

Laboratory Safety Manual

Section 2 Biological Safety

INTRODUCTION

PURPOSE

This section has been prepared to provide the laboratory personnel at Duke University with the information necessary to protect them and the surrounding environment from hazards associated with the use of biological materials. The guidelines which follow provide a means for evaluating the risks of work involving biological materials and introduce the proper handling practices which will minimize the risk of an occupational acquired infection. History has shown that if not handled appropriately, infectious agents can be transmitted to laboratory employees, and rarely, to people outside of the laboratory. Biohazardous materials are those which are either known to cause, or that present a potential risk to the health of humans or animals. Such materials would include, but are not limited to: bacteria, fungi, viruses, parasites, rickettsia, rDNA toxins, human blood and unfixed human tissues or cell lines. *Work with biohazardous material(s) requires consultation with the OESO – Biological Safety Division (919-684-8822).*

Examples for Safe Handling of Biohazardous Materials

Material	Precautions/ Containment	Employee Health Services	Waste Disposal	Shipping	Training Requirements
Human-derived (e. g. blood, body fluids, tissue, cells, etc.)	<ul style="list-style-type: none"> • BBP Exposure Control Plan (see Appendices for this section) • BSL 2 (see BMBL*) • BSL2 SOP 	<ul style="list-style-type: none"> • Hepatitis B vaccine • All exposures reported to EOHV Exposure Hotline, 115 (on campus) and 684-8115 (off campus) • Complete the Report of Work Related Injury or Illness form. 	<ul style="list-style-type: none"> • All waste decontaminated according to Duke Medical Waste Management Policy. 	<ul style="list-style-type: none"> • Classify and package according to IATA's DGR • Receipt or shipment of "select agents" must comply with 42 CFR Part 73. • Importation of infectious substances requires a permit. 	<ul style="list-style-type: none"> • New Employee Orientation • General Lab Safety (annual updates) • Shipping training every 2 years • BSL2 training • Bloodborne Pathogens training

Section 2 Biological Safety
Chapter 1
Introduction

Material	Precautions/ Containment	Employee Health Services	Waste Disposal	Shipping	Training Requirements
Known (or suspect) human or animal pathogens (e. g. HIV, Rickettsial agents, Salmonella, etc.)	<ul style="list-style-type: none"> • Refer to BMBL* or "Biosafety Levels" of this section for appropriate BSL or ABSL. • Refer to infectious agent PSDS** • BSL2 SOP 	<ul style="list-style-type: none"> • Contact EOHW at 684-3136 for vaccination information. • All exposures reported to EOHW Exposure Hotline, 115 (on campus) and 684- 8115 (off campus) • Complete the Report of Work Related Injury or Illness form. 	<ul style="list-style-type: none"> • All waste decontaminated according to Duke Medical Waste Management Policy. 	<ul style="list-style-type: none"> • Classify and package according to IATA's DGR • Receipt or shipment of "select agents" must comply with 42 CFR Part 73. • Importation of infectious substances requires a permit 	<p>New Employee Orientation</p> <ul style="list-style-type: none"> • General Lab Safety (annual updates) • Shipping training every 2 years • BSL2 training
Recombinant DNA/Transgenics	<ul style="list-style-type: none"> • Follow NIH Guidelines (also see "Recombinant DNA" in this section) • Visit Duke's IBC website to complete appropriate registration • If material is a biological vector, use appropriate BSL for wild type agent. See rDNA/viral vector webpage for more information and the rDNA registration form for IBC registration information. If BSL2 or above, must have SOP. 	<ul style="list-style-type: none"> • Contact EOHW at 684-3136 for vaccination information. • All exposures to human pathogens are reported to EOHW Exposure Hotline, 115 (on campus) and 684-8115 (off campus). Exposures to rDNA are reported to OESO-Biological Safety 684-8822. • Complete the Report of Work Related Injury or Illness form. 	<ul style="list-style-type: none"> • All waste decontaminated according to Duke Medical Waste Management Policy. 	<ul style="list-style-type: none"> • Classify and package according to IATA's DGR 	<p>New Employee Orientation</p> <ul style="list-style-type: none"> • General Lab Safety (annual updates) • Shipping training every 2 years

* Biosafety in Microbiological and Biomedical Laboratories, 5th ed., CDC/ NIH, 2009: (<https://www.cdc.gov/labs/BMBL.html>)

** Health Canada, Laboratory Center for Disease Control; Office of Biosafety: (<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php>)

ROUTES OF EXPOSURE

There are four main routes of exposure that one must try to avoid when working with biohazardous agents in the laboratory. These would include percutaneous injuries, inhaling infectious aerosols, exposure to mucous membranes, and ingestion.

Percutaneous injuries

Percutaneous injuries can result from needlesticks, cuts or abrasions from contaminated items. These exposures are particularly serious because of the potential for immediate entry of the agent into a normally sterile bloodstream. All sharps items should be handled and disposed of as noted in the [Waste Management](#) section.

Inhalation of aerosols

Many laboratory procedures can cause the aerosolization of infectious agents. Some of these procedures include the use of vortexes, blenders and sonicators. Proper work practices must be implemented to minimize the aerosolization of all materials, especially those which are known to be transmitted by the aerosol route (e.g., Adenovirus, Vaccinia virus, *Mycobacterium tuberculosis*, etc.). See Chapter 7, [Laboratory Equipment](#) for more information about minimizing and containing aerosols in the laboratory.

Mucous membrane

Exposure of mucous membranes to infectious agents can lead to occupationally acquired infections. Mucocutaneous exposures can result from splashes to the eyes, nose or mouth, or by inadvertent inoculation via contaminated hands. [Face protection](#) should always be used if there is a likelihood of splash or splatter.

Ingestion

Accidental ingestion of biohazardous materials can result from improper personal hygiene in the laboratory. Food and drink are prohibited in all areas of the laboratory in which work is conducted with potentially infectious materials. Hands must always be washed before leaving the laboratory, and immediately if visible contamination occurs.

STANDARD LABORATORY PRACTICE AND TECHNIQUE

Biosafety Levels and Warning Signage

Four biosafety levels (BSLs) are summarized in the table below for proper handling of biohazardous materials. BSLs consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed the documented or suspected routes of transmission of the infectious agents, and for the laboratory function for activity.

