in the regular trash.

D.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 should be conducted. There are several steps that should 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 should be considered when determining the monitoring area. If a known breach of containment has occurred, volunteer monitoring should 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.

D.12 Standard Operating Procedures (SOPs)

SOPs need to be prepared for all transgenic experiments that will be conducted in the greenhouse facilities. SOPs should 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 should also be accessible in the notebook.

F. 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. 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 to ensure that containment measures compatible with the material are implemented and that personnel involved have access to a facility-specific Exposure Control Plan and have received annual bloodborne pathogens training.

Samples from patients with a known infectious disease, such as HIV, Hepatitis B, Hepatitis C or COVID-19, typically require higher levels of containment and additional engineering and work practice controls that must be registered with and approved by the IBC. Requirements for work with samples from patients with HIV or Hepatitis B virus are specified in the OSHA Bloodborne Pathogens standard. The University of Utah IBC has established guidelines for research involving samples from patients with COVID-19 (Appendix L.)

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, EHS 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 EHS 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 EHS. Registration for these courses is available through Bridge.

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.

G. 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. Note, the product information sheet for some human cell lines from ATCC indicate BSL-1 containment. However, ATCC states that testing is not comprehensive and the containment designation is only for shipping purposes: BSL-2 containment is still required for work with these cells.
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.

H. 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 must be developed and implemented by the IBC. The IBC has developed spill procedure and post-exposure procedure templates that can be adapted by labs. These are found in Appendix B.
3. Research that is carried out at physical containment level BSL-2 or higher requires the principal investigator to prepare and adopt a laboratory-specific biosafety manual. Templates are available here.

The IBC has established Fact Sheets documents for working with recombinant viral vectors to assist investigators with their Risk Assessment when registering their work with the IBC. These can be found on the IBC website.

I. Human Gene Transfer

All protocols involving the use of rsNA or pathogenic agents in human subjects must be registered and approved by the IBC. Registrations are submitted in conjunction with the University of Utah IRB application using ERICA. Prior approval by the IBC, IRB and FDA is required before commencing gene therapy in humans.

J. Biological Toxins

Work with acute biological toxins must be registered with and approved by the IBC. 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 EHS staff prior to IBC approval. Detailed SOPs must be provided to the IBC as part of the registration process: a template is available in Appendix E. 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 E).

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): when working in a biosafety cabinet these are recommended, but not required.

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

Decontamination of a biological toxin(s) means the toxin is rendered inactive and is no longer capable of exerting its toxic effect. Toxin stability varies considerably outside of physiological conditions depending upon the temperature, pH, ionic strength, presence 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. 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 denaturing toxins in aqueous solutions are not necessarily applicable for inactivating dry, powdered toxin preparations.

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. Bleach decontamination solutions should always be prepared *fresh* (i.e., <24 h).

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
Alpha conotoxins	≥ 0.5% [g]	10 N[g]	ND	No[g]
Abrin	≥ 0.7% [h]	ND	ND	ND
Shiga toxin	≥ 0.5%	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.

[g]Conotoxins can also be inactivated using reducing agents such as dithiothreitol β- mercaptoethanol, or tris (2-carboxyethyl) phosphine (100 mM) at 65–100° C for 15 min, followed by alkylation with 100 mM maleimide in isopropanol at 65° C for 15 min. Alternatively, alpha conotoxins can be inactivated by hydrolysis in 10 N NaOH or HCl at 100° C for 30 min.

[h]Exposure of crude abrin solution and dried abrin to 0.67% NaOCl eliminated over 90% of cytotoxicity within 5 min.

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 10-15 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 or biohazardous 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 or C	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 materials to be removed from the cabinet must be decontaminated prior to removal.
2. All contaminated equipment to remain in the cabinet is segregated from materials to be removed.
3. The cabinet blower must be left on for at least five minutes to purge the air.
4. After removal of materials from the cabinet, decontaminate the work surfaces.
5. 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 and NSF-certified 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.)

EHS 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.

E. Decontamination

Prior to removal from a facility, such as relocation to another room or disposal through University of Utah Surplus, the biological safety cabinet must be de-contaminated by the NSF-certified technician. Surface decontamination is not sufficient. Once the cabinet has been relocated it must also be re-certified by the same technician. Contact EHS to request contact information for scheduling the de-contamination with the NSF-certified technician.

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). 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-1, 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. 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 EHS 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 Hospital Acute Care Building for laundering (See Section V): alternatively commercial companies may be used for cleaning.

M. Training

Depending on the agents being used and the requirements of the facility where the lab(s) are located, training may be required for approval of your IBC protocol. Listed below are some of the commonly required training programs, as well as links to information regarding the type of training offered and how to register for the specific training. If you have any questions regarding biosafety training that you may be required to complete, please contact the IBC administrator (biosafety@ehs.utah.edu).

1. **Bloodborne Pathogens (BBP) Training**: This is a 1.5 hour class required for anyone working with human or non-human primate cell lines, blood, tissue, body fluids, as well as other potentially infectious material (OPIM) of human origin: this includes work with established cell lines. **Training must be completed prior to working in the laboratory and repeated annually**. Information regarding the class, and how to register for a class, can be found using this link (https://utah.bridgeapp.com/learner/category/100).
2. **Biosafety Level-2 (BSL-2) and Bloodborne Pathogens (BBP) Training.** This is a 2 hour class required for anyone working with recombinant or synthetic nucleic acids or pathogenic/toxic materials requiring BSL2 containment. This class also includes information on Bloodborne Pathogens for those working with human or non-human primate cell lines, blood, tissue, body fluids, as well as other potentially infectious material (OPIM) of human origin. This includes work with established cell lines. **Training must be completed prior to working in the laboratory and repeated whenever IBC registrations are submitted (initial and renewal)**. However, personnel working with human or non-human primate samples or cell lines must repeat BBP training annually by either retaking this class or taking the BBP Training class (See

#1). Information regarding the class, and how to register for a class, can be found using this link (https://utah.bridgeapp.com/learner/category/100).

3. **BSL-2, Viral Vectors, and Bloodborne Pathogens Training.** This is a 2 hour class required for anyone working with recombinant or synthetic nucleic acids or pathogenic/toxic materials requiring BSL2 containment and includes information on commonly used recombinant viral vectors. This class also includes information on Bloodborne Pathogens for those working with human or non-human primate cell lines, blood, tissue, body fluids, as well as other potentially infectious material (OPIM) of human origin: this includes work with established cell lines. **Training must be completed prior to working in the laboratory and repeated whenever IBC registrations are submitted (initial and renewal)**. However, personnel working with human or non-human primate samples or cell lines must repeat BBP training annually by either retaking this class or taking the BBP Training class (See #1). Information regarding the class, and how to register for a class, can be found using this link (https://utah.bridgeapp.com/learner/category/100).
4. **Animal Biosafety Level 2 Training.** This is a 30 minute online class required for anyone working with animals requiring ABSL-2 containment. **Training must be completed prior to working in the laboratory and repeated whenever IBC registrations are submitted (initial and renewal)**. Information regarding the class, and how to register for a class, can be found using this link (https://utah.bridgeapp.com/learner/category/100).
5. **Laboratory/Protocol-Specific training.** All personnel working at BSL-2 or higher must receive training from the Principal Investigator, at the time of assignment and at least annually thereafter. This training must include a risk assessment for the agents being used in the lab (including routes of infection, signs and symptoms of exposure and options for vaccinations or post-exposure prophylaxis), engineering and work practice controls, PPE requirements, spill clean-up procedures, and post-exposure procedures. Laboratories with a comprehensive, IBC-approved Biosafety manual may use this for training, with signatures documenting review. In person trainings, for example during lab meetings, can be documented using "Site-Specific Training Checklist and Record SOP" in the IBC Fact Sheets and SOP library.
6. **Shared-Space Training**. All BSL2 (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. See "Training on Biohazards in Shared Spaces SOP" in the IBC Fact Sheets and SOP library.

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 EHS 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 B). 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 EHS.

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 biological or chemical hazards. 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 BSL-2+ and BSL-3 and whenever a spill is cleaned up, but is recommended at all times. Gloves long enough to cover the cuff of the lab coat are recommended (required at BSL-2+ and BSL-3). 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 and splash protection, but not inhalation 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 experiencing an 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 B) 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 EHS 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 (see Appendix B for a template).

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 EHS Associate Environmental Specialist at 801-581-6590 for specific instructions.

1. Biowaste Disposal – Solids

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

b. Waste containers obtained from EHS 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 SAM.

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 EHS through the SAM website. **NO DRAIN DISPOSAL unless approved by EHS.**
- 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 EHS through the Safety Administrative System (SAM) webpage.
- Locate sharps containers in areas in which needles are commonly used. Make containers easily accessible.
- Replacement sharps containers may be obtained through the SAM 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

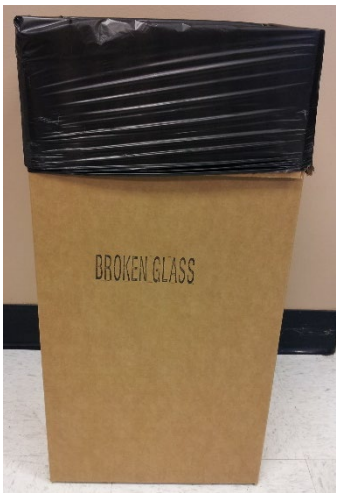
Serological pipets (glass and plastic) and disposable pipet tips are considered puncture hazards and shall be disposed of as sharps. Contaminated pipets and tips must be discarded in approved sharps containers, as described above.

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 EHS through the SAM. These will be picked up by EHS 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 EHS. 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 D** 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*). **See Appendix H**.

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. Kits can be purchased from the EHS website, https://oehs.utah.edu/resource-center/forms/spillkitorderform. 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. A template that can be adapted by the investigator is included in Appendix B.

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 EHS.

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: contact Todd Nilsen, Export Control Officer (801-581-8948) prior to export of any materials from the University of Utah. For any questions regarding shipment of the following items, please contact the Biosafety Officer and/or the Shipping Team at EHS ((801) 581-6590):

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 and/or the Shipping Team at EHS (801) 581-6590 regarding shipment of these items.

V. Laundry

The University of Utah Health Laundry and Linen Services can be used to clean contaminated clothing and other articles that require laundering. They can be found on the A Level of the Acute Care Building of

the University of Utah Hospital, 801-581-2582. 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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ACKNOWLEDGEMENTS

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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 EHS.

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 EHS. University of Utah Facilities Management do not perform maintenance on biological safety cabinets. If the BSC needs to be serviced, contact EHS.

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 EHS through the SAM 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 EHS and provided by an outside vendor. A sticker on the BSC will list when certification is due. If certification is past due, please contact EHS.

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 EHS (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: Spills and Exposure Procedures Templates

All spills or breaks involving Recombinant DNA or Synthetic Nucleic Acid Molecules and hazardous biological materials should be cleaned up using appropriate biosafety procedures, described below. If there is any doubt about what to do, call the PI (*Telephone #*), or the Biosafety Officer 1-6590, or the University's internal emergency number: 5-2677.

The following items should be included in a biological spill kit:

- **Disinfectant** – Prepare a fresh 1:10 bleach solution. In other words, a pre-measured amount of bleach in a spray bottle is placed in the spill kit, but the cold water required to dilute the bleach is not added until right before use. Otherwise, use an EPA-registered disinfectant (effective against HIV and HBV) following manufacturer's instructions. Examples are Cavicide, Cidex OPA and Clidox-S. Note the date of manufacture and/or expiration.
- **Absorbent material** (paper towel, absorbent powder)
- **Personal protective equipment** (e.g., disposable gloves (2 pairs), eye protection, face shield or surgical mask, lab coat, shoe covers). It is necessary to review the PPE in the spill kit on a regular basis to verify quality. Gloves can degrade due to exposure to UV or fluorescent lighting, temperature extremes, and the effects of time. At the first sign of degradation (e.g., discoloration, brittleness, stickiness, tearing), replace the gloves in the spill kit with new ones. Likewise, the strap on splash goggles can undergo similar degradative processes.
- **Mechanical tools** (forceps or tongs, broom and dustpan) – Dispose of biohazardous waste after spill response. Purchase inexpensive plastic tools for this purpose.
- **Waste container** (biohazard bags) – By assembling all of the spill materials in a bucket or other leak-proof and puncture-proof container, you will have a secondary container readily available for proper containment of your biohazard bag.

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. Prepare the disinfectant*.*
    f. Saturate spill with XXXXXXX *(fill in the appropriate decontaminant).* Let sit for 20 minute exposure time.
        i. For large spills (greater than 10ml) use undiluted bleach or disinfectant.
        ii. 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.
        iii. *Note: due to its evaporative nature alcohol is not recommended as the primary disinfectant but can be used to remove bleach/disinfectant residue.*
        iv. *If working with human blood or OPIM (such as human cell line) spills must be disinfected with an EPA-approved disinfectant (alcohol is not on the approved lists).*
    g. Wipe up spill, disposing of towels in biohazard bag.

h. Spray spill area with XXXXXXX *(fill in the appropriate decontaminant).* Allow to air dry.
i. Disinfect all other materials used in the biosafety cabinet by disinfecting the surface with XXXXXXX *(fill in the appropriate decontaminant)* with a 20 minute contact time. Do not attempt to disinfect contaminated cardboard or other paper items that absorb liquid: contaminated items should be disposed of.
j. If bleach or other corrosive disinfectant used, wipe spill area and disinfected equipment with alcohol or water.
k. Place all towels or absorbent materials into a designated container for biohazardous waste.
l. Remove PPE, discard disposable PPE as biohazardous waste and wash hands.
m. 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 should 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 60 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 face shield or mask, 2 pair of gloves, shoe covers. If the lab coat does not have cuffed sleeves, disposable sleeve covers should be worn.
   g. Contain the spill by covering with paper towels (to avoid splashes or aerosols)
   h. Saturate spill with XXXXXXX *(fill in the appropriate decontaminant).* Let sit for 20 minute exposure time.
      i. For large spills (greater than 10ml) use undiluted bleach or disinfectant.
      ii. Wipe areas around the spill that may have splatter and any reusable equipment with XXXXXXX *(fill in the appropriate decontaminant).*
      iii. *If working with human blood or OPIM (such as human cell line) spills must be disinfected with an EPA-approved disinfectant (alcohol is not on the approved lists).*
   i. 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.
   j. Spray spill area with XXXXXXX *(fill in the appropriate decontaminant).* Allow to air dry.
   k. If bleach or other corrosive disinfectant used, wipe spill area and disinfected equipment with alcohol or water.
   l. Remove PPE, discard disposable PPE as biohazardous waste and wash hands.
   m. Remove the "Do Not Enter" sign and inform others that it is safe to re-enter the room.
   n. Once the spill has been contained, complete the "**SPILLS OR EXPOSURE EVENT REPORTING PROCEDURE"** form (below) 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. Prepare the disinfectant*: consult the instructions of the centrifuge rotor to identify suitable disinfectants*.
c. 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.*
d. Gather supplies needed, such as a sharps container for broken glass and bins filled with disinfectant and place into the BSC.
e. 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 XXXXXXX (*fill in the appropriate decontaminant*) for at least 20 minutes. Wipe carrier/bucket with disinfectant.
f. After disinfection, carrier, bucket, or rotor must be washed with a mild soap and water.
g. Spray the interior of the centrifuge chamber with XXXXXXX (*fill in the appropriate decontaminant*), let sit for at least 20 minutes and then wipe down with soap and water.
h. Dispose of all clean-up materials (except sharps) in an appropriate biohazardous waste container. Dispose of sharps in a biohazard sharps container.
i. 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 should 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 can 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 the lab supervisor or PI.

e. If exposed to BSL-2/RG2 (or above) agent, notify the Biosafety Officer and 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 RedMed Clinic, Redwood Occupational Medicine 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 RedMed Clinic, Redwood Occupational Medicine 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 should be evacuated. Post a “Do Not Enter” notice on the door. Notify the PI or lab supervisor.
    c. Call OEHS (801-581-6590). Provide information on the nature of the material spilled.
    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

Actions in the Event of an Exposure

Definition of Exposure

1. Direct skin, eye or mucosal membrane exposure to the agent or materials potentially containing the agent, such as tissue culture media or cells, bodily fluids from infected animals.
2. Parenteral inoculation by a syringe needle or other contaminated sharp (needlestick),
3. Ingestion of liquid suspension of an infected material or by contaminated hand to mouth exposure, or
4. Inhalation of infectious aerosols.

Information for Lab Personnel

1. Remove exposed PPE taking care to avoid contact of unexposed areas to infectious agents on the PPE.

2. Inform others in area about any biohazardous materials out of containment to prevent further exposure. If possible, contain with absorbent pads, decontaminate with bleach, and/or seal off the site. **ALL exposed individuals should leave the area.**

3. Immediately wash 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. See exposure procedures for further information.