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause diseases in immunocompetent adult humans	Standard microbiological practices	None required	Open bench top, sink required
2	Associated with human disease. Hazard: percutaneous injury, mucous membrane exposure, ingestion	BSL-1 practices plus: <ul style="list-style-type: none"> • limited access • biohazard warning signs • sharps precautions • biosafety manual defining waste decontamination or medical surveillance policies 	Primary barriers: Class I or II biosafety cabinets or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPE: laboratory coats, gloves, face protection as needed	BSL-1plus: <ul style="list-style-type: none"> • non-fabric chairs and other furniture easily cleanable • autoclave available • eyewash readily available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practices plus: <ul style="list-style-type: none"> • controlled access • decontamination of all wastes • decontamination of lab clothing before laundering • baseline serum 	Primary barriers: Class I or II biosafety cabinets or other physical containment devices used for all manipulations of agents; PPE: laboratory coats, gloves, respiratory protection as needed	BSL-2 plus: <ul style="list-style-type: none"> • physical separation from access corridors • hands-free handwashing- sink • self-closing double door access • exhaust air not recirculated • negative airflow into laboratory • eyewash readily available in lab
4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission	BSL-3 practices plus: <ul style="list-style-type: none"> • clothing change before entering • shower on exit • all material decontaminated on exit from facility 	Primary barriers: All procedures conducted in Class III biosafety cabinets or Class I or II biosafety cabinets in combination with full-body, air supplied positive pressure suit	BSL-3 plus: <ul style="list-style-type: none"> • separate building or isolated zone • dedicated supply/exhaust, vacuum and decon system <p>There are no BSL-4 labs at Duke</p>

Summarized from **Biosafety in Microbiological and Biomedical Laboratories**, 5th Edition, 2009.

<http://www.cdc.gov/biosafety/publications/bmbl5/>

Biohazard Warning Signage

A sign incorporating the universal biohazard symbol **must be posted** at the entrance to the laboratory when infectious agents are present.



Biosafety Level 1 (BSL-1): The sign may include the name of the agent (s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel.

BSL-1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. All bacterial, parasitic, fungal, and viral agents which have been assessed for risk but do not belong to a higher risk group can be safely handled at BSL-1. Be aware that many agents not ordinarily associated with disease are opportunistic pathogens and may cause infection in the young, the aged and immunocompromised individuals.

Biosafety Level 2 (BSL-2): Posted information on the sign must include the name of the agent(s), laboratory's biosafety level, supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory.

BSL-2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that: 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) **all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.**

Biosafety Level 3 (BSL-3): Posted information on the sign must include the name of the agent(s), laboratory's biosafety level, supervisor's name (or other responsible personnel), telephone number(s), and required procedures for entering and exiting the laboratory.

BSL-3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures. All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices. A BSL-3 laboratory has special engineering and design features.

Classification of Agents According to Risk -

Biological agents are assigned to biosafety levels (BSL) based on the risk they pose to human health and the environment. Such factors as severity of disease caused by the agent routes of exposure, and virulence are used when determining the most appropriate BSL. The partial list below is provided to assist laboratories in making preliminary decisions on the appropriate biosafety level for particular agents. Ultimately, the Occupational and Environmental Safety Office (OESO) will make the final BSL assignment. If a particular agent is not listed below, or if further assistance is needed in interpreting BSL requirements, contact the OESO-Biological Safety Division at 919-684-8822. There are no Biosafety Level 4 labs at Duke.

BSL-1 Viral Agents

Baculovirus
Murine Leukemia virus (ecotropic)

BSL-1 Bacterial Agents

Bacillus subtilis
Eschericia coli -K12

BSL-2 Viral Agents:

Adenovirus	Respiratory syncytial virus
Creutzfeld-Jacob agent	Sindbis Virus
Cytomegalovirus	Monkeypox virus
Epstein-Barr virus	SIV
Hepatitis A, B, C, D, E	Spongiform encephalopathies
Herpes simplex viruses	Vaccinia virus
HIV	VSV (lab adapted strains)
HTLV types I and II	

BSL-2 Bacterial/Rickettsial Agents:

Campylocacter fetus, coli, jejuni	Shigella spp
Chlamydia psittaci, trachomatis	Treponema pallidum
Clostridium botulinum, tetani	Staphylococcus aureus
Corynebacterium diphtheriae	Streptococcus spp
Legionella spp	Vibrio cholera
Neisseria gonorrhoeae	Vibrio parahemolyticus
Neisseria meningitidis	Vibrio vulnificus
Pseudomonas pseudomallei	Klebsiella spp
Salmonella spp	

BSL-2 Fungal Agents:

Candida albicans	Fonsecaea pedrosoi
Cryptococcus neoformans	Sporothrix schenkii
Microsporum spp	Trichophyton spp
Exophiala dermatitidis (wangiella)	

BSL-2 Parasitic Agents:

Entamoeba histolytica
Cryptosporidium spp
Giardia spp
Naegleria fowleri
Plasmodium spp

Strongyloides spp
Tania solium
Toxoplasma spp
Trypanosoma spp

Other material at BSL-2:

Human and Non-human primate cell lines, blood, and body fluids (because of potential contamination with bloodborne pathogens)

BSL-3 Viral Agents:

Rift Valley Fever (Zinga)
VSV exotic strains (PIRY)
Yellow Fever 9wild type)
West Nile Virus

BSL-3 Bacterial/Rickettsial Agents:

Bacillus anthracis
Francisella tularensis
Mycobacterium tuberculosis
Mycobacterium bovis (non-BCG strain)
Rickettsia ricettsii
Yersinia pestis (resistant strain)

BSL-3 Fungal Agents:

Coccidioides immitis
Histoplasma capsulatum

Strongyloides
Tania solium
Toxoplasma
Trypanosoma

Personal Protective Equipment

Once a biological hazard has been identified, the supervisor and employee must agree on the appropriate personal protective equipment (PPE) to be worn as the primary barrier of protection. PPE may include, but is not limited to face protection, lab coats and gowns, respirators, and shoe-covers/booties. Supervisory personnel are responsible for the initial demonstration and periodic follow-up of proper use. Appropriate PPE should be donned before handling potentially hazardous biological materials and removed immediately and replaced if gross contamination of the equipment occurs. PPE is removed before exiting the laboratory and is not worn in non-lab areas.

Face Protection: When splash or splatter of infectious substances or other biological materials is anticipated, appropriate face protection is worn if work is performed outside a biological safety cabinet. Such equipment would include but is not limited to goggles, side-shielded safety glasses and chin length face shields.

Lab Coats and Gowns: Long sleeved lab coats or gowns must be worn to protect skin and street clothes from contamination. In circumstances when splash or splatter is anticipated, the garment must be resistant to liquid penetration. A cuffed lab coat or gown (or lab coat and cuffed disposable sleeve covers) must be worn when working with potentially infectious materials.

Laundering: Reusable lab coats should be laundered on-site or by a laundering service set up by the employer, at no cost to the employee. Personnel must never launder lab coats or gowns at home.

Soiled clothing being collected for laundering should be placed in leak-proof container (e.g., biohazard bag). If minor contamination is present, laboratory clothing should be decontaminated (i.e. disinfecting, neutralizing, autoclaving) in the laboratory before being sent to the launderer. If grossly contaminated, disposing with laboratory waste may be the best option. Soiled laundry should only be handled by individuals wearing appropriate PPE and should never be taken home. Reusable laboratory clothing worn in BSL-3 areas must be decontaminated before being laundered. **Discuss options for outside laundry service with your departmental business manager.**

Gloves: Gloves are worn when handling biohazardous materials. Disposable gloves can provide an adequate barrier between the lab employee and most biohazardous materials. Double gloves and/or cut-resistant gloves should be considered when handling sharp items and biohazardous materials. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste.