4. After washing, Notify lab supervisor or Principal Investigator of the exposure (PI's 24-hour Emergency Contact Number: (XXX) XXX-XXXX).

5. Go immediately to the RedMed Employee Health Clinic at the University Union Building or the Occupational Medical Clinic at the Redwood Health Center for medical evaluation and follow-up; contact information is below. After 5pm you will be seen by an Urgent Care Physician. After 8pm, or if the injury is serious/life threatening, go to the University of Utah Hospital Emergency Department or call an ambulance (911).

    a. **NOTE**: In the event of an exposure to SARS-CoV-2 or a sample from a patient with COVID-19, call the clinic in advance.

6. Ensure that the physician is aware of all materials that were being used at the time of exposure (e.g., virus, bacteria, human tissue, animal tissue, other potentially infected material). **Take a completed copy of the risk assessment and treatment options of this SOP with you!**

7. Follow up with the physician at Occupational Medicine, as requested.

RedMed Employee Health Clinic

200 Central Campus Dr.

Salt Lake City, UT 84112
Phone: (801) 213-3303
Hours: M-TH: 8:00AM – 5:00PM, Friday: 9:00AM – 3:30PM
Closed from 1:30 to 2:00PM

Redwood Health Center
Occupational Medicine Clinic
1525 West 2100 South
Salt Lake City, UT 84119
Phone: (801) 213-9777
Hours: M-F 8:00AM – 5:00PM

After Hours
Redwood Urgent Care
1525 West 2100 South
Salt Lake City, UT 84119
M-F 5:00PM – 9:00PM
Sat.-Sun.: 9:00AM – 9:00PM
(801) 213-9700

After 9 PM
Emergency Department at University Hospital
(main floor northeast side of the hospital)
50 N. Medical Drive
Salt Lake City, UT 84132
(801) 581-2292

8. **Post exposure prophylaxis must be initiated as soon as possible after exposure.**

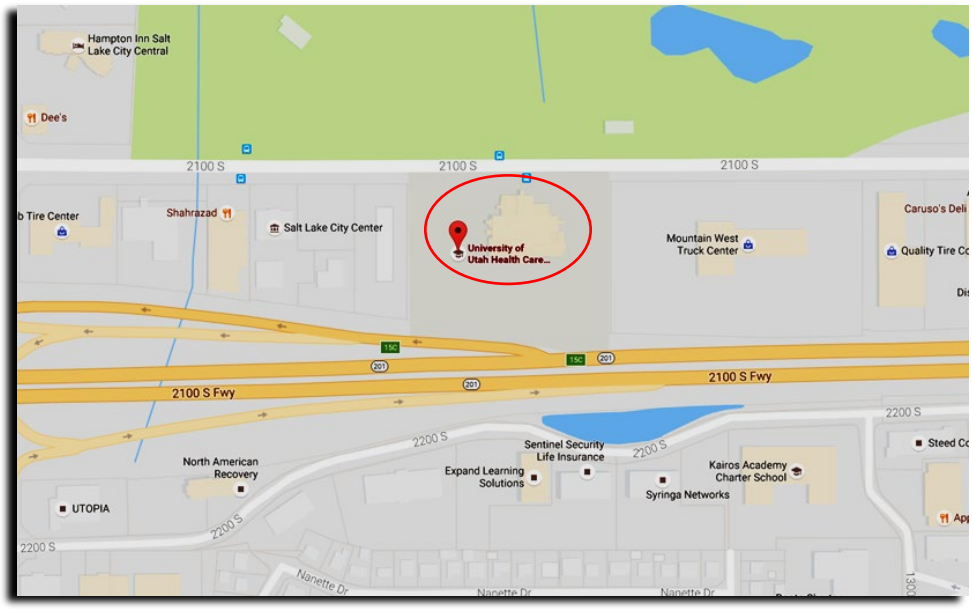
9. Inform the Healthcare Provider of any medical conditions, such as pregnancy or immunosuppression, or drug treatment that you currently have or take. The Healthcare Provider must have this information to evaluate and develop a proper post treatment evaluation.

10. Upon returning to work, fill out the Employers First Report of Injury E1 Form. This form can be downloaded from the human resources website under "Forms" (https://www.hr.utah.edu/forms/index.php).

11. After medical care, ensure that the incident is immediately reported to the Biosafety Officer (801-581-6590).

12. Have the PI/Supervisor complete a "**SPILL OR EXPOSURE EVENT REPORT,**" using the template below, and submit to the Biosafety Officer (801-581-6590).

Maps of Occupational Medicine Clinics



UNIVERSITY OF UTAH REDWOOD HEALTH CENTER OCCUPATIONAL MEDICINE

1525 W. 2100 S. Salt Lake City UT, 84119

REDMED EMPLOYEE HEALTH CLINIC

200 Central Campus Dr. Salt Lake City, UT 84112

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 should 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).

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)

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 C: Safer Sharps Devices Annual Review Form

This form (or equivalent) must be completed on an annual basis by any University of Utah Facility/laboratory that performs sharps-related procedures on human samples or other potentially infectious material. Contact the Biosafety Office at (801) 581-6590 if you have questions or need further information.

Reviewer's Name: Job Title:

Department/Clinic: Date:

Supervisor/PI Name: Telephone #:

In accordance with OSHA's application of the "Needlestick Safety & Prevention Act", all sharps that are being used where there is exposure to human blood or OPIM must be reviewed on an annual basis. This includes all needles, syringes with needles, scalpels, capillary tubes, and lancets. During your annual review of devices, you must inquire about new or prospective safer options.

The purpose of this form is to document:

- Sharps devices currently in use;
- The criteria used in the selection of the safer sharps devices in use, and;
- Annual consideration of new safer sharps devices.

Complete the table below as completely as possible to document the sharps devices that are being used. Use multiple pages if necessary.

This review form must be maintained with your safety records.

	Device #1	Device #2	Device #3
Name of Sharps Device			
Manufacturer			
Model/Size in Use			
Procedures Performed			
***Safer Sharps Device? (Y/N)**			
Description of Safety Feature			
Justification for Selection (must consider newly marketed safer sharps devices			

*A justification must be documented (below) for any device that does **not** meet the criteria of a safer sharps device (see *Sharps with engineered sharps injury protection* definition below). Acceptable justifications include, but are not limited to:

- Use of a safer sharps device will jeopardize patient or employee safety.
- Use of a safer sharps device is medically inadvisable.
- Market unavailability of an appropriate safer sharps device.

Note that cost is not typically an acceptable justification.

Sharps with engineered sharps injury protection: This includes non-needle sharps or needle devices containing built-in safety features that are used for collecting fluids or administering medications or other fluids, or other procedures involving the risk of sharps injury. This description covers a broad array of devices, including:

- Syringes with a sliding sheath that shields the attached needle after use;
- Needles that retract into a syringe after use;
- Shielded or retracting catheters
- Intravenous medication (IV) delivery systems that use a catheter port with a needle housed in a protective covering.

Description of procedure and justification for not using safer sharps device:

Appendix D: 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, EHS 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

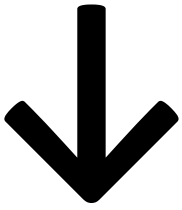
The OSHA Bloodborne Pathogen standard CFR 1910.1030 requires an EPA-registered disinfectant effective against HIV-1 and Hepatitis B virus. Therefore, diluted ethanol or isopropanol may not be used to disinfect materials and surface contaminated by human or non-human primate blood or other potentially 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. EHS 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. Descending Order of Relative Resistance to Disinfectant Chemicals

	Type of Microbe	Examples
More Resistant	Prions	
	Bacterial or Fungal Spores	*Bacillus subtilis, Clostridium difficile/perfringens, Cryptococcus*
.	Mycobacteria	*Mycobacterium tuberculosis, Mycobacterium bovis*
	Non-enveloped and small viruses	Coxsackievirus, Rhinovirus, Adenovirus, Poliovirus
	Fungi	*Aspergillus., Candida sp.*
. .	Vegetative Bacteria	*Streptococcus pneumoniae, Staphylococcus aureus, E. coli, Pseudomonas spp., Klebsiella spp.*
Less Resistant	Enveloped or medium viruses	Herpes Simplex virus, Cytomegalovirus, HIV (Lentiviruses), Respiratory syncytial virus, Hepatitis B virus, Hepatitis C virus, Hantavirus, Ebola virus, Coronavirus

Note: There are exceptions to this list. *Pseudomonas* spp. are sensitive to high-level disinfectants. However, in biofilms, the protected cells and those within free-living amoeba, or existing as persister cells (viable but not culturable) within the biofilm, can approach the resistance of bacterial spores to the same

disinfectant. The same is true for the resistance to glutaraldehyde by some nontuberculous mycobacteria, some fungal ascospores of *Microascus cinereus* and *Chaetomium globosum*, and the pink-pigmented Methylobacteria. Prions are also resistant to most liquid chemical germicides

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 Good against: Vegetative Bacteria	Free available chlorine combines with contents within microorganism,	Kills hardy viruses (e.g. hepatitis) Kills a wide range of organisms	Corrodes metals, such as stainless, aluminum Organics may reduce activity	Follow spill procedure and dilution instructions	Bleach solutions (sodium hypochlorite) Clorox

Class	Recommended Use	How They Work	Advantages	Disadvantages	Comments & Hazards	Examples
	Fungi Enveloped Viruses Non-enveloped Viruses Good at >1000ppm Sodium Hypochlorite: Spores Good with extended contact time: Mycobacteria	reaction byproducts cause its death Need 500 to 5000 ppm Produce chemical combination with cell substances Depends upon release of hypochlorous acid	Inexpensive Penetrates well Relatively quick microbial kill May be used on food prep surfaces	Increase in alkalinity decreases bactericidal property Unpleasant taste and odor Tuberculocidal, with extended contact time	Make fresh solutions before use Eye, skin and respiratory irritant Corrosive Toxic	Cyosan Purex
Iodophors (Iodine with carrier)	Disinfecting some semicritical medical equipment Very Good: Fungi Viruses Bacteria Some Spores Good with extended contact time: Mycobacteria	Free iodine enters microorganism and binds with cellular components Carrier helps penetrate soil/fat Probably by disorder of protein synthesis due to hindrance and/or blocking of hydrogen bonding	Kills broad range of organisms Highly reactive Low tissue toxicity Kills immediately rather than by prolonged period of stasis Not affected by hard water May be used on food prep surfaces	May stain plastics or corrode metal May stain skin/laundry Stains most materials Odor Some organic and inorganic substances neutralize effect Tuberculocidal, with extended contact time Sporicidal: Some	Dilution critical Follow directions! Use only EPA registered hard surface iodophor disinfectants Don't confuse skin antiseptic iodophors for disinfectants Skin and eye irritant Corrosive Toxic	Wescodyne Bactergent Hy-Sine Ioprep Providone (iodine/betadine)
Alcohols	Good Against: Vegetative Bacteria Fungi	Changes protein structure of microorganism	Fairly inexpensive	< 50% or >90% Solution not very effective	Flammable Eye Irritant Toxic	70% Ethanol Cavicide

Class	Recommended Use	How They Work	Advantages	Disadvantages	Comments & Hazards	Examples
	Enveloped Viruses	Presence of water assists with killing action		Not active when organic matter present Not active against certain types of viruses Evaporates quickly Contact time may not be sufficient for killing Alcohol solutions not EPA-registered disinfectants		
Glutaraldehyde	Good Against: Vegetative Bacteria Fungi Mycobacteria Viruses Spores	Coagulates cellular proteins	Non-staining, relatively noncorrosive Useable as a sterilant on plastics, rubber, lenses, stainless steel and other items that can't be autoclaved	Not stable in solution Has to be in alkaline solution Inactivated by organic material	Eye, skin and respiratory irritant Sensitizer Toxic	Cidex Calgocide 14 Vespore
Quaternary Ammonium compounds (QUATS)	Ordinary housekeeping (e.g. floors, furniture, walls) Good Against: Vegetative Bacteria Enveloped Viruses Fungi	Affects proteins and cell membrane of microorganism Releases nitrogen and phosphorous from cells	Contains a detergent to help loosen soil Rapid action Colorless, odorless Non-toxic, less corrosive Highly stable	Does not eliminate spores, TB bacteria, some viruses Effectiveness influenced by hard water Layer of soap interferes with action	Select from EPA list of hospital disinfectants Skin and eye irritant Toxic	Coverage 258 End-Bac Hi Tor Bacdown

Class	Recommended Use	How They Work	Advantages	Disadvantages	Comments & Hazards	Examples
			May be used on food prep surfaces			
Phenolic Compounds	Good Against: Vegetative Bacteria Fungi Enveloped Viruses Some non-enveloped Viruses Mycobacteria	Gross protoplasmic poison Disrupts cell walls Precipitates cell proteins Low concentrations inactivate essential enzyme systems	Nonspecific concerning bactericidal and fungicidal action When boiling water would cause rusting, the presence of phenolic substances produces an anti-rusting effect	Unpleasant odor Some areas have disposal restrictions Effectiveness reduced by alkaline pH, natural soap or organic material Not Sporicidal	Skin and eye irritant Sensitizer Corrosive Toxic	Hil-Phene Amphyl LpH se Metar Vesphene Decon-Cycle

3). Disposal

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

Appendix E: 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 BMBL Appendix I: Guidelines for work with Toxin of Biological Origin for more information.}

Standard Operating Procedures for *[Toxin]*	
#1 Chemicals/Hazards	[*Obtain specific toxin hazard information from MSDS/SDS.*] CAS number: *[XXX]* Routes of exposure: *[XXX]* How exposure might occur: *[XXX]* Target organs: *[XXX]* 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. Appropriate inactivation method(s) for [*toxin*] will be determined and supplies for inactivation and spill cleanup of *[toxin]* will be readily available. *[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).* In-line HEPA filters will be used on vacuum lines. 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]* • Laboratory coat or gown with long cuffed sleeves

	• Disposable lab coat • Disposable sleeves • Safety glasses with side shields or chemical safety goggles • Face protection such as a face shield if splash/spatter possible • Gloves *[type]* that are impervious to *[toxin]* and diluent • Respiratory protection *[if aerosol hazard is present] If respirators are used the worker must be enrolled in the Respiratory protection program; contact EHS for information (http://d2vxd53ymoe6ju.cloudfront.net/wp-content/uploads/sites/4/20160922155338/Resp-Prot-Program-rev.2016.pdf).* Gloves must be changed immediately if contaminated, torn, or punctured.
#5 Special Handling Procedures & Storage Requirements	**HANDLING** <u>Prep</u> • Sign will be posted on the room door when toxin is in use stating: "Toxins in Use -- Authorized Personnel Only." • 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. • 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.]* <u>Use</u> • Only needle locking (Luer-Lock type) syringes or disposable syringe units will be used for injection or aspiration of *[toxin]*. • A sharps container will be in the immediate vicinity for safe sharps disposal. • Containers will be decontaminated before they are removed from *[fume hood/BSC]*.

	• The *[fume hood/BSC]* will be decontaminated upon completion of tasks with *[decontaminant and concentration]* for *[contact time]*. • All potentially contaminated disposable items will be placed in a hazardous waste bag and decontaminated before disposal. • Hands will be washed upon completion of tasks. **STORAGE** • *[Toxin]* will be stored in locked *[freezer/refrigerator/cabinet/box/other]* in *[secure location room #]*. **TRANSPORT** • *[Toxin]* will be transported in labeled and sealed non-breakable secondary containers.
#6 Spill and Accident Procedures *[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 EHS 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. **Liquid spills:** 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. **Powder spills inside of [*fume hood/BSC]*:** 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. **Powder spills outside of a *[fume hood/BSC]*:** 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 EHS (at 801-581-6590). Tell them that a spill has occurred, and you need EHS to assist with the spill cleanup. Be prepared to provide the following information: • Name and phone number of knowledgeable person that can be contacted: *[emergency contact name and phone number]* • *[Toxin name]*, concentration and amount spilled, liquid or solid spill • Number of injured, if any • Location of spill This information can also be used in reporting to the Emergency Department after potential exposure. 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. For questions on spill cleanup, contact EHS at 801-581-6590 for guidance.

	Wash hands thoroughly after completing any spill clean-up.
EXPOSURE PROCEDURES In Case of Emergency	**1. Provide First Aid Immediately** • For sharps injury (needlestick or subcutaneous exposure), scrub exposed area thoroughly for 15 minutes using warm water and soap. • 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. • For eye exposure, use the eye wash for 15 minutes while holding eyelids open. • For inhalation, move out of contaminated area. Get medical help. **2. Get Help** • 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. • Notify your supervisor as soon as possible for assistance. • Secure area before leaving. **3. Report Incident to Occupational and Environmental Health & Safety** • If serious accident, hospitalization or fatality, notify EHS immediately after providing first aid and/or getting help. 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 EHS for disposal whenever possible. 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. 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 EHS at 801-581-6590 for disposal instructions. For chemical waste pick up complete a request through the Safety Administrative 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)* *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. *[Give detailed procedures for safely completing tasks, containment, decontamination information, and any special disposal requirements.]* *[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.]* After procedures are complete, the restraining apparatus and surrounding work station will be decontaminated *[inactivating agent + concentration]*. All reusable lab equipment will be autoclaved. *[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 F: 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 Environmental Health and Safety (EHS) 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 EHS at 801-581-6590 or biosafety@ehs.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 EHS biosafety office at biosafety@ehs.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 EHS. 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. EHS 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 EHS, arranged through the EHS Safety Administrative 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 EHS, 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 EHS. 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 EHS 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 EHS 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. EHS 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 EHS, arranged through the EHS Safety Administrative 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 EHS, 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 EHS 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. 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

Appendix G: Cell Sorter Guidelines and Standard Operating Procedures

1. Purpose

By design, stream-in–air cell sorters produce aerosols. Therefore, the use of these instruments with certain biological agents constitutes a potential procedure hazard. This OEHS Standard Operating Procedure establishes requirements for the design of laboratories housing cell sorters, the creation of laboratory or instrument-specific Standard Operating Procedures (SOPs), and the procedures for the safe operation of cell sorters and validation of their aerosol containment systems.

2. Scope

The objectives and responsibilities set forth in this SOP are applicable to all University of Utah employees. University of Utah employees will comply with this policy and perform their duties in the safest possible manner.

3. Background

Flow cytometric cell sorting is an important technology in basic and clinical research laboratories. However, samples that are sorted may contain infectious biological agents, and standard procedures must be implemented to minimize risk of exposure to these potentially hazardous agents.

Laboratory procedures that generate aerosols are classified as the most important operational risk factor supporting the need for containment equipment and facility safeguards. The likelihood of aerosol production by cell sorters is high due to the possibility of fluid exiting a small orifice (usually 70µm) at high pressure (up to 70psi) impacting a hard surface.[1] Aerosol production is highest in the event of a partial obstruction of the nozzle orifice and subsequent stream deviation.

The fundamental objectives of any laboratory biosafety program should be containment of hazardous materials, and the development and implementation of procedures designed to reduce exposure based upon a thorough risk assessment. This policy is meant to reduce or eliminate exposure of the outside environment and laboratory personnel to potentially hazardous agents during the operation of cell sorters.

This policy is derived and adapted from established biosafety principles as outlined in the BMBL, the *National Institutes of Health* and the current *International Society for the Advancement of Cytometry (ISAC) Cell Sorter Biosafety Standards*.

4. Definitions

a. **Flow Cytometric Analyzer** - Scientific instrument used to characterize cells or particles in a fluid stream, based upon their measured fluorescence and light scatter characteristics, but are incapable of sorting the cells.

b. **Fluorescence-activated Sorter (FACS)** - FACS is a trademarked name for BD Biosciences instruments.

Note: The scope of this document is for Cell Sorters**, not analyzers**. The SOP's used as examples in this document are for BD FACS Aria cell sorters, hence the use of the name FACS. **It is important to distinguish a Cell Sorter from a Flow Cytometric Analyzer, since the risk of aerosol production from analyzers is much lower.**

c. **Cell Sorter** - Scientific instrument used to isolate cells or particles based upon their measured fluorescence and light scatter characteristics. There are two classes of Cell Sorters: electrostatic droplet-based (also known as jet-in-air or stream-in-air) and mechanical cell sorters. Electrostatic droplet-based sorters employ a liquid stream at high (up to 70 psi) pressure, carrying cells through a nozzle. The stream is not confined and therefore is open to the air. Mechanical sorters utilize fluid streams that are confined within tubing or microfluidic channels. The term cell sorters used in this SOP refer to electrostatic droplet-based sorters.

d. **Biosafety Levels** - A biosafety level is the level of the biocontainment precautions required to work with dangerous biological agents in an enclosed facility. The levels of containment range from the lowest biosafety level 1 (BSL-1) to the highest at level 4 (BSL-4). In the United States, the Centers for Disease Control and Prevention (CDC) have specified the requirements for each of these levels.

e. **Infectious Biological Agent** - A microorganism (including, but not limited to, bacteria (including rickettsiae), viruses, fungi, or protozoa) or prion, whether naturally occurring, bioengineered, artificial, or a component of such microorganism or prion that is capable of causing communicable disease in a human, animal, or plant.

f. **Personal Protective Equipment (PPE)** - Items of clothing (i.e. lab coats, shoe covers, safety glasses, facemasks, gloves, etc.) or equipment (i.e. face shields, eye goggles, etc.) designed to prevent or limit exposure to potentially harmful agents.

g. **Standard Operating Procedure (SOP)** -Written procedures that describe, in detail, how to perform a particular task or overall duty/responsibility.

h. **Mucous membrane protection** - A device or combination of devices, such as a full face shield or surgical face mask combined with form fitting goggles or approved protective glasses, etc., which protect the mouth, nose and eyes from splash or droplet contamination.

i. **Cell Sorter in certified Biological Safety Cabinets (BSC) -** Class II BSC: manufactured to meet functional certification criteria for personnel and product protection as defined by NSF 49; Class I BSC: manufactured to meet functional certification criteria for personnel protection as defined by the BMBL and have an inward airflow velocity of 100 linear feet per minute. High Efficiency Particulate Air (HEPA) filters are to be tested for leakage annually.

j. **Select Agents** – Select Agents are bio-agents which have been declared by the U.S. Department of Health and Human Services (HHS) or by the U.S. Department of Agriculture (USDA) to have the "potential to pose a severe threat to public health and safety." These bio-agents are divided into three broad categories: 1) HHS select agents and toxins (affecting humans); 2) USDA select agents and toxins (affecting agriculture); and 3) Overlap select agents and toxins (affecting both).

k. **Institutional Biosafety Committee (IBC)** - The Institutional Biosafety Committee reports to the Vice President of Research or his or her designee on matters pertaining to the control of biological hazards. The IBC is the primary reviewing and biosafety approval body for all proposed research associated with the intramural use of microbiological agents and recombinant or synthetic nucleic acid research subject to the NIH Guidelines at the University of Utah.

l. **Human Pathogens** - Human Pathogens are agents (such as viruses, bacteria, prions, or fungi) that cause disease in humans.

m. **High Efficiency Particulate Air (HEPA) filter** -A throwaway, extended-media, dry type filter with a rigid casing enclosing the full depth of the pleats. The filter shall exhibit a minimum efficiency of 99.97% when tested at an aerosol of 0.3 µm diameter.

n. **Agent Risk Group**-The classification of an infectious microorganism according to its capability to infect and cause disease in a susceptible human or animal host, its virulence as measured by the severity of disease, and the availability of preventative measures and effective treatment for the disease. Four Risk Groups are defined in the BMBL, ranging from least likely to cause human disease (Risk Group 1) to highly likely to cause serious or lethal disease (Risk Group 4).

5. Procedures:

A. Risk Assessment

A risk assessment should be conducted for all samples/agents prior to sorting by the Principal Investigator/Facility Director, and the appropriate biosafety level determined in collaboration with OEHS Biosafety Specialists, subject matter experts, and the Institutional Biosafety Committee. The purpose of a risk assessment is to recognize and identify hazards and measure the risk or probability that something will happen because of that hazard.

The results of a comprehensive risk assessment determine the appropriate procedures and practices for cell sorting. The designation of safety measures is dependent upon the risk and the severity of the consequences if exposure occurs. Risk analysis takes into account the Risk Group of the agent and the procedures performed with the agent.

Risk Assessment consists of five steps:

1. Identify and evaluate agent hazards: To aid in the identification of risks associated with biohazardous agents, microbiological agents have been classified into one of four Risk Groups (RG) by the WHO, Canada, Australia, the European Union and the NIH Recombinant DNA Advisory Committee and the American Biosafety Association (ABSA). Although these classifications differ dependent upon the country or organization, they generally will take into account factors such as pathogenicity of the organism, virulence, mode of transmission, infectious dose, communicability and availability of effective vaccines or effective treatment. There is a wide range of risk within each risk group classification, underscoring the importance of conducting a risk assessment for each biohazard. Agent characteristics to consider may involve the degree of attenuation, fixatives used to inactivate the agent, route of infection, and how a pathogen may have been rendered defective. Other characteristics that could elevate risk include the use of strains for which immunization is not protective, prior LAIs with the agent via the airborne route, and agents with a high consequence of infection.
2. Identify laboratory procedure hazards: It is important to emphasize that the second major factor to consider in risk assessment are the laboratory procedures in agent handling. Procedures with biohazards involving the use of sharps, those involving research animals, and those that may generate splash, splatter or aerosols can elevate risk. For example, human pathogens that are designated as Risk Group 2 agents under normal laboratory procedures and practices may be classified at a higher biosafety containment level because of the potential for aerosol and/or splash exposure. Cell sorting is therefore considered a laboratory procedure hazard due its potential for aerosol production.
3. Make final determination of biosafety level (BSL) (See Table 11): BSL2 with enhanced precautions is not a biosafety level, but reflects procedures and practices at the BSL2 level together with additional procedures as specified in Table 11. The guidelines for risk assessment in Table 11 take into account both agent hazards and sample origins for assignment of biosafety containment levels and procedures. This therefore combines agent Risk Group classifications, coupled with the recognition of cell sorting as a laboratory procedure hazard. In this regard, handling of all human and non-human primate specimens and primary human cell cultures as infectious is recommended, unless comprehensive pathogen screening has been performed and demonstrated the absence of adventitious agents. Although impractical for most cell sorting experiments, samples may be fixed in order to reduce the biocontainment level required. However, in this case, appropriate methods

must be selected to reliably inactivate potentially biohazardous agents. Concerns exist about the effectiveness of standard fixation methods to reduce the level of infectivity in samples containing high titers of known viruses or unknown infectious agents resistant to inactivation. Fixation procedures must be performed carefully within well-defined standard operating procedures; otherwise, samples that are presumed inactivated, may not be and therefore could pose a serious health risk to laboratory personnel. Cell sorting operators or managers and IBCs may require proof of inactivation for higher risk biohazards to ensure that the biosafety level selected is appropriate for the proposed sorting experiment.

4. Evaluate proficiencies of staff and integrity of safety equipment: It is critical to evaluate the level of proficiency of the cell sorter operator in conducting a risk assessment. This includes an evaluation of cell sorter operating skills, as well as techniques for safe handling of specimens and use of any safety equipment. Proficiency in the operation of the cell sorter is particularly important in the event of a nozzle obstruction with subsequent aerosol production. In this case, an inexperienced operator will focus on instrument operation and thus will be more likely to ignore or circumvent biosafety features and procedures resulting in potential exposure. Training of cell sorter operators is therefore an essential component of the cell sorting laboratory's operational procedures. The amount of training deemed sufficient for independent operation of a cell sorter is dependent upon several factors, but must include the results of the risk assessment process. Specifically, for sorts requiring higher biosafety containment levels (BSL2 with enhanced precautions or BSL3) the degree of training and experience must be correspondingly greater. For independent operation of cell sorters at these biosafety containment levels, a checklist of requirements of experience/training is essential to ensure safe operation. These must include required institutional biosafety training such as bloodborne pathogen training, BSL2-specific training, BSC training, etc., but also instrument experience, such as hours of supervised and independent cell sorting operation. Ideally, before sorting samples at a higher biosafety containment level, initial training should include sorting on cell sorters of similar design using non-infectious samples of the same type that will contain the known biohazard. In addition, when procedures are changed, all operators should be required to review these procedures and documentation of this review must be maintained. All safety equipment must be inspected or tested to verify functionality. For cell sorters, evaluation of safety equipment includes visual inspection of sort/collection chamber doors to ensure integrity, or absence of dirt and/or salt crystals on seals; presence of an aerosol management system and validation of containment (see below) and verification that all other supplied safety features are intact.

5. Review risk assessment with OEHS Biosafety Specialists and the Institutional Biosafety Committee

The Risk Group of a given agent can be determined from a variety of sources, most notably the BMBL. Cell sorting is considered a laboratory procedure hazard because of the potential for aerosol and/or splash exposure. Agents that may be worked with at BSL-2 under normal laboratory procedures and practices, may require greater precautions as defined in this document as BSL-2 with enhanced precautions. Due to the risk of aerosol exposure in cell sorting, an aerosol management system is required at all biosafety levels and usually consists of a sort chamber evacuation pump equipped with a High Efficiency Particulate Air (HEPA) filter. All aerosol management systems require validation (as indicated below), although the frequency of testing increases with increased biosafety levels.

6. Standard Operating Procedure (SOP) Development for Cell Sorter Laboratories

An important outcome of any risk assessment process is the creation of standard operating procedures (SOPs). An SOP must take into account hazards (agents and laboratory procedures) and specify practices and procedures designed to minimize or eliminate exposures to those hazards. For cell sorters, the design of the instrument, especially containment or aerosol evacuation components, must be considered in the development of the SOP. Each instrument must be evaluated for deficiencies in containment or aerosol evacuation design and appropriate procedures adopted to minimize risk. An important example of this is that most cell sorters do not possess an interlock designed to prevent the operator from opening the sort chamber after a nozzle obstruction with subsequent stream deviation. Therefore, the SOP should clearly address the procedures for evacuating the sort chamber of aerosols prior to opening the sort chamber, including a stated time period to wait after a clog induced stream deviation.

The general considerations for SOP development are outlined below:

1. Preparation before the sort
    a. Check fluids, empty waste
    b. Cover control surfaces with plastic wrap, including keyboards and mouse (or use washable keyboards).
    c. Perform containment testing. A detailed procedure can be found here
        i. Validation of containment and evacuation of aerosols is essential for operator safety. Testing on individual cell sorters may differ due to variation in cell sorter design among the available models, but it is essential that the following considerations are incorporated into the SOP:
        1. Fail mode testing: test is designed to mimic a nozzle obstruction with stream deviation and the subsequent generation of aerosols.

2. Testing frequency: dependent upon risk assessment, and biosafety containment level.
3. Containment testing for sorter in BSC: aerosol containment and evacuation of sorter independent of BSC operation must be performed.
4. Record keeping.

d. Verify sorting operation and sample introduction system

i. Before each sort, verify the proper operation of the sort mechanism and the stability of the sort streams and droplet break-off. If the streams and the droplet break-off do not remain stable during the sort setup, correct the problem before sorting.

ii. Cell sorters pressurize the sample tube once it is secured on the sample introduction port. While newer generation instruments are equipped with completely enclosed sample introduction chambers for operator safety, some older sorters have an open port requiring careful operator handling. Each time a sample tube is placed on the instrument, the operator must check the tube seal and its secure fit onto the sample introduction port. Otherwise, once the sample tube is pressurized, it could blow off and splash sample onto the operator or others involved in the experiment. Make sure that the tube material provides sufficient strength to tolerate high instrument pressure. On some instruments, when the tube is removed, the sample line back-drips, creating a potential biohazard through splattering of sample droplets on hard surfaces. To avoid this hazard, allow the back-drip to go into a tube until the sample is flushed out of its introduction line to avoid splashing of sample droplets. Alternatively, a soft absorbent pad soaked in disinfectant can collect the back drip without splattering. Installation of a plastic shield around the sample introduction port can block droplet spraying from the sample back-drip. The catch tray or trough should be decontaminated carefully after each sort.

iii. Select the appropriate nozzle size for the cell size to be sorted. Smaller nozzle sizes provide optimal signal resolution and easy sort setup, however, to avoid clogs, it is recommended that the nozzle orifice be at least four times larger than the cell diameter, but ideally it should be at least six times larger.

e. Verify any automated decontamination functions

i. If these systems are not used on a regular basis, it is possible that valves, connectors or pumps may fail due to buildup of salts, etc.

f. Preparation of disinfectant solutions

i. Disinfectants should be made before starting the sort, especially for those that have limited shelf life, such as solutions of sodium hypochlorite.

g. Sample preparation, i.e. staining, centrifugation, pipetting or manipulations that may generate aerosols should be performed in a manner to maximize containment and protect the worker

2. Procedures in the event of a nozzle obstruction

   a. Turn off stream

   b. Evacuate sort chamber prior to opening; increase Aerosol Management System (AMS) evacuation rate

   c. Attempt to clear nozzle clog by stream flush routines, with sort chamber door closed. If clog is not cleared, remove the nozzle and dependent upon sample risk assessment, decontaminate nozzle before sonication

3. Decontamination procedures

   a. All decontamination procedures should be validated and documented per Institution Guidelines.

   b. Decontaminate and clean sample lines, sort chamber and collection chamber.

   c. Decontaminate and clean surfaces around cytometer, especially near the sort chamber after each sort. The instrument should be decontaminated with a disinfecting agent, taking into account the biohazards under study. Sort collection tube holders are heavily exposed to sample droplets and must be carefully decontaminated before handling. Before designing a cell sorter-specific decontamination protocol, the operator or laboratory manager should consult the instrument manufacturer for compatible disinfectants and refer to more complete resources for decontamination. Two disinfectants commonly used in cell sorters are alcohols and bleach. Alcohols are not classified as high-level disinfectants, because they cannot inactivate bacterial spores and penetrate protein-rich materials, and isopropanol is not able to kill hydrophilic viruses. Aqueous solutions of sodium hypochlorite are widely used because they have a broad spectrum of antimicrobial activity, are inexpensive, fast acting, are unaffected by water hardness and do not leave a toxic residue. They can be corrosive to metals and therefore should be rinsed with water following decontamination. All surfaces inside the sort chamber, the sample introduction port and holder, are wiped down with appropriate disinfectant. Disinfectant is also run through the instrument for the appropriate exposure time and then followed with distilled water to completely remove the disinfectant as some disinfectants are corrosive to instrument components (consult manufacturer), and residual disinfectant solution can affect the viability of sorted samples. Make sure that the water used for removal of the disinfectant is sterile and does not introduce new contaminants into the instrument.