Respirators: When engineering controls (i.e. BSCs) are not available to provide adequate protection against aerosolized agents or when mandated by federal regulations, respirators shall be worn. Duke's Respiratory Protection Program requires that employees be medically cleared, fit-tested, and trained on proper usage and care before allowed to wear a respirator. Details of the Program can be viewed [here](#).

Disposable Shoe-covers/Booties: When significant splash and splatter are anticipated, shoe-covers/booties should be considered. Prior to exiting the laboratory, these must be removed and disposed of properly.

Handwashing

Hands should be washed as soon as possible when they come in contact with potentially infectious materials. A vigorous handwashing with a mild soap for 20 full seconds is appropriate. Hands should also be washed as soon as feasible after gloves are removed, and before exiting the laboratory.

Eating, Drinking, Smoking, Applying Cosmetics and Handling Contact Lenses

Eating, drinking, smoking, applying cosmetics and handling contact lenses is prohibited in work areas in which potentially infectious materials are being manipulated. Food and drink must not be stored in refrigerators in which laboratory materials are kept.

Housekeeping

Good housekeeping in laboratories can reduce the risk of accidents occurring. Work benches should be kept as clutter-free as feasible, and aisles should always be free of trip hazards. Benches should be wiped down with an approved disinfectant at least once a day and immediately after a spill of potentially infectious materials.

Pipetting

Pipetting infectious agents can lead to personnel exposures by inhalation, contact, or ingestion if not performed properly. The following are a few safety precautions to be followed when pipetting in the laboratory: 1) Never mouth pipette; pipetting aids should always be used, 2) Pipette contents should be allowed to run down the wall of the container, making sure not to release the contents from a height, 3) Place absorbent paper on benchtops to reduce the risk of aerosols being generated by accidental dripping of infectious materials from pipette tips, and 4) Place disposable pipettes into pipette disposal boxes which have been lined with an autoclave bag, and then steam sterilize (autoclave) (see [Waste Management Section](#)).

Sharps

The use of needles, glass pipettes, glass slides and cover slips, scalpels and lancets should be eliminated, when possible. Appropriate precautions should be taken to avoid percutaneous injuries. These items should be disposed of immediately after use by placing them in an appropriate puncture-resistant container. Bending, recapping or clipping of needles is prohibited. If recapping is absolutely necessary, a mechanical device or the one handed scoop method must be used. Plasticware should be used whenever possible, such as plastic graduated cylinders, funnels, aspirators, etc. Safety devices should be used when available (e.g. mylar-coated capillary tubes, Eclipse safety needles).

Decontamination

The purpose of decontamination is to make a hazardous material safe for further handling. A decontamination procedure can range from sterilization to simple cleaning with soap and water. The following includes a description of the four main categories of physical and chemical means of decontamination.

Heat: Wet heat is the most dependable method of sterilization. Steam autoclaving is the most convenient method available to the Duke laboratories for decontaminating biological waste and sterilizing glassware and media. **Note: Autoclaves that are used for decontamination of biohazardous wastes should be monitored for the efficacy of treatment. This is accomplished by the use of biological indicators.** The generator of the waste (the lab) is responsible for performing and documenting this testing. See *Waste Management in [Section VII](#) of the [University Safety Manual](#).*

Liquid Disinfection: Many types of liquid disinfectants are available under a variety of trade names. The most practical use of liquid disinfectants is for surface decontamination. Agents included in the category include, but are not limited to, quaternary ammonium compounds, phenolic compounds, halogens, aldehydes, alcohols and amines. **A tuberculocidal disinfectant or diluted household bleach should always be used for decontamination when human materials are handled.**

NOTE: When **household bleach** is used for the decontamination of spills, a fresh solution (at least 10% household bleach) must be prepared. Bleach solutions used for routine surface decontamination must be *made up at least weekly*. **Each solution container must be labeled with either a made-on or an expiration date and the word corrosive.**

Vapors and Gases: The use of vapors and gases as decontamination methods usually involve the decontamination of biological safety cabinets, but can also be used for whole building or room decontaminations. Agents used in this category include ethylene oxide, formaldehyde, gas, hydrogen peroxide and peracetic acid.

Radiation: Ultraviolet radiation (UV) is sometimes used in biological safety cabinets for inactivating contaminants, but because of the low penetrating power of UV, dusty or soiled areas may limit its usefulness in the laboratory. Because UV can cause serious burns to eyes and skin, it must not be used when work areas are occupied. Whole room UV is not recommended. Do not rely on just radiation for your disinfection process.

Decontaminants and Their Use in Laboratories

Decontaminant	Active Ingredient/ Concentration	Temp (°C)	Contact time (min.)	Vegetative bacteria	Lipo viruses	Tubercle bacilli	Hydrophilic viruses	Bacterial spores
Autoclave	Steam	121	50–90	+	+	+	+	+
Incinerator	Heat	649-929	1-60	+	+	+	+	+
Phenolic compounds	0.2-3%		10-30	+	+	+	±	—

Chlorine compounds	0.01-5%		10-30	+	+	+	+	±
Alcohol (ethyl or isopropyl)	70-85%		10-30	+	+	+	+	+
*Formaldehyde	4-8%		10-30	+	+	+	+	+
*Gluteraldehyde	2%		10-600	+	+	+	+	±
Hydrogen peroxide	6%		10-600	+	+	+	+	+

+ very positive response

± less positive response

— negative response

*irritating characteristics of agent precludes use for routine spill cleanup

HUMAN BLOOD, BLOOD PRODUCTS, TISSUES AND BODY FLUIDS

In 1991, the Occupational Safety and Health Administration (OSHA) promulgated a standard to minimize the risk for occupational exposure to bloodborne pathogens (e.g., HIV, Hepatitis B). The regulation, titled Occupational Exposure to Bloodborne Pathogens mandates several provisions for those working with materials that are human-derived such as human blood, blood products, other bodily fluids and any unfixed tissues. The full text of the Duke University Bloodborne Pathogen Exposure Control Plan, including a copy of the Bloodborne Pathogen Standard can be found in the Appendices of this section. The Plan must be readily available to all employees working with those materials mentioned above. *This includes all employees working with primary human cell lines, or human cell lines that have not been tested for human pathogens.* The following are a few highlights of the Plan.

Universal Precautions

Universal precautions are defined as handling all human blood, body fluids, and tissues as if they are infectious. This calls for the use of appropriate protective measures to reduce or eliminate the risk of occupational exposure.

Hepatitis B Vaccination

All employees working with human blood, blood products, fresh tissues or bodily fluids shall be offered the Hepatitis B vaccine at no cost to them. If an employee should decline the vaccine, they must sign a waiver which is kept on file in the Employee Occupational Health and Wellness (EOHW). For more information about the vaccine, contact the EOHW at 919-684-3136.

EOHW Blood and Body Fluid Exposure Hotline

All occupational exposures to potentially infectious materials are to be reported immediately by calling 115 from a landline campus phone and 919-684-8115 from any phone. An EOHW representative will connect you with a healthcare provider to discuss with the employee the appropriate follow up of the exposure. It is important that exposures are reported as soon after the incident as possible because some post exposure treatments are considered time sensitive.

Safety Training

All employees who work with materials (primary and well-characterized human cells, tissues, blood) covered by OSHA's Bloodborne Pathogen Standard are to receive initial safety training and annually thereafter. Bloodborne Pathogens, General Laboratory Safety, and Biosafety Level 2 (BSL2) are available as [online training](#) modules. On-site general laboratory safety training can be requested. In fact, credit for this may be given for those who participate in the annual lab safety audit. Laboratory-specific training is the responsibility of the Primary Investigator. Written [standard operating procedures \(SOP\) for agents used at BSL2](#) are required and supplement this general lab safety manual for your lab-specific training.

BIOHAZARD SPILL CLEAN-UP

The following procedures should be followed to insure proper spill clean-up of blood, body fluids and cultures of biological hazards at Biosafety Level 1 or 2.

1. Alert people in immediate area of spill.
2. At a minimum, wear disposable gloves and face protection.
3. Cover spill with paper towel or other absorbent material.
4. Carefully pour a freshly prepared 1:10 dilution of household bleach (or other effective disinfectant) around the edges of the spill and then into the spill. Avoid splashing.
5. Allow a 20 minute contact period for bleach (or as indicated as effective time for different disinfectant). If broken glass is present, use forceps to remove and place glass in sharps collection container.
6. Use paper towels to wipe up the spill, working from the outer edges into the center.
7. Clean spill area again as indicated in steps 4 and 5.
8. Depending on the size and concentration of the spill, a third disinfection (steps 4 and 5) may be warranted.
9. Discard disinfected disposal materials. Items that do not contain large amounts of bleach may autoclaved according to the [Medical Waste Management Policy](#) before disposal.

Spill Involving Concentrated Microorganisms Required BSL 3 Containment (e.g. *Mycobacterium tuberculosis*, (TB) cultures)

Attend to injured or contaminated persons and remove them from exposure. Alert people in the area to evacuate. Close doors to affected area; do not enter area for at least one hour. Have a person knowledgeable of the incident and area assist in proper clean-up. Wearing gowns, gloves, respirator and shoe covers, clean up spills as indicated for **BIOHAZARD SPILL CLEAN-UP**.

WASTE MANAGEMENT

Appropriate waste handling practices at Duke University and Medical Center are based on compliance with OSHA regulations for protection of personnel who have to handle the waste, and the North Carolina Medical Waste Regulations for appropriate disposal.

There are three primary methods for disposing of biological waste at Duke. These methods include autoclaving, incineration, and chemical disinfection.

Autoclaving is usually the most convenient choice for labs since autoclaves are readily available throughout most research laboratory buildings. Red bags ARE NOT approved for autoclaving at Duke. See *Medical Waste Management* in [Section VII of the University Safety Manual](#) for instructions.

Incineration of biological waste is a viable option for all biological waste; however, coordination with other departments is necessary to utilize this option. Red bags are used for incineration. For incineration of medical lab waste, contact Environmental Services' Biomedical Waste Division (919-681-9700) for pick-up. The Division of Laboratory Animal Resources must be contacted (919-684-5212) for animal carcass disposal.

Chemical disinfection is a treatment option for liquid biological waste. An example is household bleach, but [do not mix bleach with incompatible chemicals](#). The disinfectant must be effective against the biological material it is treating. The appropriate contact time must be allowed for effective disinfection/inactivation. Follow manufacturer instructions.

Categories of Biological Waste and Acceptable Treatment

Sharps — Needles, syringes with attached needles, capillary tubes, slides and cover slips, scalpel blades, razor blades, and broken glassware that are contaminated with biological material should be placed in a plastic puncture-resistant container (needlebox). There are two acceptable methods for disposal of needleboxes: 1) place in autoclavable bag that has the biohazard symbol on it and autoclave before disposal or, 2) contact Environmental “Room” Services' Biomedical Waste Division for pick-up (919-681-9700).

Pipettes — Plastic pipette tips and serological pipette tips used to process human body fluids or cultures of infectious agents, should be placed in a puncture-resistant “pipette” box (cardboard) that is labeled with the biohazard symbol and lined with an autoclavable bag that has the biohazard symbol on it. Once filled, these boxes should be placed in an autoclavable bag with the biohazard symbol on it and autoclaved before disposal. Non-infectious pipettes should also be placed in a puncture-resistant container before disposal; however, it is not necessary to autoclave.

Microbiological/Molecular Waste — Includes cultures and stocks of etiologic agents and recombinant DNA/transgenics. Solid waste should be placed in an autoclavable bag that has the biohazard symbol on it and steam sterilized (autoclaved) before disposal. Liquid biological waste (no

hazardous chemicals) can be autoclaved or chemically treated (i.e. bleached) before disposal down the drain. [Do not mix bleach with incompatible chemicals](#).

Specimens of human blood/body fluids that are OPIM — Containers of blood/body fluids less than 20 mls and tissue cultures can be placed in an autoclavable bag that has the biohazard symbol on it and autoclaved before disposal. Greater than 20 mls should be treated as applicable using a different method listed in this chapter.

Tissue Culture Wastes (Animal and Human) — All solid waste should be discarded in autoclavable bags that have the biohazard symbol on them and autoclaved before disposal. Liquid waste can be chemically disinfected (bleach) before disposal down the drain. The waste should not contain other chemicals that are incompatible with bleach or other disinfectants used.

Anatomical/Pathological Waste — Organs, limbs, animal carcasses etc., which must be incinerated (Not Autoclaved!) for proper treatment. All large, human-derived anatomical/pathological waste should be submitted to Environmental Services' Biomedical Waste Division (919-681-9700). Animal carcasses should be disposed of through the Division of Laboratory Animal Resources (919-684-5212).

Non-contaminated glass — Items should be discarded in a bag-lined heavy-duty cardboard box and taped shut before disposal. Do NOT use cardboard boxes with “biohazard” symbols printed on them, which implies biohazardous waste requiring special treatment. Usually, these are labeled “broken glass” by the manufacturer. **Keep in mind that these boxes are very heaving if filled to the rim. Consider smaller glass disposal boxes or disposing the boxes when the box is a manageable weight and not full.**

Solid Disposal Supply Wastes — Disposable gloves, gauze, paper wrappings, parafilm, etc., that are minimally contaminated. Decontamination is not required before disposal; however these items should be placed in leakproof containers (i.e., a sturdy, plastic bag).