Development of the SOP should also include consultation with OEHS Biosafety Specialists who can provide guidance on general biosafety procedures as well as information on University of Utah policy. Examples of SOPs for cell sorters are included in Section 10 to serve as templates for development of individual laboratory SOPs. **Finally, the SOP should be reevaluated at least on an annual basis or whenever there is a change in instrument configuration that may affect biosafety.**

7. Specific Requirements for Operation of Cell Sorters in University of Utah Laboratories

Biosafety Level 2 (BSL-2) Laboratory – General:

a. The laboratory must meet all criteria for BSL-2 containment and be surveyed and posted by EHS.
b. Air flow in the room is balanced to create negative airflow into the room. The door must remain closed at all times.
c. Laboratories must have a sink for hand washing. The sink may be operated manually, hands-free, or automatically. It should be located near the exit door.
d. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
e. Vacuum lines should be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
f. An eyewash station must be readily available.

Biosafety Level 2 (BSL-2) Laboratory – With Enhanced protections:

a. Ideally, the cell sorter is located in a separate, lockable room where no other laboratory activity is performed. If the sorter is located in shared laboratory space, all Personal Protective Equipment **(**PPE) requirements should be followed by all personnel in the laboratory (NOT just those using the sorter) during sorting procedures. The cell sorter should be placed in a location in the lab so that directional air flow is toward the cell sorter and away from other areas of the lab. If the cell sorter is enclosed within a certified BSC (Class I or Class II), the requirement for placement of the cell sorter in a separate room may be abrogated dependent upon the overall risk assessment.

b. Air flow in the room is balanced to create negative airflow into the room. The door must remain closed at all times.

c. The sorting room is locked to restrict access to allow the operator to concentrate on the sort and to maintain regular air flow and negative air pressure in the room.

d. During sorting procedures, a sign should be placed on the outside of the door to indicate that a potentially biohazardous sorting process is in progress. This sign also should contain all necessary information for entering the room safely, including warning for Class IIIb or IV lasers, if applicable.

Biosafety Level 3 (BSL-3) Laboratory:

a. The laboratory must meet all criteria for BSL-3 containment and be surveyed and posted by EHS.

b. The cell sorter must be located within a Class II certified BSC (can be recirculated).

There are no Cell Sorters in BSL-3 Laboratories at the University of Utah presently.

Biosafety Level 4 (BSL-4) Laboratory:

a. The laboratory must meet all criteria for BSL-4 containment and be surveyed and posted by DOHS.

b. The cell sorter must be located within a Class II or Class III certified BSC.

There are no BSL-4 Laboratories at the University of Utah presently.

8. Cell Sorter-Specific Equipment and Practices

1. **Aerosol Containment:**

Aerosol management System (AMS): All cell sorters must be equipped with an aerosol management or evacuation system that is designed to evacuate the sort chamber and sort collection area of the cytometer. It consists of an evacuator that creates negative pressure within

those chambers, and transports aerosols through a HEPA or an ultra-low penetration air (ULPA) filter before exhausting to the room. The AMS should be operated under all biosafety levels, BSL-2, BSL-2 with enhanced precautions, BSL-3, and BSL-4.

2. Validation of Aerosol Management Systems:

Currently, the most widely accepted method of containment testing utilizes fluorescent plastic beads that are run on the instrument as a sample (See references 2-4 below).

The AMS must be tested under simulated worse-case "failure mode." In this mode, the instrument is set to high pressure (usually 70psi), and fluorescent particles are concentrated to approach speeds of approximately 20,000-50,000 particles/second. The stream is forced to glance off of the waste catcher shield to create a large plume of aerosols and aerosols concentrated on a slide for subsequent analysis on a microscope. Tolerance of aerosol escape is zero particles when the AMS is active and sort chamber door is closed. This test (or other validated test for containment) is performed periodically (monthly or only when filters are exchanged) for BSL-2 labs and labs performing sorts under BSL-2 with enhanced precautions. The test is performed prior to every sort for BSL-3 labs. Frequency of testing will be dependent upon the risk assessment and consultation with biosafety professionals and/or the IBC. However, containment testing must be performed in the following circumstances:

a. Following instrument service or maintenance involving the sort chamber and/or AMS hose connections.
b. Following initial instrument installation or relocation.
c. Following change out of the standalone AMS filter.
d. For BSL-3 or 4 labs:
    i. Prior to every sort if the frequency of sorting is once/week or less
    ii. Weekly, if the frequency of sorting is multiple sorts/week

3. Cell sorters in biological safety cabinets:

Class II BSC's enclosing cell sorters must be manufactured to meet functional certification criteria for personnel and product protection as defined by NSF 49. Class I BSC's enclosing cell sorters must be manufactured to meet functional certification criteria for personnel protection as defined by the BMBL, although it is recommended that the inward airflow velocity be 100 linear feet per minute or greater; HEPA filters must be tested for leakage annually. Cell sorters placed in BSC's must have an AMS in which aerosol containment validation can be performed independent of the BSC blowers,

i.e. with the BSC directional air current system turned off. This is done to provide greater sensitivity when performing the cell sorter AMS containment tests. The BSC must be validated initially at installation.

Frequent retesting and monitoring proper functioning of the cabinet is mandatory, as per NSF 49 requirements.

4. **User Specific Safety Equipment:**

   a. **Personal Protective Equipment (PPE) for Biosafety Level 2 (BSL-2) Laboratory:**

      i. **Lab coat**

      ii. **Gloves**

      iii. **Eye Protection**: Safety glasses (Impact resistant and side protection)

   b. **Personal Protective Equipment (PPE) for Biosafety Level 2 (BSL-2) with enhanced precautions:**

      i. **Isolation-style solid-front or wrap-around gown**, with cuffed sleeves or disposable sleeve covers

      ii. **Gloves (double pair)**. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Gloves and protective clothing must not be worn outside the laboratory and must be disposed of with other contaminated waste.

      iii. **Eye protection:** Safety goggles, face shield, splatter guard, or integral respirator/face shield that provide mucous membrane protection as required for anticipated splashes or sprays of infectious agents or other hazardous materials.

      iv. **Respirator:** National Institute for Occupational Safety and Health (NIOSH)-approved respirators must be worn during operation of the cell sorter under BSL-2 with enhanced precautions conditions. Approved respirators include N-95, N-99, or N-100 filtering face-piece respirators or powered air-purifying respirators (PAPR) with integral face shield. Respirators must remain on during all procedures associated with sample manipulation, including sample tube cap removal and loading of sample on instrument, or when removing collection tubes or other procedures where the sort or collection chamber is opened. For non-primate samples containing agents that do not pose respiratory risk, mucous membrane protection may be substituted for respirators. For example, the human pathogens

leishmania and mouse models of toxoplasma infection are included in this category.

- All individuals using respirators must be enrolled in the University of Utah Respiratory Protection Program. Questions should be directed to OEHS at 381-581-6590

v. **Cell Sorters enclosed in a certified BSC:** use of respirators as outlined above is recommended during instrument/sample manipulation within the BSC but can otherwise be removed during sorting procedures providing the BSC is operational, aerosol management system is active and all sort chamber and collection chamber doors are closed. If the BSC-enclosed Cell Sorter is in a shared laboratory, respirators are not required for other laboratory personnel.

c. Disinfection:

The choice of disinfectant is dependent upon a variety of factors including the agent in use, the chemical resistance of the cell sorter components, and potential of exposure of lab personnel to the chemical disinfectant. Broad-spectrum disinfectants are desirable in a facility in which agent use is varied. For work involving human or non-human primate cell lines it must be an EPA-Registered disinfectant.

Sodium hypochlorite solutions (1:10 dilution of household bleach in H_2O; final concentration of 5,250-6,150 ppm of chlorine) offer several advantages over alcohols and other disinfectants; bleach has broad-spectrum antimicrobial activity, does not leave toxic residues, is unaffected by water hardness and is inexpensive and fast acting. However, because of the corrosive nature to metals, exposure to instrumentation should be limited to times determined to be maximally efficacious to microbial killing. In addition, bleach solutions must be prepared fresh due to loss of free available chlorine. However, there are commercially available sprayers that mix the bleach and water when sprayed, eliminating the need to make fresh solutions daily.

9. References

1. Holmes KL. Characterization of aerosols produced by cell sorters and evaluation of containment. Cytometry A 2011:79;1000-8.
2. Holmes KL, Fontes B, Hogarth P, Konz, R, Monard S, Peltcher CH, Wadley RB, Schmid I, Perfetto S. International Society for Advancement of Cytometry Cell Sorter Biosafety Standards. Cytometry A 2014:85;434-53.
3. Oberyszyn AS. Method for visualizing aerosol contamination in flow sorters. Curr Protoc Cytom 2002: Chapter 3; Unit 3.5

4. Perfetto SP, Ambrozak DR, Koup RA, Roederer M. Measuring containment of viable infectious cell sorting in high velocity cell sorters. Cytometry A 2003:52;122-30.

Table 11: Biosafety Level Determination for Cell Sorting

	BSL-2	BSL-2 with enhanced precautions (during sorting operations)
Risk Assessment Condition	Pathogen-free Human /NHP cells Uninfected non-primate cells	Unscreened Human /NHP cells Infectious but with low risk assessment (e.g. Risk Group 2)
Example: Sample type or Agents[1]	Normal murine cells 3rd generation Lentivirus transduced cells (non-human cells or pathogen-free human cells: >72 hours post transduction)	Normal human blood Unscreened Human cell lines[1] An example agent is: Influenza A[1] 2nd generation Lentivirus transduced cells or 3rd generation lentivirus in unscreened human cells
Containment System Validated	Periodically (monthly or with filter change)	Periodically (monthly or with filter change)
Aerosol Containment Operational	Required	Required
Respirator	Optional	N-95 or better[2]
Eye protection	Safety Glasses	Safety Glasses or goggles plus face shield or mask
Lab Coat	Lab coat	Wrap around rear closure, cuffed or disposable sleeves
Separate Room and Environmental controls	Optional	Required or limited access to room[3]

[1]Example: Sample type or Agents - the samples and/or agents listed represent only a partial list of agents which may be included in each category. A risk assessment should be conducted for all samples/agents prior to sorting, and the appropriate biosafety level determined in collaboration with Biosafety specialists, subject matter experts and the IBC. For additional information please consult the following web sites: http://www.phac- aspc.gc.ca/msds-ftss/index-eng.php; https://www.cdc.gov/biosafety/publications/bmbl5/index.htm

[2]Respirators must remain on during all procedures associated with sample manipulation, including sample tube cap removal and loading of sample on instrument, or when removing collection tubes or other procedures where the sort or collection chamber is opened. Note that respirator protection may otherwise be removed during the sorting process providing the aerosol management system is active and all sort chamber and collection chamber doors are closed. For human pathogens with a containment recommendation of BSL-2 and are not respiratory hazards, but which may pose a risk if exposed to mucous membranes, only mucous membrane protection is required. Examples of agents in this category include Leishmania and toxoplasmosis in murine cells.

[3]Enclosure of the cell sorter within a certified BSC may abrogate the need to house the sorter in a separate room within the BSL-2 lab space; PPE (as detailed above) is optional, but strongly encouraged for the operator during procedures requiring manipulation of instrument. Cell sorters located within a shared laboratory may be operated under BSL-2 with enhanced precautions if during the operation of the sorter, access to the room is limited and PPE, as detailed above, is worn by all occupants.

Table 12: Example Agent List with Biosafety Level for Cell Sorting

Agent	Recommended Biosafety Level[1]	Restrictions or Comments	MSDS Link
Hepatitis C	BSL-2+[2]		https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/hepatitis-c-virus.html
Human Metapneumovirus	BSL-2+		
Human Parainfluenza Virus type 3	BSL-2+		https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/human-parainfluenza-virus.html
Influenza A	BSL-2+	Influenza (seasonal) vaccine required	https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/influenza-virus-type-a.html
Klebsiella pneumonia	BSL-2+		https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/klebsiella.html
LaCrosse virus	BSL-2+		
LCMV	BSL-2+ or BSL-3	Ensure that HVAC system does not exhaust near vivarium housing mice; BSL dependent upon strain; pregnant women should consult	https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/lymphocytic-choriomeningitis-virus.html

		Occupational Medical Service (OMS) or their personal physician prior to performing a procedure with this agent.	
Leishmania	BSL-2+[3]		https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/leishmania.html
Malaria	BSL-2+[3]		
PVM (Pneumonia Virus of Mice)	BSL-2+		
Respiratory Syncytial Virus	BSL-2+		https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/respiratory-syncytial-virus.html
Toxoplasma gondii	BSL-2+	Pregnant women should consult OMS or their personal physician prior to performing a procedure with this agent.	https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/toxoplasma-gondii-pathogen-safety-data-sheet.html
Vaccinia	BSL-2+	vaccine required	https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/vaccinia-virus.html
HIV	BSL-2+ or BSL-3		https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-

			assessment/human-immunodeficiency-virus.html

[1]This list represents examples of biosafety level determination for cell sorting of specific agents. The final determination of the biosafety level is dependent upon the risk assessment conducted in collaboration with OEHS Biosafety, subject matter specialists and the University of Utah IBC.

[2]BSL-2 with enhanced precautions is abbreviated BSL-2+ for this table.

[3]Respirator PPE optional (mucous membrane protection required) for this agent except where the sample also contains human/NHP blood cells or fluids.

The following is a list of agents that may require BSL-3 containment for sorting. Currently no such facilities are available at the University of Utah:

Agent	**Recommended Biosafety Level**	**Restrictions or Comments**	**MSDS Link**
LCMV	BSL-2+ or BSL-3	Ensure that HVAC system does not exhaust near vivarium housing mice; BSL dependent upon strain; pregnant women should consult Occupational Medical Service (OMS) or their personal physician prior to performing a procedure with this agent.	https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/lymphocytic-choriomeningitis-virus.html
1918 Influenza	BSL-3	Influenza (seasonal) vaccine required	
Avian influenza	BSL-3	Influenza (seasonal) vaccine required	

HIV	BSL-2+ or BSL-3		https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/human-immunodeficiency-virus.html
Monkeypox	BSL-3	Vaccine required, every 3 years	
SARS-CoV-2, including unfixed samples from patients with COVID-19	BSL-3		
TB, *Mycobacterium tuberculosis*	BSL-3		https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/mycobacterium-tuberculosis-complex.html

10. Examples of Standard Operating Procedures

These can be used as guidelines for formulating a Standard Operating Procedure for individual laboratories. Procedures and practices will vary dependent upon risk assessment and instrument designs. Product or company names used in these examples do not in any way constitute explicit or implicit endorsement of these products or companies by the University of Utah.