LABORATORY EQUIPMENT

Biological Safety Cabinets (BSCs)

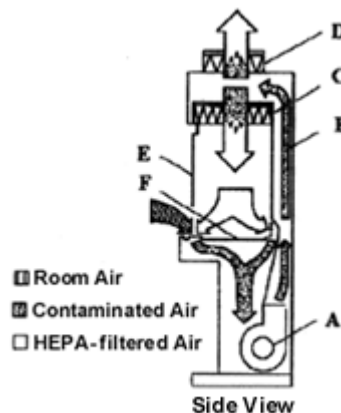
BSCs are the most commonly used primary containment devices in microbiological laboratories. There are three classes of BSCs (Class I, II, and III). When combined with appropriate microbiological techniques, each Class provides different levels of protection.

Class I BSC — provide both personnel and environmental protection, however, they do not provide product protection such as that needed for sterile tissue culture work. Class I BSCs are suitable for work with low to moderate risk agents.

Class II BSC —these are the most commonly used BSCs at Duke. Class II BSCs provide environmental, personnel and product protection. The main difference between Class I and II cabinets is the HEPA filtration of the air flow down across the work surface of a Class II cabinet.

Class II, Type A BSC

- A. Blower
- B. Rear plenum
- C. Supply HEPA filter
- D. Exhaust
- E. Sash
- F. Work surface



Things to Remember When Using a Class II BSC

Keep front and rear perforated grills free of clutter. Cluttered grills can cause a disruption of air flow which can compromise personnel, environmental and product protection. Avoid sudden movements in and out of the cabinet. Also, avoid installing BSCs near windows or frequently used doorways. Each of these can disrupt airflow. Gas burners should not be used in the BSC. The heat disrupts air flow, the flame can damage the HEPA filter and gas can build up inside the work space due to recirculation of air. Volatile chemicals and volatile radionuclides should not be used unless approved by the Occupational and Environment Safety Office. Don't store items on top of the cabinet. The HEPA filter could be damaged and the balance of air flow could be disrupted. Do not eat, drink, and chew gum or smoke near the cabinet. Doing this could result in ingestion of hazardous materials. Wipe down the cabinet interior with a surface disinfectant before and after all manipulations.

Class III BSC — Gas tight BSCs provide the highest level of environmental, personnel and product protection. A Class III BSC, (also referred to as a glove box), provides a complete physical barrier between the product and personnel. These cabinets are used for high risk biological agents when absolute containment is required.

A high efficiency particulate air (HEPA) filter is the main functional unit of a BSC. The HEPA filter is a device which removes particulates and microorganisms from the air. These filters remove 99.97% of all particulates 0.3 microns in diameter and have a greater efficiency for particles < or > 0.3 microns. HEPA filters are made of boron silicate fiber sheets which are pleated to increase surface area. In order to direct the airflow in the filter, aluminum baffles separate each pleat.

Certification of Biological Safety Cabinets

BSCs are to be certified by one of the [Duke Procurement](#) Office “approved vendors”. These vendors are National Sanitation Foundation certified, and have demonstrated expertise in maintaining BSCs. For more information on certification of BSCs, contact the OESO Biological Safety Division at 919-684-8822. All cabinets in which human materials, infectious agents, or other potentially infectious materials are being used must be certified annually. Cabinets in which non-infectious materials are manipulated (i.e. sterile tissue culture) should be certified at least every two years, but annually is encouraged. All newly purchased or moved cabinets must be certified before they can be used for any type of work.

Relocating a BSC — Before a BSC is moved to another Duke Campus location, the need for gas decontamination is determined by the work that has been conducted in the BSC.

If Risk Group 2 only biological agents were used, then the following must be done:

1. Double wipe down with an appropriate disinfectant, inside and outside.
2. With the exception of the feet/legs, the BSC must not be dismantled during the move.

When is a gas decontamination required?

Before a BSC is moved to surplus or dismantled, gas decontamination must be completed by the certified vendor.

If the BSC was used for work with infectious agents with the potential for aerosol transmission (i.e. vaccinia virus), the BSC must be decontaminated by the certified vendor with a disinfecting gas prior to maintenance or relocation of the BSC.

Clean Benches

Horizontal laminar-flow clean benches are designed to protect the product from contamination and should never be confused with BSCs! Vertical flow clean benches may be useful, for example, in hospital pharmacies when a clean area is needed for preparation of intravenous solutions. These pieces of equipment discharge HEPA-filtered air from the back of the cabinet across the work surface and toward the user. Clean benches should never be used when handling cell culture materials, drug formulations, potentially infectious materials, or any other potentially hazardous materials. The worker will be exposed to the materials being manipulated on the clean bench potentially resulting in hypersensitivity, toxicity or infection depending on the materials being handled. Horizontal airflow “clean benches” must never be used as a substitute for a biological safety cabinet. Users must be aware of the differences between these two devices.

Centrifuges

Centrifuges (including microhematocrit centrifuges) are commonly used in the laboratory environment. Centrifuges must be properly used and maintained to ensure safe operation. The following are suggested practices:

- Refer to the owner's manual for routine maintenance requirements.
 - Perform a visual inspection prior to each use (note unusual cracks, irregularities or wear).
 - Verify proper loading of specimens to maintain balance.
 - After starting, listen for unusual noises or vibrations until programmed speed is reached.
 - Perform routine decontamination of interior surfaces using an appropriate disinfectant. Immediate decontamination is required when visible contamination is noted.
-
- Prevent the release of aerosols when centrifuging infectious materials that are spread via the aerosol route or with high titer/concentrated infectious materials by using "safety devices", (i.e. sealed buckets, safety trunnion cups, and sealed heads). Safety cups must be opened in a BSC after centrifuging such materials to avoid the release of aerosols into the room.
 - Spills should be addressed immediately by following established [biological spill procedures](#). Special precautions should be taken when broken glass or other sharps may be involved. Use mechanical device to pick up sharps. Do not use hands.

Homogenizers and Blenders

These items are commonly used in laboratories, and both are considered producers of aerosols. Safety sealed homogenizers and blenders are commercially available and should be used when working with those agents known or suspected of being transmitted through aerosols. The purpose of these items is to contain any aerosols created during work procedures. These safety devices may be used on the open benchtop; however, they must be opened in a BSC. All non-sealed devices must be used exclusively in a BSC.

RECOMBINANT DNA (rDNA)

Since the inception of rDNA technology, scientists have been concerned over the possibility that artificially constructed rDNA could be biologically hazardous if not handled appropriately or released into the environment. These concerns prompted the development of the NIH Guidelines on rDNA research in May of 1976. The most recent revision is available for review at <https://osp.od.nih.gov/biotechnology/biosafety-and-recombinant-dna-activities/>.

Researchers at Duke University who construct and/or handle materials containing recombinant DNA molecules must comply with the requirements of the [National Institutes of Health \(NIH\) Guidelines for Research Involving Recombinant DNA Molecules](#). The following information and procedures are developed to assist Duke University researchers with the documentation of this compliance.

Generally, experiments requiring the use of recombinant biological agents should be handled under the same BSL requirements as the wild type agent. For example, handling of adenoviral vectors should be performed under BSL 2 conditions.

NIH Guidelines for Research Involving rDNA molecules are applicable to all rDNA research conducted or sponsored by an institution that receives any support for rDNA research from the NIH. rDNA research at Duke must be registered with the Duke Institutional Biosafety Committee (IBC) whether or not the Principal Investigator received funding from NIH for the project.

All rDNA research receiving NIH funding through Duke University but conducted outside of the US must be registered with the Duke IBC and comply with any rules of the host country. The NIH Guidelines provide guidance for containment and safe practices of various categories of rDNA research.

What is rDNA?

The NIH rDNA Guidelines defines rDNA as 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 and 2) Molecules that result from the replication of those described in (1) above.

At Duke, rDNA work may include:

1. Plasmids and viral vectors
2. Any synthetic DNA or RNA
3. Any RNA produced from rDNA, including messenger RNA (mRNA), small interfering RNA (siRNA), micro RNA (miRNA), etc.
4. Genetically-modified organisms (animals, plants, bacteria, viruses, fungi, etc.). This includes creation, cross-breeding, or manipulation of transgenic animals and plants.
5. Any such material obtained from another researcher or source

The Institutional Biosafety Committee (IBC)

The NIH rDNA Guidelines requires that an IBC be established at any institution receiving NIH funding for rDNA research to oversee all rDNA research at that institution, and insure that such work is compliant with the Guidelines.

The mission of the Duke University IBC is to:

1. ensure that all recombinant DNA research conducted at the institution or sponsored by the institution is conducted in compliance with the National Institutes of Health Recombinant DNA Guidelines, and
2. ensure that protocols of research involving Select Agents (defined by the Centers for Disease Control and Prevention), including but not limited to recombinant DNA, are reviewed and found to comply with all national, state, and local requirements
3. ensure that all laboratory work conducted at Biosafety Level 3 containment and/or Animal Biosafety Level 3 is reviewed and found to comply with all national, state, and local requirements

The Duke University IBC has responsibility for such research throughout the Duke Health System, Medical Center, and University. IBC members are appointed by the Chancellor of Health Affairs and the Provost of Duke University. The Duke Occupational and Environmental Safety Office staff will support the IBC in carrying out its mission. The IBC is authorized to inspect research facilities, approve research practices and procedures, and to take actions, such as enforcement of cessation of laboratory or clinical research activities, in the event of an unsafe workplace situation.

Principal Investigator (P.I.) Responsibilities

The P.I. makes an initial determination of the required levels of physical and biological containment, and practices and procedures in accordance with the NIH Guidelines. Determine whether or not your research must be registered with the IBC by completing the **rDNA survey** found at <https://sms.duhs.duke.edu/onlinetraining> under the complete list of “Courses Available Online”. If your research is not exempt submit the appropriate paperwork for the proposed work (see rDNA Registration Process below).

The Principle Investigator:

1. Is responsible for adherence to all requirements of the NIH Guidelines, including required safety practices.
2. Submits an annual update of the continuing protocols to the IBC.
3. Trains all laboratory workers regarding the potential hazards of the work and precautions to be taken.
4. Investigates and reports any significant problems or illnesses pertaining to the operation and implementation of containment, or any adverse reactions occurring during clinical studies to the Biological Safety Office for review by the IBC.
5. Ensures that all lab workers experiencing occupational exposures to rDNA material will report such exposures to Employee Occupational Health and Wellness (EOHW) and the IBC.
6. Complies with any shipping requirements for rDNA molecules.
7. Ensures that laboratory workers who work with animals involved in the work participate in the [Duke Health Surveillance for Animal Handlers](#).

The rDNA Registration Process - All research that is not exempt from compliance with the [NIH Guidelines for Research Involving rDNA Molecules](#) must be registered with the [IBC](#). Non-exempt

manipulation of recombinant DNA molecules includes, but is not limited to cross-breeding to create a new strain of animal or plant, rDNA in viral vectors or human cells, and rDNA in clinical human trials.

1. Review the table "[Required Documents for Protocol Submission to the Institutional Biosafety Committee](#)".
2. Submit the appropriate documents to the IBC. Templates are below.
 - a. [rDNA form](#)
 - b. [SOP for BSL2/ABSL2 labs](#), and
 - c. [Plasmid/Vector Table](#).
3. Gene Transfer/Human Subject: **every** human trial requires its own review by the [NIH OBA](#) the [Duke Institutional Review Board](#) and the IBC, even if the same rDNA material is used in multiple trials. See guidance information [here](#).

General Laboratory Procedures - Review the general laboratory procedures for biosafety and rDNA work. These procedures include physical containment, standard practices and training. The procedures can be found in

1. The CDC Guidelines on Biosafety in Molecular and Microbiological Laboratories ([BMBL5](#)). The BMBL has general descriptions (Section IV) of appropriate laboratory biosafety levels.
2. Appendix G of [NIH Guidelines](#). The appendix provides rDNA-specific descriptions.

Recombinant DNA Waste Management - rDNA and transgenic organisms must be treated the same as medical or infectious waste before disposal. Organisms must be rendered inviable before disposal. See the [waste management policy](#) for more information.

Incident Response and Reporting - The NIH requires institutions to report incidents involving rDNA materials including loss, theft, or release. This includes both NIH exempt and non-exempt rDNA materials.

1. Report any loss, theft, or release involving rDNA materials to the Occupational and Environmental Safety Office at 919-684-8822.
2. Report any human exposure to rDNA to Employee Occupational Health and Wellness (EOHW, 919-684-8115) and to the Occupational and Environmental Safety Office (OESO, 919-684-8822). Complete the [Report of Occupational Injury or Illness form](#).

Training - A variety of training is essential to ensure good lab practices. OESO training is available at the www.safety.duke.edu (Training & Reports) website.

1. General Laboratory Safety is relevant to all lab workers.
2. Lab-specific orientation and training is provided by the P.I.
3. Biosafety Level 2 (BSL2) training is for those who handle infectious material or [other potentially infectious material](#) (OPIM) that poses a splash, splatter, or percutaneous exposure hazard.
4. Bloodborne Pathogens training is required for those who handle materials of human origin (i.e. primary and well-established cell lines). This training is included in BSL2 training for lab workers.
5. Biosafety Level 3 (BSL3) training for higher containment
6. Plant containment training ([Phytotron website](#))
7. Animal handlers training ([IACUC Website](#))

SELECT AGENTS AND TOXINS

(OESO Select Agent Website: <http://www.safety.duke.edu/biological-safety/select-agents-biological-toxins>)

All Select Agents and Toxins (see Page 25) are ordered and obtained through the OESO - Biological Safety Division 919-684-8822.