BSL-2 SOP

1. Wear Lab Coat, Eye Protection and Gloves
2. Turn on Aerosol Management System (biohazard vacuum) and operate at 20% or as recommended by instrument manufacturer:
    a. Check vacuum reading. If vacuum is >2.4 inches of H_2O, change HEPA filter. Note: HEPA filter must be changed every 6 months, regardless of vacuum reading.
    b. Procedure for changing HEPA filter on AMS unit:
        i. While wearing gloves, lab coat, N-95 rated face mask (respirator) or PAPR and goggles/safety glasses, place the Buffalo unit HEPA filter inside an orange biohazard plastic bag. Disconnect hose from the Aria and also place within the bag. Seal the bag and place within a Medical Pathological Waste (MPW) box. Install a new HEPA filter and hose.
3. Make sure collection chamber door and sort chamber door are closed during sorting procedures
4. Do not eat or drink in laboratory
5. Remove gloves before answering phone
6. Remove lab coat and gloves and wash hands before leaving lab

BSL-2 with enhanced precautions SOP - FACS Aria II

1. **Preparation before the sort**
    a. If not using a sealed keyboard and mouse, cover keyboard, mouse and other instrument control surfaces w/ plastic wrap; clear surfaces of clutter, use absorbent pads for samples.
    b. Using a damp paper towel(s), wipe up dried bleach residue from instrument areas, paying particular attention to the sample uptake area, O-rings, charge plates and the side

stream viewing window. Warning: Failure to remove salt residue from the sample uptake system may cause the pressurized seal to fail and release potential aerosols!

c. Prepare sort collection chamber as necessary. Install the correct collection tube holder. Close sort collection chamber door.

d. If the Aria is contained within a BSC, turn the BSC blower fan on and turn the evacuation vacuum on low.

e. If not using a BSC, turn biohazard vacuum (Buffalo Filter Whisper Unit) on and operate at 20%. Check vacuum reading. If vacuum is >2.4 inches of H_2O, change HEPA filter. Note: HEPA filter must be changed every 6 months, regardless of vacuum reading.

   i. Procedure for changing HEPA filter on AMS unit:

      - While wearing gloves, closed front lab coat, N-95 rated face mask (respirator) or PAPR and goggles/safety glasses, place the Buffalo unit HEPA filter inside an orange biohazard plastic bag. Disconnect hose from the Aria and also place within the bag. Seal the bag and place within a Biohazardous waste container. Install a new HEPA filter and hose.

f. Make sure sheath tank is filled and standard waste tank contains enough bleach to give a final 10% (1:10 dilution of household bleach) solution when filled. Fill a spray bottle with a freshly made 1:10 dilution of bleach solution for work area decontamination.

g. Wear two pairs of gloves, closed front lab coat, N-95 rated face mask (respirator) and goggles/safety glasses or PAPR before handling samples.

h. Lab door must be closed and investigators are to remain outside of the lab until data files of the experimental controls and samples have been collected and tubes are no longer being manipulated.

i. For areas within a BSC, wear gloves, closed front lab coat, and goggles/safety glasses (and N-95 mask, optional) before handling samples. Notification of a potential biohazard must be posted outside the lab entrance. Investigators may remain in the room during data file collection.

j. Respirators must remain on during all procedures outside of the BSC associated with sample manipulation, including sample tube cap removal and loading of sample on instrument, or when removing collection tubes or other procedures where the sort or collection chamber is opened as outlined below. Note that respirator protection may otherwise be removed during the sorting process except during procedures as outlined above.

k. Have a spare nozzle, with new O-ring installed, available in case of a clog.

2. **Procedures during sorting/analysis**

a. Filter samples prior to sort to avoid clogs

b. Fill sample tube with as much sample as possible to minimize loading and unloading sample. DO NOT fill higher than ¼ inch from the top of the tube.

c. Make sure the "Sweet Spot" is enabled.

d. Close sort collection chamber door before starting sample.

e. When changing collection tubes:

   i. Stop the sample flow and close the aspirator drawer by clicking the Acquire button.

   ii. Wait at least 60 seconds before opening sort collection chamber door.

f. When removing collection tubes, be aware that the outside of the tube is potentially contaminated, use alcohol swab or bleach to wipe outside of tubes.

3. Procedures in the event of a nozzle obstruction

a. If during the sort the stream is deflected (due in part to a clogged nozzle), the sort is designed to stop automatically and block the sort tubes. The sort will not restart until the operator has cleared the clog. In the event of a nozzle clog , DO NOT open sort collection chamber door or sort block door before following this procedure:

   i. If the system has not already shut down automatically, turn off the stream using the button labeled with an '✓' on the Breakoff window. This will shut off the stream, unload the sample and close the aspirator door. Remove and cap the sample tube.

      - With the sort block chamber door, aspirator drawer and collection chamber door all closed, turn the stream on and off several times or perform the 'Clean flow Cell' procedure with DI H20 followed by turning the stream on to see if the clog will clear itself.

   ii. Turn stream off.

   iii. Open aspirator drawer using software controls.

   iv. Increase the air evacuation rate on the AMS unit to 100% or if using a BSC, push the high evacuation button (low button must also remain on).

   v. Wait at least 60 seconds. This procedure will clear aerosols from the sort chamber. Close the aspirator drawer.

vi. The sort block chamber door and sort collection chamber door can now be opened.

vii. If it is necessary to change nozzles, remove nozzle and O-ring and place in tube with a 1:10 dilution of bleach for 30 minutes. Thoroughly rinse nozzle in water and let air-dry. Discard O-ring if not using nozzles with integrated O- rings. Spare integrated nozzle or spare nozzle with O-ring may be installed while obstructed nozzle is soaking in bleach.

viii. With stream turned off, open the sort block chamber door and dry plates and surfaces as needed.

ix. When removing collection tubes, be aware that the outside of the tube is potentially contaminated, use alcohol swab or bleach to wipe outside of tubes.

x. Set AMS unit to 20% vacuum or toggle the high evacuation button off if using a BSC.

xi. Make sure that all chamber doors are closed and restart the stream.

4. Aerosol Release/Spill Response Procedures

a. In the event of an aerosol release or a spill of infectious sample outside of Biological Safety Cabinet, the following protocol must be followed.

i. Aerosol Release Definition: The engineering controls on the Aria (Sort Chamber door, Collection Chamber door and Aerosol Management system) and the SOP in this document are designed to prevent aerosol release into the room. Failure of these systems or failure to follow the SOP may result in an aerosol release. The most likely scenario for an aerosol release is opening the sort chamber door, during, or immediately following a nozzle obstruction.

ii. In the event of an aerosol release or spill of infectious material:

1. Push the Emergency Stop Button, and immediately exit the lab, closing the door as you leave. (All personnel must immediately exit the room)
2. Wait 30 minutes, and then put on respirator, gloves and lab coat as detailed above.
3. Enter the lab and clean any spill using 10% bleach HypeWipe pads. Clean horizontal surfaces near the cell sorter, or near the spill location using HypeWipe pads. Respirator may be removed after all cleaning procedures have been performed.

5. Decontamination Procedures:

a. Disengage “Sweet Spot” and turn the stream off.

b. Disinfect sample lines using a freshly made 1:10 dilution of bleach solution as follows:
    i. Fill a tube with a volume of 1:10 diluted bleach equal to or greater than the volume of sample that was sorted and place on the sample stage.
    ii. Select from the menu - Instrument > Cleaning Modes > Clean Flow Cell. Perform this step three times or until a bleach drop is visible in the stream camera view.
    iii. Wait 30 or more minutes with 1:10 diluted bleach in flow cell.
    iv. Fill a tube with DI water, Select from the menu - Instrument > Cleaning Modes > Clean Flow Cell.
    v. Fill a tube with 70% ETOH, Select from the menu - Instrument > Cleaning Modes > Clean Flow Cell. Perform this step three times or until an ETOH drop is visible in the stream camera view. Shutdown instrument.
c. Clean all surfaces around optical bench, sort block chamber and charge plates, sort collection chamber, sample introduction area and sample tube holder(s) with a prepackaged 10% bleach wipe and/or 1:10 dilution of bleach from a spray bottle. Clean keyboard cover, remove any plastic wrap that may have been used and discard in MPW box.
d. When leaving the lab:
    i. Make sure all sample tubes and containers are closed.
    ii. Remove gloves, respirator & lab coat (remember outside of gloves are contaminated!).
    iii. WASH HANDS!

Appendix H: SOP for Autoclaving Infectious Waste: Use and Testing.

1. **Purpose**

The purpose of this Standard Operating Procedure (SOP) is to ensure that potentially infectious waste materials are adequately sterilized when subjected to autoclaving (steam sterilization). State law requires that all autoclaves used for decontamination of biohazardous materials be tested weekly for efficacy following the parameters described in these operating procedures.

2. **Scope**

This SOP applies to all University of Utah facilities where autoclaving is used to decontaminate biohazardous materials prior to release to the normal waste streams (sanitary sewer for liquids, permitted landfill for solid wastes).

The Utah Administrative Code, Rule R315-316, describes infectious waste treatment and disposal requirements, including those required to use and maintain an autoclave (steam sterilizer).

3. **Definitions:**

"Potentially infectious wastes" needing to be autoclaved include the following:

- "Biological waste", which includes blood and blood products, excretions, exudates, secretions, suctionings and other body fluids that cannot be directly discarded into a municipal sewer system, and waste materials saturated with blood or body fluids, but does not include diapers soiled with urine or feces.

- "Cultures and stocks," which includes etiologic agents (of disease) and associated biologicals, including specimen cultures and dishes and devices used to transfer, inoculate and mix cultures, wastes from production of biologicals, and serums and discarded live and attenuated vaccines. "Cultures" does not include throat and urine cultures.

- Gloves and other disposable personal protective equipment used as barriers when handling biological wastes or cultures and stocks.

5. **References:**

The Utah Administrative Code, Rule R315-316. Infectious Waste Requirements.

Rutala, W. A., Stiegel, M. M. and F. A. Sarubbi, Jr. *Decontamination of laboratory microbiological waste by steam sterilization*. App. Env. Microbiol. 43: 1311-1316 (1982).

Lauer, J. L., Battles, J. R. and D. Vesley. *Decontaminating infectious laboratory wastes by autoclaving*. App. Env. Microbiol. 44: 690-694 (1982).

Ozzane, G., Huot, R. and C. Montpetit. *Influence of packaging and processing conditions on decontamination of laboratory biomedical wastes by steam sterilization*. App. Env. Microbiol. 59: 4335-4337 (1993).

Adapted from Oregon State University and University of Nebraska Standard Operating Procedures.

6. Procedures:

Autoclaves are used in many areas to sterilize materials. Due to the high heat and pressure created in autoclaves during operation, proper loading, use, and unloading procedures must be followed to prevent burns and other accidents. Burns can result from physical contact with the structure of the autoclave and steam burns can occur from contact with steam leaving the apparatus. Burns can also result from careless handling of vessels containing hot liquids. Explosive breakage of glass vessels during opening and unloading due to temperature stresses can lead to mechanical injury, cuts, and burns. Autoclave performance for sterilization is dependent on proper use. This SOP provides guidance related to prevention of injuries as well as effective sterilization.

A. Run Settings

Autoclave manufacturers generally provide several pre-set cycle options.

- Gravity
  In this mode, evacuation of air from the autoclave chamber prior to the sterilization portion of the run is accomplished by gravity air purge. A gravity cycle is appropriate for loads where air removal from porous materials or penetration of steam into wrapped or packaged items is not required.
- Vacuum cycle
  In this mode, evacuation of air from the autoclave chamber prior to the sterilization portion of the run is accomplished by pulsing between pressure and vacuum. As the number of pulses (prevacs) increases, so does the efficiency of the air removal and subsequent steam penetration. A vacuum mode is suitable for hard goods, with a minimum of 3 prevacs for wrapped or difficult to penetrate hard goods.
- Liquids cycle

This mode is similar to gravity cycles in that air is evacuated from the autoclave chamber by gravity air purge. Pulling a deep vacuum is not conducted since liquids to be autoclaved would be drawn out of their vessels.

Often, the autoclave manufacturer will provide two pre-set cycles for each type of run option presented above (e.g., gravity, vacuum, and liquids). The pre-set cycles for each type of run will vary in the pre-set sterilization temperature, sterilization time, and dry time.

General Precautions (Do's and Don'ts)

Things to do when operating an Autoclave

- Read and follow the recommendations made by the manufacturer in the owner's manual. Ensure regular maintenance of autoclaves and ancillary equipment in accordance with the manufacturer's specifications.
- Understand the temperature and pressure readings on the printed autoclave tape reflect the chamber temperatures, and may not reflect the temperatures achieved in the material that is autoclaved.
    - Most autoclaves are equipped to provide a printed tape documenting the conditions of the run. The chamber temperature and material temperature will correlate only when the ideal run parameters (i.e., pressure, prevac, etc.) have been established for the load conditions (i.e., load size, load distribution and configuration, depth of the autoclave pan, wetness of the load, etc.).
- Use only those types of containers, bags, and lids that are designed for autoclaving. Inspect vessels for cracks or chips. Only use unblemished containers. ***Note: not all biohazard bags are rated for autoclave use. Also, some are rated only to a certain temperature; make sure the bags you use are compatible with your waste cycle.***
- Autoclave disposable plastic labware (e.g., culture plates, petri dishes, pipette tips, etc.), paper and gloves, etc. If autoclaving dry biohazardous waste containing little liquid, add a glove or Ziploc bag containing water when the bag is half-full.
- Place soiled glassware and lab ware in secondary containers and autoclave them in the solids cycle.
- Loosen lids on containers of liquids and closures on autoclave bags.
- Arrange loads to allow free circulation of steam. If items that can hold water are in the load (e.g., trays, pans), place them in such a way to allow for condensed steam to drain (i.e., on their sides).
- Report all malfunctions to your supervisor and clearly tag the equipment as "**Out-of- Service.**"
- ALWAYS use secondary containment when sterilizing or decontaminating. Steel autoclave containers achieve appropriate temperatures quicker than polypropylene containers and thus are recommended. If you use plastic containers, ensure that they are certified for use at the temperatures in the autoclave.
- **Autoclave waste must be clearly marked that it has been sterilized appropriately:**
    - **Use chemical indicator tape, it is very important to use a lead-free tape to avoid potential implications under the hazardous waste regulations.** Examples of lead-free autoclave tape include:
        - Fisher brand, catalog number 15-903
        - VWR brand, catalog numbers 10127-464 and 10127-460
    - Use autoclave bags with sterilization indicator. Examples include:
        - Fisher brand, catalog number 01-814

Things NOT to do when operating an Autoclave

- Do not autoclave flammable or corrosive liquids. Never autoclave bleach solutions; this can damage the plumbing of the autoclave.
- Do not place loose sharps (e.g., glass pipettes, broken glass, razors, slides, etc.) in autoclave bags.
- Do not stack items and "stuff" the chamber. There must be sufficient clearance on all sides of the load to allow for steam to circulate.
- Do not autoclave sharps waste containers: arrange for collection through the EHS Safety Administrative Management System
- Do not stack or store combustible materials next to an autoclave (cardboard, plastic, volatile or flammable liquids).
- Do not autoclave materials containing hazardous wastes or radioactive materials.
    - Hazardous waste and ionizing radiation regulations pertain to autoclaved waste as well, so it is imperative to consult with EHS if your run contains any agars or other materials that may contain a regulated substance (e.g., heavy metal such as Pb, Hg, Ag, Se, Ba, As, Cd, Cr, or other potentially toxic constituent). Consult with EHS prior to autoclaving radioactive materials.

DO NOT use an autoclave unless you have received specific operation instructions or are working under the direct supervision of an experienced autoclave worker.

B. Specific Instructions for Autoclaving Contaminated Plant Material and Soil

Autoclaving plant material and soil requires special consideration to the parameters used as the organic nature of the materials can sometimes affect the effectiveness of the sterilization cycle. Soil is often autoclaved prior to use to ensure that it is sterile and no undesired pathogens or pests are present. The parameters discussed below are specifically for treatment of plant and soil waste materials that contain recombinant or synthetic nucleic acid molecules, pathogens or pests, or plants and soil that may be contaminated by these materials.

- Recombinant nucleic acid-containing plant material
    - Standard autoclave parameters should be used (121°C at 15-17psi for 60 minutes)
    - Do not overfill bags
- Contaminated soil (pathogens and/or genetically modified plants)
    - Soil is much denser than mixed soil and plant material so containers must be filled much less than their capacity.
    - EHS has determined through a validation study that the following parameters result in successful decontamination of soil.
    - A maximum depth of 9” in a trash can or similar container.
    - 121°C and 16 psi for 180 minutes or two (2) 90 minute cycles.
    - Soil autoclaved in plastic or metal trays and less than 6” deep, may only require a single cycle of 90 minutes @ 121°C to achieve sterilization. Use of these parameters should be verified by use of biological indicators.
    - Smaller quantities of contaminated soil may, by extrapolation, require shorter autoclave times.
- Consider the materials you are trying to sterilize when selecting the autoclave program parameters.
- Deviation from these parameters should be done in consultation with Environmental Health and Safety (EHS) to ensure that effective decontamination/sterilization is achieved, particularly for waste materials.

Loading the Autoclave

- Plan the load in accordance with the autoclave settings and load configurations that will ensure proper autoclave performance.

- Use a chemical indicator (e.g., autoclave tape) in every load to demonstrate that the chamber temperature was greater than 121°C. Place autoclave tape in an “X” pattern over the biohazard symbol on waste bags or sharps containers (Figure 1).

Figure 1 Autoclave tape utilizes a chemical reaction to indicate a temperature of greater than 121°C was achieved. This color change is usually displayed with hash marks or words that appear on the tape when proper temperature is reached.