Background

On December 13, 2002, new regulations were published to implement the Public Health Security and Bioterrorism Preparedness and Response Act of 2002, Public Law 107-188. The regulations apply to possession, use, and transfer of certain biological agents and toxins, and to recombinant DNA experiments involving those agents and toxins which pose a threat to public health and safety. The regulated materials are referred to as "select agents" and lists of these potential bioterrorist agents have been developed by 21 governmental agencies. The lists are segregated by their potential targets: humans, humans and animals, animals only, and plants only.

How was the [Select Agents and Toxin list](#) determined?

CDC prepared the Select Agents list for 42 CFR 73 after receiving extensive input from scientists representing 21 Federal government entities. The Health and Human Services (HHS) Secretary considered the following criteria for establishing the list:

- The effect on human health of exposure to the agent or toxin;
- The degree of contagiousness of the agent or potency of the toxin and the methods by which the agent or toxin is transferred to humans;
- The availability and effectiveness of pharmacotherapies and immunizations to treat and prevent any illness resulting from infection by the agent or toxin.

Regulations:

- 42 CFR Parts 72 and 73, 42 CFR Part 1003 RIN 0920-AA09 Possession, Use and Transfer of Select Agents and Toxins; Final Rule.
This regulation covers select agents which target humans.
- 7 CFR Part 331 and 9 CFR Part 121 Agricultural Bioterrorism Protection Act of 2002; Possession, Use and Transfer of Biological Agents and Toxins; Final Rule.
This regulation includes the "overlap" list in the HHS regulation, as well as a list of select agents affecting only animals and plants.

Dual Use Research of Concern (DURC)

US Government Policy, effective September 2015

Dual Use Research of Concern (DURC) is life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security. The United States Government's oversight of DURC is aimed at preserving the benefits of life sciences research while minimizing the risk of misuse of the knowledge, information, products, or technologies provided by such research.

Research that directly involves any of the following 15 agents and toxins is subject to the policy*

Avian influenza virus (highly pathogenic)	Marburg virus
Bacillus anthracis	Reconstructed 1918 Influenza virus
Botulinum neurotoxin (<u>in any quantity</u>)	Rinderpest virus
Burkholderia mallei	Toxin-producing strains of Clostridium botulinum
Burkholderia pseudomallei	Variola major virus
Ebola virus	Variola minor virus
Foot-and-mouth disease virus	Yersinia pestis
Francisella tularensis	

**Except attenuated strains of the agents that are excluded from the Select Agent list and inactive forms of botulinum neurotoxin: <http://www.selectagents.gov/SelectAgentsandToxinsExclusions.html>*

Training Requirements

The institution and the Investigators must ensure that all lab personnel conducting research with any of the 15 listed agents have received training on DURC, whether or not the research has been deemed as DURC. **Contact OESO-Biological Safety Division for more information (919)684-8822.**

Who reviews this research for DURC?

- The PI reviews his/her own research with the following concerns in mind:

Is the research DURC?

Does the work...

- Enhance the harmful consequences of the agent or toxin?
- Disrupt immunity or the effectiveness of an immunization against the agent or toxin without clinical and/or agricultural justification?
- Confer to the agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitate their ability to evade detection methodologies?
- Increase the stability, transmissibility, or the ability to disseminate the agent or toxin?
- Alter the host range or tropism of the agent or toxin?
- Enhance the susceptibility of a host population to the agent or toxin?
- Generate or reconstitute an eradicated or extinct agent or toxin listed in the policy?

If yes or probable, then:

- The PI submits the research protocol for review by the [Institutional Review Entity \(IRE\)](#)

Transfer, Receipt, and Storage of Select Agents

Responsibilities and Procedures

“Responsible Official” (RO):

The Final Rule requires that a RO be designated at each institution where select agents are shipped, received, and/or possessed. The Director of the Biological Safety Division of the Occupational and Environmental Safety Office shall serve as Duke’s RO. An alternate may be designated by the RO if deemed necessary. The primary responsibility of the RO is to oversee the registration of the laboratory with the CDC and/or the APHIS, and assure that all requirements of compliance are met. The RO must give final approval of the facility registration application prior to it being submitted to the applicable federal department.

Researcher:

The principal investigator (PI) is held responsible for assuring that he registers all possession, transfer, and receipt of select agents through the Duke University Biological Safety Office. He is also responsible for assuring that his laboratory fully complies with all prescribed safety policies and procedures. Consequently, the PI must work closely with the RO to assure compliance with this standard.

Compliance with the regulations requires that the “Responsible Official” obtain a permit for the procurement, storage, and work with select agents, and that the Principal Investigator agrees to conduct all activities as described in the permit application. The documentation required is described in the regulations, and includes security plans, background checks on those authorized to access select agents, laboratory inspections and inventory recordkeeping.

The CDC / APHIS have developed a helpful website that provides guidance on the Select Agent Rule, helpful FAQs, Required Forms and Resources:

<http://www.selectagents.gov/>

How Do Researchers Register Select Agents?

Researchers planning on handling any of the listed Select Agents or Toxins (*see Page 25*) **must** contact the Duke University Responsible Official (currently the Director of Biological Safety) to begin the registration process with the CDC or APHIS. Call 919-684-8822 for assistance.

What toxins are regulated as select agents and what quantities are exempt?

Work with the toxins listed below is regulated by the [CDC/APHIS](https://www.cdc.gov/aphis/) unless the aggregate amount under the control of a principal investigator does not, at any time, exceed the amount specified. **Although only amounts greater than the maximum permissible limit must be registered with CDC/APHIS, any amount of these toxins must be ordered through the OESO - Biological Safety Division Select Agent Ordering Website: (<http://www.safety.duke.edu/biological-safety/select-agents-biological-toxins>).**

Maximum Permissible Limit

<i>HHS Toxins</i>	<i>Amount</i>
Abrin	100 mg
Botulinum neurotoxins	0.5 mg
Clostridium perfringens epsilon toxin	100 mg
Conotoxin	100 mg
Diacetoxyscirpenol (DAS)	1000 mg
Ricin	100 mg
Saxitoxin	100 mg
Shiga-like ribosome inactivating proteins	100 mg
Shigatoxin	100 mg
Staphylococcal enterotoxins	5 mg
T-2 toxin	1000 mg
Tetrodotoxin	100 mg

HHS AND USDA SELECT AGENTS AND TOXINS
7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73
HHS SELECT AGENTS AND TOXINS