- Biohazard waste bags must NEVER be placed directly on the floor. Transfer the waste bag directly to a solid, autoclave compatible pan large enough to contain any leaks.
- Use a cart to transfer items to the autoclave. To avoid overexertion injuries, push the cart up to the autoclave door and gently slide the load into the autoclave.
- Prior to loading the autoclave, visually inspect the drain strainer to ensure it is clean.
- Use the appropriate autoclave setting as described above. Recommended settings for waste are at least 121°C, 15-17 psi for at least 30 minutes.
- Firmly lock autoclave doors prior to starting the run to prevent sudden release of high-pressure steam.

Unloading the Autoclave

- After the run is completed, check the pressure gauge. If pressure is not released, do not open the door. Contact the Building Maintenance/Facilities Staff for malfunctions; do not use the pressure relief override valve.
- While wearing eye protection and insulated gloves or mitts, carefully open the autoclave using the door to shield your body from the contents of the autoclave. Hot condensate may drip from the door so ensure your feet are protected.
- Use caution when removing liquids. Liquids, especially large volumes, may continue boiling for a time after autoclaving.
- Slide a cart to the opening of the autoclave and pull the autoclave secondary container onto the cart. Place the cart in a low traffic area while additional cooling occurs.

Disposal of Autoclaved Waste

- Liquids that have been autoclaved may be poured down the sink if all chemical components are listed on the EHS sewer disposal list. If the liquid contains chemicals that are not approved for sewer disposal, the vessel must be tagged for pick-up by EHS.
- Non-toxic solids (including lead-free autoclave tape) that do not contain any chemical constituents regulated under the hazardous waste laws or radioactive material may be disposed in the regular trash following autoclaving and demarcation.
    - Seal the bag by taping or tying the bag shut.
    - Place the autoclaved waste bag in a black (opaque) trash bag or ensure that it is obvious that the waste has been autoclaved (autoclave tape).
    - If any free liquids (i.e., condensate) are present, add sufficient absorbent to the bag.
- Tag autoclaved toxic and/or potentially regulated solids (including autoclave tape containing lead) for collection by EHS.

C. Autoclave Performance Checks

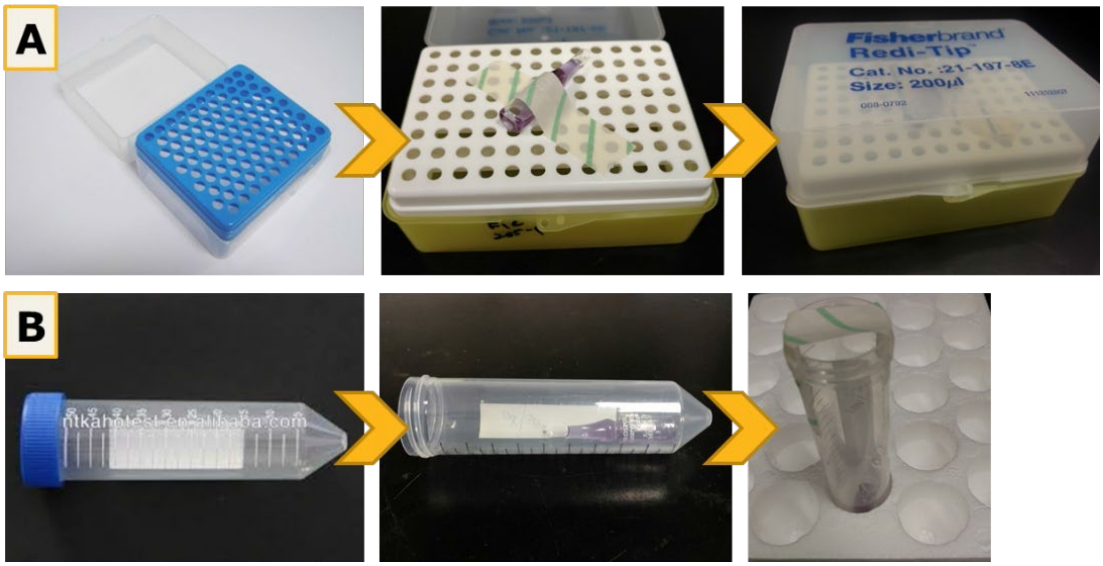
Autoclaves that are used to sterilize biohazardous waste must be tested at least weekly to ensure they are functioning correctly:

Testing Procedure

1. Many companies sell *Bacillus stearothermophilus* or *Geobacillus stearothermophilus* that can be used for verification (e.g. VWR, and Fisher Scientific). In most cases, these come in the form of ampoules or paper strips. Follow the manufacturer's instructions.
2. To perform the test, use the following procedures:

    2.1 For dry loads (gravity or vacuum cycle), obtain a pipet tip storage box (Figure 2A) and (1) place the ampoule, on its side, or strip inside the box. (2) Use autoclave tape to secure the ampoule. (3) Close the lid of the box and place the box under your load (i.e., under the autoclave waste bag(s)).

Figure 2: Autoclave Biological Indicator Testing Methods



2.2 For liquid loads, using a piece of string tied around the ampoule, suspend the ampoule in the liquid and secure it to the outside of the container with autoclave tape.

2.3 For tabletop and top-loading portable autoclave/sterilizers, (Figure 2B) (1) Obtain a 50 mL conical centrifuge tube. (2) Place the ampoule/strip in the tube. (3) Place one piece of autoclave tape over the mouth of the tube to prevent the ampoule/strip falling out. It is important to allow for steam to enter the tube.

IMPORTANT: ***Do not place the lid back on the tube.***

Using another piece of autoclave tape, secure the tube to the bottom of the autoclave bag or simply place the tube under the biohazard bag(s).

2.4 For plant material and soil in bags, use either method 4.1 or 4.3 outlined above and shown in Figure 3.

3. Autoclave using the appropriate cycle/settings (*a typical decontamination cycle is 15-60 min at 121°C and 15-17 psi*).
4. Allow time for the autoclave to cool down and for pressure to return to atmospheric.
5. Using insulated gloves or mitts, remove load from autoclave.
6. Remove pipet tip box from bottom of tray and open box to remove ampoule or remove cover from liquid container and retrieve ampoule from liquid.
7. Incubate test ampoules/strips according to the manufacturer's instructions and check for growth. Typically, these require incubation at 55-50°C for 2 to 7 days. If the autoclave is

functioning properly, no growth will be observed. If there are shows signs of growth, the test has failed. Repeat the test to confirm.

8. If the second test also fails, place an “Out of Order, Do Not Use!” sign clearly on the autoclave and submit a repair order. Be sure to indicate an alternate autoclave to use until repairs are made. A sample sign is included in Appendix i of this document.

D. Record Keeping

In accordance with State requirements, records must be kept for each autoclave. These records serve two purposes, (1) they aid in autoclave malfunction investigation and (2) they provide documentation to regulatory agencies that materials were properly decontaminated/sterilized prior to disposal. Records should contain the following information:

- ☐ On-site maintenance records
- ☐ Documentation of testing with spores of *B. stearothermophilus*. An example log can be found in Appendix ii of this document
- ☐ Log of autoclave use. Each load of material inactivated shall be logged as follows:
  - Date, time, and operator's name
  - Type and approximate amount of waste
  - Confirmation of sterilization
  - Record the temperature, pressure, and length of time the load is sterilized.
  - Save the autoclave printout, if the autoclave has a working printer. An example autoclave use log can be found in Appendix iii of this document.

E. Routine Maintenance

The best way to ensure effective autoclave operation is regular maintenance. Consult the owner's manual for autoclave-specific maintenance recommendations.

Appendix i: Out of Order Sign

Instructions: Print sign in color, cut out along dotted line and post on autoclave in need of repair.

Appendix ii: Log for testing sterilization efficacy with *B. Stearothermophilus*.

Test Vial Information
Test vial #:
Test vial type: e.g. *BT Sure / Barnstead Thermolyne Test* Vial Lot #:
Expiration Date:

Each vial contains e.g., *2.0 x 10^5 endospores, Geobacillus stearothermophilus*
Incubation conditions: e.g.,*55°C for >24 hours*

Autoclave Information
Building:
Room: Manufacturer: Model:
Serial:

Test Conditions
Time:
Chamber Pressure: Buried in waste bag: Date of test:

Results:

User Information Department: *Contact* Person:

Comments:

Appendix iii. Autoclave Use Log

Instructions: Post log by autoclave and record each use of the autoclave.

- Principal Investigator
- Lab Room – indicate in which lab the biohazard waste was generated
- Time at cycle temperature (example: 121°C)
- Chamber temperature – maximum temperature reached
- Cycle Type – list type of cycle (e.g., Gravity, liquid, waste, etc.)
- Operator – indicate person who autoclaved waste
- Comments – list comments

	Date	PI	Lab Room	Cycle Time	Max Temp	Press	Cycle Type	Operator	Run (Pass/Fail)
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									

Run Comments: *(be sure to list run #)*

Appendix I: 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 J: Inactivation and Verification

This appendix provides a summary of inactivation methods described in Appendix K of the 6th Edition of the BMBL. These inactivation methods enable retention of characteristic(s) of interest in pathogens, viral nucleic acid sequences, or toxins in order to accommodate the intended future use(s) of the material and verification of inactivation procedures. Inactivation and verification of Select Agents and Toxins must be in compliance with current regulations from the Federal Select Agent Program.

Background

When choosing an inactivation method, consider key characteristics, including the infectious agent (e.g., pathogen, viral nucleic acid sequences, or toxin), resistance to treatment, and ability to recover from the treatment. Environmental stability is high for some agents including spores, pathogens residing within biofilms, and prions.

Different types of inactivation procedures target different components and/or systems within the agent. Inactivation targets include: bacterial cell walls; lipid envelopes or cell membranes; nucleic acids; and regulatory systems involved in the agent's virulence, replication, and/or transmissibility. Types of inactivation methods may include:

- Physical (e.g., heat, ionizing irradiation, 254 nm ultraviolet [UV] light);
- Chemical (e.g., chaotropic compounds such as guanidine hydrochloride, oxidizers such as chlorine and hydrogen peroxide, psoralen or titanium dioxide nanoparticles activated by UV-A);
- Natural antimicrobial strategies (e.g., enzymes such as lysozymes and
- virolysins [bacteriophage-encoded lytic enzymes], antimicrobial peptides such as nisin, and bacteriophages); or
- Combination (e.g., sublethal mild temperatures [<60 degrees Celsius] with various nonthermal treatments, antimicrobial compounds with ionizing radiation, and lysozyme with antimicrobial compounds).

Some traditional disinfection methods can also serve as inactivation treatments. For example, spores, vegetative bacteria, DNA viruses, and RNA viruses can be effectively inactivated with peracetic acid with minimal effects on the ability to do subsequent PCR and ELISA immunoassays. Alternatives to antibiotics for humans and animals, environmental decontamination methods, and food safety processes could potentially lead to the development of inactivation procedures.

Novel inactivation strategies include use of cell wall hydrolases, such as lysozyme, and antimicrobial peptides such as nisin.

When choosing an inactivation method, several factors need to be considered including: specific controls; the balance between efficacy of inactivation vs. the retention of desired characteristics; and the appropriate safety margin (i.e., overkill amount). Additional advantages may include low cost and broad applicability to different types of agents.

Filtration and Centrifugation

Filtration is a common pathogen removal method; filtration is also used to supplement an inactivation method by removing or reducing the amount of active pathogen, viral nucleic acid sequences, or toxin from biological fluids, culture supernatant, and other materials. Filtration may result in the loss of a significant fraction of the material to be used and will require viability testing to ensure no agent passes through any defect in the filter. Centrifugation or centrifugation combined with filtration can be used to supplement inactivation methods by separating out and removing significant amounts of the pathogen, viral nucleic acid sequences, or toxin from the material that will be used for subsequent purposes. Centrifugation may result in adverse effects on the structural integrity of the residual material and requires additional time and processing steps to recover the material for further use.

An extract (e.g., nucleic acids, antigens, lysate) is derived from a two-step process with an initial step (e.g., lysis) where the agent is subjected to a treatment, followed by a second step (e.g., filtration) to remove any residual active agent.

Development of Inactivation Procedures

The starting point for development of an inactivation procedure is deciding which inactivation method(s) is appropriate, effective, and feasible to use for the specific set of circumstances. Inactivation procedures considered can be based on:

1. A procedure developed in-house;
2. A procedure published in a peer-reviewed journal; or
3. A commonly accepted method (e.g., heat, dry or wet).

Many variables need to be considered when developing inactivation procedures; these include the type and amount (i.e., volume and titer) of agent (e.g., pathogen, nucleic acid or toxin) to be inactivated; matrix/solvent surrounding the agent; concentration of starting matrix material; treatment time,

temperature, pH, and dose of treatment; process controls; type of container being used for inactivation; and appropriate safety measures. The post-exposure environment may also play a role in the efficacy of the inactivation; therefore, the subsequent environmental conditions (e.g., temperature and nutrients in the matrix) should be controlled as well.

In cases where limited samples are available, it may be appropriate to use surrogate strains or agents to develop the inactivation procedures. If resistance information is known, the most resistant strain or agent should be used as the surrogate. Generally, suitable surrogates are bacteria from the same genus and viruses from the same family. Another type of surrogate that may be appropriate in some situations is a tissue surrogate. In this case, a sample of the tissue adjacent to the tissue of interest that has also undergone the inactivation may be used for confirmation of the inactivation procedure and verification that adequate efficacy has been achieved in the process.

Use of dose-response (e.g., survival of the pathogen, viral nucleic acids, or toxin vs. the inactivating treatment dose or time), spike-and-recovery experiments (i.e., bioburden reduction studies), and building an adequate safety margin are all important elements to incorporate into an inactivation procedure. Factors that should be considered include:

1. Testing method(s) for the specific set of circumstances involved (e.g., type, amount, and concentration of starting material);
2. Controls (process, negative, positive);
3. The limit of detection;
4. Interference of residual inactivation material and matrix materials with viability, infectivity, or toxicity testing; and
5. Appropriate safety margins.

Tables 1–8 in Appendix K of the BMBL outline the key advantages and disadvantages of four broad inactivation method categories—physical, chemical, chemical activated by physical, and natural and emerging. Tables 9 and 10 in Appendix K of the BMBL outline advantages and disadvantages of combination methods.

Physical inactivation includes heat (dry or wet), ionizing radiation, and ultraviolet light (UV-C radiation). Physical inactivation through heat involves hot-air (dry) or steam under pressure (wet), which is used to irreversibly destroy an agent's protein structure (denaturation). Ionizing radiation induces single- and double-strand breaks in nucleic acids. Ultraviolet light, especially at 254 nm, is an effective treatment for

reduction of bacteria; UV-C causes photochemical damage to nucleic acids through formation of pyrimidine dimers, inhibiting DNA replication and transcription.

Chemical inactivation includes chaotropic agents and oxidizers. Chemical inactivation through chaotropic agents utilizes guanidine-based denaturing agents to disrupt cells and liberate nucleic acids; these agents have strong protein denaturant properties when used at high concentrations. Oxidizing agents oxidize cell membranes resulting in loss of structure leading to cell lysis and death. Examples of oxidizing agents include: hypochlorous acid (HOCl), chlorine, hydrogen peroxide, and peracetic acid.

Inactivation may also be achieved via a chemical inactivation activated by physical treatment; examples include psoralen and UV-A radiation and titanium dioxide (TiO_2) and UV-A radiation. Psoralens, in the presence of UV-A (320–400 nm) radiation, inactivate viral agents. TiO_2 is a stable and inert material that can continuously exhibit antimicrobial effects when illuminated. Photocatalysis increases cell permeability with efflux of intracellular contents leading to cell death.

Inactivation may also be achieved through natural and emerging antimicrobial strategies including lysozyme, antimicrobial peptides (AMP), and bacteriophages. Bacterial killing by lysozyme occurs through hydrolysis of cell walls. It is effective against Gram-positive bacteria and is an important component in the prevention of microbial growth in foods. Bacteriocins (i.e., bacterial proteins or peptides) are AMPs widely used in food bio-preservation. Antimicrobial peptides are the cornerstone of innate immunity. AMPs have various intracellular and extracellular targets, but AMPs primarily bind to and form pores in cell membranes. Bacteriophages (phages) are viruses capable of infecting and killing bacteria. Phages are among the most abundant organisms in nature and are not known to infect eukaryotes. Use of multiple closely related phages (i.e., cocktail) has been shown to be more effective in killing microbial pathogens.

Finally, inactivation may be achieved through combination methods including sub-lethal mild temperatures (<60°C) with non-thermal treatments, antimicrobial compounds with ionizing radiation, and antimicrobial compounds with lysozyme. Some common non-thermal treatments include High Pressure Processing (HPP), Pulsed Electric Field (PEF), and ultrasound (US). The use of anti-microbial compounds, such as AMPs, can facilitate reduction of the dose of ionizing radiation treatment necessary for inactivation of pathogens. Synergistic effects of antimicrobial compounds, such as AMPs with lysozyme, effectively inactivate and/or kill Gram-positive bacteria. Antimicrobial compounds with lysozyme are effective against a broader spectrum of pathogens. Resistance mechanisms to antimicrobial compounds are well known and must be considered as a potential risk.

Validation of Inactivation Procedures

Conditions of an inactivation procedure must be optimized for efficacy and tailored to the specific materials and circumstances present in that setting. A validated inactivation procedure will designate a set of conditions that have been determined to adequately render:

1. A pathogen non-viable, with efficacy established by viability testing data;
2. The isolated viral nucleic acid incapable of producing infectious forms of virus, with efficacy established by infectivity testing data; or
3. A toxin no longer capable of exerting a toxic effect, with efficacy estab- lished by toxicity testing data.

Viability testing procedures may include cell viability assays, growth analysis, in vivo exposure, or a combination of these methods. A common viral infectivity testing procedure consists of introducing the positive (+) strand RNA into permissive cells to determine if that strand can produce an infectious virus.

Toxicity testing may include functional activity assays and in vivo exposure assays.

The potential for incomplete inactivation, including errors that might result from exceeding the capacity of the inactivating process to kill the pathogen, lack of specificity, detection limits, and run-to-run variation should be considered when setting specifications for confirmed inactivation procedures. Sufficient replicates of the testing must be performed in order to determine the underlying variability within the procedure in the hands of the laboratorians performing it. In addition to the factors considered during development of an inactivation procedure, elements that should be evaluated when confirming an inactivation procedure include:

1. Any chemical inactivation treatments that need to be neutralized or diluted prior to the confirmation testing; and
2. The statistical probability of inactivation (i.e., was the sample subject to sufficient inactivating material/process to provide a statistically significant probability of complete inactivation).

Process Verification

The validated inactivation procedure should be verified in the hands of the laboratorian performing the procedure while using the reagent sources and equipment intended for the routine process; verification occurs regardless of procedure source (i.e., commonly accepted, published, or in-house procedure).

Run-to-run variability is due to the cumulative effect of variation, sometimes slight, in a number of factors including materials, equipment, pathogen concentration, environmental conditions, and the personnel performing that particular procedure. Verification of a validated inactivation procedure is necessary because run-to-run variations may result in somewhat different levels of efficacy.

Verification will need to be risk-based. For lower risk organisms, verification may be the printout from an autoclave that demonstrated adequate time and temperature for inactivation or results of a biological indicator. For higher risk organisms, verification involves testing for the absence of viability, infectivity, and toxicity. The purpose of process verification is to demonstrate that adequate efficacy is achieved despite these normal variations in run-to-run conditions.

Conclusion

Inactivation and verification procedures need to be tailored to the specific procedural circumstances and based on a risk assessment. In-house testing is recommended for all methodologies due to the wide variability in conditions at different institutions; the inevitability of differences in assay conditions, equipment and/or reagent sources; and the varied conditions used for the different types of inactivation procedures. Gaps in knowledge of inactivation and verification methods mean there is often improvisation at the institutional level. One useful way to ensure that information on effective inactivation and verification methods is broadly shared with the scientific community is through inclusion of this important data in the “Materials and Methods” sections of publications.

Novel inactivation methods that enable retention of desired agent characteristic(s) are an area of active research in the field of biosafety, but additional work is needed. Advances in inactivation and verification procedures can improve safety and security, enable reduction of the Biosafety Level used and the costs, and allow forward movement in some valuable research projects that might otherwise face obstacles.

Appendix K: Large Scale Biosafety

Introduction

This appendix is a summary of Appendix M of the 6th Edition of the BMBL: references can be found there.

When working with biological agents in large-scale quantities, there are unique considerations that must be addressed in order to ensure worker and environmental protection. Large-scale biological production facilities should use the laboratory scale principles of risk assessment set forth in the BMBL and by ISO 35001, Biorisk Management for Laboratories and Other Related Organizations.

In addition to laboratory scale risk assessment requirements, the utilization of larger equipment and volumes of chemicals or raw materials requires risk management strategies beyond biological safety alone. The following sections apply risk management steps to give readers the most pertinent information for managing risk in large-scale production. The recommendations assume that those performing risk assessments for large-scale work will involve industrial hygienists and other process safety specialists when implementing risk assessment and control measures for large-scale operations.

Appendix K of the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)* prescribes safety practices and containment procedures for large-scale (i.e., >10 liters per container) facilities. These guidelines can be applied to all large-scale work with biological materials (e.g., genetically modified organisms [GMO] and non-GMO, human, and animal/ zoonotic pathogens).

Risk Assessment

Integrate the steps and processes utilized in laboratory biological risk assessment for any large-scale project. Risk assessment should be done during planning, when elements of the process change, and during periodic reviews of existing biological production processes, particularly after incidents or process failures.

Risk control measures must be installed to mitigate unacceptable risk. Systems must be evaluated to determine their contribution to risk. The Good Practice quality guidelines and regulations (GxP) include three commonly used GxPs: Good Clinical Practices (GCP), Good Laboratory Practices (GLP), and Good

Manufacturing Practices (GMP); GxP product Impact Assessment (IA) analysis can be extended to evaluate biosafety and laboratory biosecurity-related systems that govern exposure control, process room and environmental protection, decontamination, access control and accountability. Risk assessments should focus on the biological, chemical, physical, product, and equipment biosafety and laboratory biosecurity risk points. Production technologies and equipment with the potential for misuse (laboratory biosecurity/dual-use/export control) may also be included in the risk assessment. Subject matter experts in engineering; Heating, Ventilation, and Air Conditioning (HVAC); quality control; occupational health; security; and health, safety, and environment (HSE) should always be consulted when making risk-based determinations.

Hazard Identification

The first step of risk assessment is hazard identification. Review additional factors that are unique to large-scale biological processes. Additional factors include but are not limited to:

1. Unique strains utilized primarily for research or manufacturing processes (e.g., producing high titers of a toxin);
2. High volumes (>10 liters) and high concentrations of product;
3. Specialized equipment and processes with unique risk points require a Hazard Analysis of Critical Control Points and/or Hazard and Operability studies;
4. Pressurized vessels and lines for biological and chemical reactions pose a risk for aerosol generation (e.g., bioreactors, fermenters, thermal inactivation tanks); and
5. Atypical routes of transmission (e.g., inhalation of biological agents or toxins not normally transmitted via the aerosol route).

Non-biological hazards to consider when performing a risk assessment may include, but are not limited to:

1. Hazardous chemicals: formaldehyde or similar for inactivation, large quantities of detergents, disinfectants and caustics, adjuvants, preser- vatives, solvents for down-stream processing, allergens or toxins, and asphyxiants;
2. Physical hazards: noise, steam, heat, cold, and radiation including UV and lasers;
3. Life-safety hazards: confined space, working at heights, line breaking, and pressurized systems;
4. Ergonomics;
5. Process safety-relevant controls (e.g., fire/explosions; pressurized systems);

6. Preventative maintenance (PM): solid and process effluent waste streams and control measures employed, including PM of relevant equipment;
7. Processes to control release of material (i.e., human and environmental risks), including corresponding emergency procedures; and
8. Risk points associated with equipment.

Hazard Evaluation

As with laboratory risk assessment, the hazards associated with the biological agent/material and process equipment must be evaluated. In addition, the operational integrity of containment equipment and facility safeguards and the capability of area staff to effectively control potential hazards must be considered. Staff capability will depend on the training, technical proficiency, and good habits of all team members.

Large-scale research and production pose additional risks that require evaluation. Increased growth, vessel size, and enhanced aeration magnify the aerosol generation risk. By design, the biological agent concentration is greatly increased. Therefore, protection from aerosol transmission must be considered for agents normally transmitted by insect bite or injection.

Chemical risks are also increased due to handling of dry powders for media preparation, pumping of acid or base for pH control, and preparation/addition of inactivation chemicals for vaccine preparation. Closed system transfer technology may be foreign to those with experience limited to the laboratory.

Risks due to hazardous energy (i.e., electrical, steam, pressurized gases) are also magnified. Hazardous energy control procedures such as removing the power cord or closing a supply valve become complex and may be poorly under- stood by those with experience limited to the laboratory.

Risk Control

Risk mitigation strategies identified in large-scale research and production follow the same principles (i.e., hierarchy of controls) established to control HSE risks. Those performing risk assessments for large-scale work may be able to eliminate a hazard or substitute to reduce risk. When this is not possible, engineering, administrative and/or work practice controls, and PPE are utilized.

Engineering Controls

Selecting the proper engineering solution is an iterative process. The design provisions for a large-scale biological production facility will differ greatly depending on whether the work is dealing with an exotic, indigenous, eradicated, novel, or emerging disease-causing agent; a highly allergenic compound; a GMO, carcinogenic or highly toxic product; or a well-characterized and attenuated childhood vaccine.

Many controls must be considered in the process, including HSE-risk, biosafety, and laboratory biosecurity. In addition, large-scale GxP facilities must evaluate quality design controls for product as well as personnel and environmental protection. Consider state and local regulations when implementing the design of a large-scale biological production facility. A large-scale facility balancing GxP and biosafety requirements will need to evaluate the following basic facility principles:

Clean to Dirty: The process design must include controls to prevent contamination spread within the facility and to the environment. If applicable, an assessment of conflicts between GxP and biosafety requirements must also occur to achieve two different definitions of clean. If there are two competing requirements, implement controls that address the highest consequence events and identify alternate methods to meet the intent of the competing requirement. For example, if an operation requires positive-pressure environment to achieve product protection, you can create an air pressure sink in an anteroom to ensure containment of the biological agent.

Change Rooms and Barriers: Establish donning and doffing needs by creating an operational flow diagram. This will help clarify how many actions an operator must take for a given procedure or process step when passing through a personnel barrier or door. The review should cover normal operations, planned and unplanned maintenance, and emergencies. This process should identify the potential demand in PPE for the facility, the number and locations of room(s), and room size(s) necessary for storing PPE and changing. Facilities covered by GxP requirements must consider PPE and workflow requirements to achieve product protection in addition to personnel and environmental protection.

Airlocks and high/low-risk rooms (i.e., biologicals vs. cleanrooms): The design must address biosafety concerns as well as applicable GxP requirements to achieve personnel, environment, and product protection, if required.

Surfaces: Floor, wall and ceiling, door and window, and other exposed component surfaces must be impervious and easy to clean. The materials must be resistant to a host of chemicals including liquid and gaseous disinfectants, if needed, for decontamination or prevention of cross-contamination. Construction attributes of floor strength, ceiling height, segregation need, piping (i.e., materials, product, and waste) and energy lines must support and promote large-scale processes.

HVAC system, room pressure, and airflow: The design of the airflow must provide personnel and environmental protection. In the event a process area must be positive-pressure, consider designing the room airlock or changing area as a pressure sink. Exhaust air filtering systems may be required, as in the case of vaccine plants producing live attenuated vaccines, to prevent ductwork contamination. GxP requirements may also require product protection design considerations.

Gaseous Decontamination: The HVAC system, walls, and wall penetrations must be made such that the room can be decontaminated without a negative impact to adjacent spaces. The decontaminant employed must be appropriate for the process and biological agents handled. Use the same principles for gaseous decontamination of a laboratory, but the quantities used and the clearing times will differ substantially.

Spill Containment: When designing for spill containment, consider the biological, chemical, and physical processes in an area. Always review spill scenarios while designing a facility. Identify what and how much can be released, where spilled materials will flow (e.g., are there drains leading to an effluent decontamination system (EDS) or will materials released be captured within a containment dike), if manual inactivation will be required, and what emergency response activities will encompass.

Kill Tanks/EDS Systems: Ensure EDS systems can inactivate effluent from production waste and spills. It is particularly beneficial to have a facility designed with secondary failsafe systems when large amounts of material are processed. The exact method used will depend on local regulations and the materials in question. Numerous options exist, including chemical inactivation using acids or caustics, and heat inactivation (batch or continuous). Ensure holding tanks have stirrers when volumes are large. Most facilities employ hard piping, and a process to clean and decontaminate these lines between production areas and the EDS must be integrated into the plan.

Those performing risk assessments for large-scale work will also determine the type of equipment to be used by considering production needs and risk assessment results. Historically, the standard has been fixed equipment (i.e., stainless steel bioreactors) with a combination of hard and flexible hose piping for upstream (i.e., biological agent propagation) and downstream (i.e., biological agent purification, concentration, and potentially inactivation) processes. Increasingly, single-use (SU) equipment is replacing fixed equipment for upstream processes. The “ballroom” concept, where both upstream and downstream processes are in one large production facility, is now accepted for select biological processes. The ballroom concept relies on maintaining closed systems at all times.

1. Ballroom Layout Advantages
    a. More flexibility to accommodate different process trains;

b. Improved operational efficiency and oversight (e.g., avoids having to move equipment between rooms); and

c. Reduction of footprints and cost.

2. Ballroom Layout Disadvantages

a. Increased risk of contamination spread in upset conditions to downstream processes;

b. Need for typically open operations (e.g., cell expansion, column packing or powder addition) to be handled in closed system

c. Need for enhanced environmental monitoring to be conducted to detect a breach in any closed system and need to ensure contamination or cross-contamination has not occurred; and

d. Challenging area and equipment decontamination when production areas are shared.

A non-comprehensive list of containment requirements and associated risk points is provided below to assist in the assessment of risks associated with SU equipment.

Containment Requirements and Example Risk Points

1. Viable organisms should be handled in a closed system or other primary containment.

a. Ensure the bioreactor bag is compatible with maximum output temperature of heating control circuit;

b. Ensure the tubing is compatible with process media, including pH control solutions and stability testing has been performed; and

c. Implement procedures to ensure that probes are not removed during operation.

2. Culture fluids are not removed from a system until organisms are inactivated.

a. Implement procedures for removing bioreactor bag(s) containing infectious agent(s).

3. Inactivation of waste solutions and materials with respect to their biohazard potential.

a. Implement procedures for processing used bioreactor bags containing infectious agents;

b. Ensure presence of biosafety cabinet for removing reusable components before destruction;

c. Ensure the waste disposal procedure compatible with bioreactor bags;

d. Implement a procedure for safely autoclaving used bag;

e. Implement a procedure for safe packing and transport to incinerator if the used bag will be directly incinerated; and

f. Ensure the incinerator facility can burn large quantities of silicone tubing and bag film.

4. Control of aerosols by engineering or procedural controls to prevent or minimize release of organisms.
   a. Implement controls to prevent bioreactor bag overfilling during additions;
   b. Ensure proper procedure for tubing welding;
   c. Ensure proper procedure for tube weld integrity test;
   d. Ensure regular PM of tubing welders to prevent misalignment; and
   e. Ensure that plastic quick connectors (non-steamable) release viable organism(s) when released.
5. Treatment of exhaust gases from a closed system to minimize or prevent release of viable organisms.
   a. Consider exhaust gas filtration;
   b. Consider controls of exhaust filter clogging with foam and humidity; and
   c. Ensure there is an exhaust filter holder positioned to encourage condensate drainage.
6. Closed system that has contained viable organisms not opened until sterilized by a validated procedure.
   a. Ensure the bioreactor bag is compatible with inactivation chemical.
7. Closed system to be maintained at as low a pressure as possible to maintain integrity of containment features.
   a. Implement a process safety management study of gas overlay and sparging system to determine susceptibility to overpressure, including post-power failure;
   b. Ensure bag installation procedures to prevent damage;
   c. Ensure pressure control to limit aeration and overlay pressure;
   d. Ensure the pressure alarms are interlocked to the gas supply;
   e. Ensure pressure relief devices are installed on gas supplies and properly sized;
   f. Consider installing in-line pressure relief before the bioreactor to protect against gas regulator failure; and
   g. Ensure the gas supply valves fail closed upon power interruption.
8. Rotating seals and other penetrations into closed system designed to prevent or minimize leakage.

a. Consider magnetic couplings to eliminate rotary seals;

b. Implement procedures to ensure stirrer operates during pre-use integrity test;

c. Ensure rotary seals engineered to prevent infectious agent release; and

d. Consider that over-speed may result in decoupling and in-bag rupture.

9. Closed system shall incorporate monitoring or sensing devices to monitor the integrity of containment.

a. Consider bioreactor bag pressure logging;

b. Ensure that loss of pressure (low-pressure alarm) results in sparge/overlay shutdown; and

c. Ensure that the sensors respond quickly enough to pressure changes.

10. Validated integrity testing of the closed containment system.

a. Consider integrity test procedures pre-inoculation.

11. Emergency plans required for handling large losses of cultures.

a. Implement a leak detection system for bottom- or side-mounted probes;

b. Consider bottom- or side-mounted sensors guarded to prevent impact damage;

c. Consider respiratory PPE as part of operating PPE or ensure respiratory PPE availability for emergency cleanup;

d. Ensure a contaminated worker emergency procedure available;

e. Ensure a large spill clean-up procedure available, including a spill kit;

f. Ensure personnel trained in large-scale clean-up of infectious organisms; and

g. Consider gas decontamination of production suite post-incident.

12. Requirements for controlled access area.

a. Ensure aerosol-containment within skid (i.e., process module);

b. Consider a spill containment pan to contain or divert entire bioreactor contents for inactivation;

c. Ensure the pan will divert a worst-case leak scenario to biowaste without spill to the floor;

d. Consider spill containment within the suite (dike, bund, raised door threshold) to contain entire bioreactor contents for inactivation;

e. Ensure the suite exhaust HEPA filtration for fluid transfers outside bioreactor

containment; and

f. Ensure the suite is designed to prevent the release of infectious aerosols using differential pressure and sealing of room penetrations.

Those performing risk assessments for large-scale work will also need to review equipment types and assist in the evaluation of the choice that will best balance the needs of GxP and biosafety. These equipment types include:

Pumps and Pipes: The type of piping used will depend on how the process is laid out. Hard piping will need clean-in-place (CIP) and sterilization-in-place (SIP) for both GxP and biosafety reasons. Soft hoses allow for quick changes and cleaning. The type of pump will have to meet the volume demands of production. Peristaltic pumps are often used in combination with soft hoses. The risk assessment must show what type of piping and pump to use to meet GxP (if applicable), biosafety, and general HSE demands. Make sure that points where pipes penetrate walls are correctly sealed to promote safe gaseous decontamination. Additionally, pump operation should be evaluated for hearing protection implementation.

Compressed Air and Gases: Compressed air is one means of transferring fluids between vessels. The safety review will identify elevated pressure points, type of relief valve protection required, and rupture disc failure scenarios. Some processes require asphyxiants, such as CO_2 or N_2, and safety measures are to be established to mitigate associated risk.

Electrical Power: Power should be installed in a manner that prevents water ingress in all production and failure modes. Planning and construction must follow local electrical codes and the Occupational Safety and Health Administration electrical standards. Large fixed equipment fermenters and equipment often require high voltage power, which creates the need for additional safety measures including emergency stop buttons to shut down equipment and installation of water and dustproof electrical enclosures. Special care must be taken when solvents are used in production; follow applicable national codes, such as NFPA, UL, and OSHA. UPS needs must be evaluated based on the equipment and facility needs. An emergency generator may be essential to maintain biocontainment.

Production equipment including bioreactors, fermentors, filtration units and centrifuges: In all upstream and some downstream processes, equipment is used while the product is still infectious. These units must be set up to eliminate the risk of aerosol release. Prior to charging process equipment with live biological material, the integrity of the closed system should be verified. Before opening a closed system for maintenance or cleaning, in situ decontamination of the vessel is required. To prevent an aerosol release occurring as a result of an upset condition, small equipment can be placed inside a containment device such as a biological safety cabinet.

Larger equipment containing infectious agents should reside in rooms under negative pressure. If negative pressure can't be achieved, room entry and exit airlocks may be used as negative air pressure "sinks" to prevent the escape of aerosols into adjacent areas.

Work Practice and Administrative Controls

Good microbiological practices are vital and apply in the same way as they do in biological research laboratories. Chemical hygiene, hearing protection evaluations in equipment areas, ergonomic, and safety principles apply to large- scale biological production areas as they do in other research laboratories and production areas. Access should be restricted to trained personnel only. Other administrative controls include:

Occupational Health: Employers should offer workers appropriate medical surveillance programs to identify immune suppression and other underlying medical conditions, which could be risk factors that necessitate adaptations or accommodations. Occupational physicians should advise on, from a medical point of view, protection measures and procedures (e.g., fitness for duty to wear respirators or perform specific tasks). Where appropriate, the physician will offer vaccination, or provide vaccines, with follow up on titers. In addition to surveil- lance, clinical treatment procedures for accidental exposure should be developed. For biological agents susceptible to antibiotics, antimicrobial susceptibility testing results should be obtained before large-scale operations begin.

Emergency Response: Plans for different emergency situations should be estab- lished, including spill protocols. Where appropriate, post-exposure prophylaxis and policies for isolation of potentially infected people should be established. One differentiating factor between small and large spill clean-up is that, unless there is an immediately dangerous for life and health (IDLH) situation, the operator in a large-scale facility must remain in the room long enough to stop and contain the release to minimize HSE consequences.

L**aboratory Biosecurity:** The risk management strategy for a large-scale risk assessment should define both a biosafety containment strategy (refer to the BMBL, NIH Guidelines' Appendix K, and the area-specific risk assessment) and a laboratory biosecurity strategy. The biosafety containment strategy defines controls that mitigate risk from an unintentional release, and the laboratory biosecurity strategy defines controls that prevent theft of biological agents that are associated with human health and/or agricultural industry impact. Likewise, materials, equipment, technology, and knowledge of dual-use potential needs to be addressed and a strategy developed to address misuse.

Training: Biosafety, laboratory biosecurity, and GxP training (if applicable) are essential in large-scale biological production. For large-scale processes, training should review the epidemiology,

signs/symptoms of infection, mode of transmission, risk-mitigating controls including donning and doffing of PPE, and emergency response procedures, area-specific SOPs, including spill response protocols, required for the biological agent/material handled. Workers should understand when PPE is required for product protection vs. personnel protection. An understanding of the handling requirements for inactivated vs. unconfirmed inactivated materials is critical. Training should include a knowledge check.

Ergonomics: The ergonomic issues associated with large-scale operations differ from those encountered in the laboratory. Material handling in large-scale operations will present a larger risk of ergonomic injury. To address the ergonomic issues associated with material handling, include the nature of the load in the risk assessment (i.e., the weight distribution and shape of the load), the capabilities of the individual performing the task, the duration and frequency of the task, and the environment in which the material handling task is performed (e.g., space limited or extreme temperature environments). Mitigate ergonomic risks by mechanical means (e.g., lifts, hand trucks, pushcarts), redesign of the work area (e.g., ramps to replace stairs, automated transfer of materials to replace manual transfer), redesign of the work task (e.g., pushing rather than pulling), and training of personnel (e.g., proper lifting technique).

Waste Handling: The processes of waste handling are the same as for research laboratories but larger amounts require different logistics. For guidance on validation of decontamination agents and procedures, refer to Appendix B. Key considerations include inactivation of organisms in situ vs. external to process vessel or container. Consider inactivation methodologies for solid infectious waste streams as well as wastewater from production effluent (i.e., determine if there will be an impact to the site wastewater treatment permit due to the presence of organics including preservatives such as thimerosal or adjuvants).

Review and Checking of Risk Control Measures: Risk control measures need to be evaluated for efficacy in order to protect people and the environment. The organization should maintain a risk control register, which should be periodically reviewed. The strategy should address the major risk streams (e.g., chemical, physical, biological, and ergonomic).

Preventative Maintenance: Preventative maintenance is vital to avoiding process contamination and to ensuring biocontainment. Safety and security-related equipment and infrastructure should be incorporated into a preventive maintenance program that incorporates a change control process. For example, rotary seals in fermenters must be monitored for increased loss of seal water or steam pressure and should be replaced before failure; high-pressure piston seals of homogenizers must be replaced regularly to prevent aerosol release; autoclave temperature and pressure sensors require regular calibration, and steam traps must be maintained. Depending on design, autoclave bioseal or air differential seals should be tested (e.g., smoke, pressure hold, soap bubble, and helium leak testing) to determine whether they have deteriorated. When

required, HEPA filters (i.e., HVAC and equipment) should be integrity tested annually and critical barrier HEPAs should be monitored for pressure differential. Thermal or chemical inactivation systems should undergo regular inspection for corrosion and preventative maintenance of gaskets, seals, and sensors, as well as addition pumps, to ensure proper operation. Validation of inactivation parameters is also required by using spore-based indicators or the actual production organisms. Continuous flow thermal inactivation systems should undergo regular chemical clean-in-place cycles to remove coagulated protein residues, which can reduce system efficiency.

PPE/Gowning

PPE and gowning are used for both personnel and product protection. When PPE is utilized for product protection, it is designed to prevent shedding of foreign material into the production process and final product and to contain skin and respiratory shedding from the worker. Standard cotton or synthetic materials are not acceptable because they are prone to shedding. When PPE is utilized for worker protection, it should be assessed against physical, chemical, and biological hazards. Cotton laboratory coats or jumpsuits are easily saturated with chemical and biological liquids during a large release or spill and do not provide adequate protection. Man-made, water-resistant polymers are a better choice; they are less apt to become saturated. Refer to the material permeation rate or breakthrough detection time. The most protective options for personnel protection are gowns made of microporous laminated materials or jumpsuits with covered zippers.

Depending on the chemicals and/or biological materials handled, large volumes at high concentration plus the inherent increased risk of aerosol generation may require respiratory protection. Common disposable, half-face respirators (e.g., N95) may be sufficient for biological material protection, but they are not designed for chemical protection and may not be sufficient to protect against large volumes of a concentrated high-risk pathogen. Therefore, a risk assessment should be performed to identify the appropriate respirator required for the operation (i.e., filtering facepiece, tight-fitting facepiece, PAPR or SCBA).

Conclusion

Large-scale growth of biological agents is necessary in a variety of settings and requires an evaluation of both the GxP and biosafety requirements. With careful planning and a robust risk assessment of the unique requirements of a large-scale facility, it is possible to design and operate a facility that protects the product, workers, and the environment.

Appendix L: COVID-19 Biosafety Guidelines

(Adapted from Duke University and ABSA Guidelines)

Research Activities	Biosafety Level

• Storage and laboratory work with seed stocks, working stocks or specimens[1] with the intent to grow or use live virus.
  ○ Virus isolation, characterization and/or expansion
  ○ Viral cultures or isolates must be transported as Category A, UN2814, "infectious substance, affecting humans." Contact Environmental Health and Safety (801-581-6590) for shipping
• Use of live SARS-CoV-2 virus in functional assays:
  ○ Plaque/Focus Forming Unit assays
  ○ Serologic virus capture/binding assays
  ○ Therapeutic minimum inhibitory concentration (MIC) assays
	BSL-3

• Processing, aliquoting or preparing specimens[1] for research use and storage
  ○ Preparation of chemical- or heat-fixed specimens[1] for microscopic analysis
  ○ Nucleic acid extraction of specimens[1] for molecular analysis
  ○ Preparation of inactivated specimens for other laboratory assessments
  ○ Performing diagnostic tests (e.g. serology) that do not involve activities with the potential to propagate virus
  ○ Inoculating bacterial or mycological culture media
	BSL-2+ (enhanced)[2]

• Molecular analysis of already extracted nucleic acid preparations • Analysis of specimens[1] that have been inactivated by a method approved by the U. BioSafety Program or documented inactivated by a commercial vendor • Final packaging of specimens[1] already in a sealed, decontaminated primary container for transport to collaborating laboratories for additional analyses ○ Specimens from suspected or confirmed cases should be transported as UN3373, "Biological Substance, Category B • Pathologic/microscopic examination of fixed specimens[1] (e.g. formalin-fixed tissues or glutaraldehyde-fixed grids) • FACS analysis of samples/specimens that have been inactivated by a method approved by the U. BioSafety Program • Routine staining and microscopic analysis of fixed smears	**BSL-2**

The following laboratory activities cannot be performed at the University of Utah at this time due to lack of appropriate workspace:

- Live cell sorting with intact virus
- Use of live SARS-CoV-2 virus in animal

1. Specimens are defined as, but not limited to, blood, serum, plasma, tissues, feces, urine, sputum, mucosal swabs or washes/secretions collected from patients with COVID-19 or animals infected with SARS-CoV-2: see matrix below for procedures for handling and processing human samples.
2. Required Enhancements to standard BSL2:
   - Personnel will wear a closed front gown, eye protection and face mask (or face shield) and double pair of gloves. Respiratory protection (N-95s or PAPRs) may be required, based on the risk assessment.
   - The use of glassware and sharps should be eliminated wherever possible.

SARS-CoV-2 Sample Type Risk Assessment

Sample types	Potential hazards	Engineering controls	Administrative controls	PPE	Additional controls/processes
Processing blood, plasma/serum (convalescent, confirmed negative)	Droplet Fomites Aerosol	Negative air flow into laboratory (recommended) Biosafety Cabinet (BSC) (preferred) Centrifuge cup & lid or sealed rotor	Universal precautions Biohazardous waste disposal Disinfection SOP Bloodborne Pathogens (BBP) training Exposure Control Plan or Biosafety Manual	Laboratory coat Single or double gloves Eye protection (if outside of BSC) Mucosal protection (face mask) (if outside of BSC)	Hand hygiene If BSC not available, work behind a splash shield
Processing Blood (suspected or confirmed SARSCoV2)	Droplet Fomites Aerosol	Negative air flow into laboratory (recommended) BSC Centrifuge cup & lid or sealed rotor	Universal precautions Biohazardous waste disposal Disinfection SOP BSL2/BBP training Biosafety Manual	Solid front gown (not cloth) Double gloves Eye protection Mucosal protection (face mask)	Hand hygiene No glass or sharps

Sample types	Potential hazards	Engineering controls	Administrative controls	PPE	Additional controls/processes
Processing swab (suspected or confirmed SARSCoV2)	Droplet Fomites Aerosol	Negative air flow into laboratory (recommended) BSC Centrifuge cup & lid or sealed rotor	Universal precautions Biohazardous waste disposal Disinfection SOP BSL2 training Biosafety Manual	Solid front gown (not cloth) Double gloves Eye protection Mucosal protection (face mask)	Hand hygiene No glass or sharps
Urine (suspected or confirmed SARSCoV2)	Droplet Fomites Aerosol	Negative air flow into laboratory (recommended) Biosafety Cabinet (BSC) (preferred) Centrifuge cup & lid (recommended)	Universal precautions Biohazardous waste disposal Disinfection SOP BSL2 training Biosafety Manual	Laboratory coat Single or double gloves Eye protection (if outside of BSC) Mucosal protection (face mask) (if outside of BSC)	Hand hygiene If BSC not available, work behind a splash shield
Stool (suspected or confirmed SARSCoV2)	Droplet Fomites Aerosol	Negative air flow into laboratory (recommended) BSC Centrifuge cup & lid or sealed rotor	Universal precautions Biohazardous waste disposal Disinfection SOP BSL2 training Biosafety Manual	Solid front gown (not cloth) Double gloves Eye protection Mucosal protection (face mask)	Hand hygiene No glass or sharps

Sample types	Potential hazards	Engineering controls	Administrative controls	PPE	Additional controls/processes
Respiratory samples	Higher concentration of virus Droplet Fomites Aerosol	Negative air flow into laboratory BSC Centrifuge cup & lid or sealed rotor	Universal precautions Biohazardous waste disposal Disinfection SOP BSL2 training Biosafety Manual	Solid front gown (not cloth) Double gloves Eye protection Respiratory protection recommended or, if not available, mucosal protection (face mask): may be required based on the risk assessment	Hand hygiene No glass or sharps
Environmental samples (wastewater/ sewage) **Collection, surface sampling and routine processing**	Droplet Fomites Aerosol	Processing: BSC Negative air flow into laboratory (recommended) Centrifuge cup & lid or sealed rotor	Universal precautions Biohazardous waste disposal Disinfection SOP BSL2 training Biosafety Manual	Solid front gown (not cloth) Double gloves Eye protection Respiratory protection recommended or, if not available, mucosal protection (face mask)	Hand hygiene No glass or sharps

Sample types	Potential hazards	Engineering controls	Administrative controls	PPE	Additional controls/processes
Concentrated Environmental samples (Filtration/ Precipitation)	Higher concentration of virus Droplet Fomites Aerosol	BSC Negative air flow into laboratory Centrifuge cup & lid or sealed rotor	Universal precautions Biohazardous waste disposal Disinfection SOP BSL2 training Biosafety Manual	Solid front gown (not cloth) Double gloves Eye protection Respiratory protection	Hand hygiene No glass or sharps Separate area for donning and doffing PPE

1. https://www.cdc.gov/coronavirus/2019nCoV/lab/labbiosafetyguidelines.html#testing
2. https://www.cdc.gov/coronavirus/2019ncov/php/water.html
3. https://www.cdc.gov/coronavirus/2019nCoV/lab/guidelinesclinicalspecimens.html

Appendix M: Contact Information and EHS Guidance

Environmental Health and Safety Mainline: 801-581-6590

E Mail: Biosafety@ehs.utah.edu

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

IBC Website: https://ibc.utah.edu