Abrin
 Botulinum neurotoxins*
 Botulinum neurotoxin producing species of *Clostridium**
 Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence X₁CCX₂PACGX₃X₄X₅X₆CX₇)
Coxiella burnetii
 Crimean-Congo haemorrhagic fever virus
 Diacetoxyscirpenol
 Eastern Equine Encephalitis virus¹
 Ebola virus*
*Francisella tularensis**
 Lassa fever virus
 Lujo virus
 Marburg virus*
 Monkeypox virus¹
 Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
 Ricin
Rickettsia prowazekii
 SARS-associated coronavirus (SARS-CoV)
 Saxitoxin
South American Haemorrhagic Fever viruses:
 Chapare
 Guanarito
 Junin
 Machupo
 Sabia
 Staphylococcal enterotoxins A,B,C,D,E subtypes
 T-2 toxin
 Tetrodotoxin
Tick-borne encephalitis complex (flavi) viruses:
 Far Eastern subtype
 Siberian subtype
 Kyasanur Forest disease virus
 Omsk hemorrhagic fever virus
 Variola major virus (Smallpox virus)*
 Variola minor virus (Alastrim)*
*Yersinia pestis**

OVERLAP SELECT AGENTS AND TOXINS

*Bacillus anthracis**
Bacillus anthracis Pasteur strain
Brucella abortus
Brucella melitensis
Brucella suis
*Burkholderia mallei**
*Burkholderia pseudomallei**
 Hendra virus
 Nipah virus
 Rift Valley fever virus
 Venezuelan equine encephalitis virus¹

USDA SELECT AGENTS AND TOXINS

African horse sickness virus
 African swine fever virus
 Avian influenza virus¹
 Classical swine fever virus
 Foot-and-mouth disease virus*
 Goat pox virus
 Lumpy skin disease virus
*Mycoplasma capricolum*¹
Mycoplasma mycoides^{1,2}
 Newcastle disease virus^{1,2}
 Peste des petits ruminants virus
 Rinderpest virus*
 Sheep pox virus
 Swine vesicular disease virus

USDA PLANT PROTECTION AND QUARANTINE (PPQ) SELECT AGENTS AND TOXINS

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

*Denotes Tier 1 Agent

¹ C = Cysteine residues are all present as disulfides, with the 1st and 3rd Cysteine, and the 2nd and 4th Cysteine forming specific disulfide bridges; The consensus sequence includes known toxins α-MI and α-GI (shown above) as well as α-GIA, Ac1.1a, α-CnIA, α-CnIB; X₁ = any amino acid(s) or Des-X; X₂ = Asparagine or Histidine; P = Proline; A = Alanine; G = Glycine; X₃ = Arginine or Lysine; X₄ = Asparagine, Histidine, Lysine, Arginine, Tyrosine, Phenylalanine or Tryptophan; X₅ = Tyrosine, Phenylalanine, or Tryptophan; X₆ = Serine, Threonine, Glutamate, Aspartate, Glutamine, or Asparagine; X₇ = Any amino acid(s) or Des X and; "Des X" = "an amino acid does not have to be present at this position." For example if a peptide sequence were XCCHPA then the related peptide CCHPA would be designated as Des-X.

² A virulent Newcastle disease virus (avian paramyxovirus serotype 1) has an intracerebral pathogenicity index in day-old chicks (*Gallus gallus*) of 0.7 or greater or has an amino acid sequence at the fusion (F) protein cleavage site that is consistent with virulent strains of Newcastle disease virus. A failure to detect a cleavage site that is consistent with virulent strains does not confirm the absence of a virulent virus.

³ Select agents that meet any of the following criteria are excluded from the requirements of this part: Any low pathogenic strains of avian influenza virus, South American genotype of eastern equine encephalitis virus, west African clade of Monkeypox viruses, any strain of Newcastle disease virus which does not meet the criteria for virulent Newcastle disease virus, all subspecies *Mycoplasma capricolum* except subspecies capripneumoniae (contagious caprine pleuropneumonia), all subspecies *Mycoplasma mycoides* except subspecies mycoides small colony (Mmm SC) (contagious bovine pleuropneumonia), and any subtypes of Venezuelan equine encephalitis virus except for Subtypes IAB or IC, provided that the individual or entity can verify that the agent is within the exclusion category. 9/10/13

- ☐ I verify that I do not store or use any of the select agents or toxins listed above.
☐ I verify that I do store or use the following select agents or toxins:

Agent/toxin name; amount stored: _____

Contact name/signature: _____

PI/Lab name: _____

Date: _____

PACKAGING AND SHIPPING BIOLOGICAL MATERIALS

Although several agencies have published regulations or guidelines for the proper packaging and shipment of biological materials, the International Air Transport Association's (IATA) Dangerous Goods Regulations (DGR) governs all international shipments. Furthermore, all air transport of regulated biological materials (including domestic flights) must strictly adhere to the DGR. For this reason, the OESO provided training is primarily focused on compliance with these regulations.

Training

All personnel involved in the process of shipping biological materials must receive proper training initially and at least every two years thereafter. Training is provided through the OESO website's "[online-training](#)" link. The program is titled *Shipping Biological Materials*. The [Training Supplement Guide](#) includes checklists and a summary of the most relevant training content for properly classifying, packing and labeling a shipment. **Note:** The information provided in the Training Supplement Guide may not include all relevant shipping criteria, and are not intended to be used without first completing the official training.

Permitting import or export of agents or vectors of human disease

Importation and exportation of infectious materials and vectors that may contain them is regulated by federal law. When an infectious agent is being imported into or exported out of the United States, it may need to be accompanied by a permit which is issued by the United States Public Health Service (USPHS). Permits are issued only to the importer/exporter that is located in the United States. Permit applications are available through the Duke University Export Controls Office: <https://export.duke.edu/>

The permit, along with the proper packaging and labeling, will expedite clearance of the package of infectious materials through the USPHS Division of Quarantine and release by US Customs.

Import or export of etiologic agents of animals and plant pests

The United States Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) regulates the importation and domestic transfer of agents, which may pose a risk to animals or plants. A permit must be obtained prior to the receipt of any material that could pose a potential risk to animals or plants. The permitting procedures are coordinated through the Duke University Export Controls Office: <https://export.duke.edu/>.

Select Agents

The Department of Health and Human Services' 42 CFR Part 73, titled *Possession, Use, and Transfer of Select Agents and Toxins*, became law on February 7, 2002. All researchers who possess or plan to possess select agents must be registered with the Centers for Disease Control and Prevention. For a list of restricted agents and other Select Agent Program requirements, see the following: <http://www.cdc.gov/od/sap/>

The Director of OESO's Biological Safety Division will serve as Duke's Responsible Official (RO) for select agents. All CDC registrations must be facilitated through the RO. To contact the RO, call 919-684-8822.

Proper Shipment of Non-Regulated Liquids

Provisions must be made to ensure that all non-regulated liquids (i.e. buffers, water, etc.) are properly packaged to prevent leakage during transport. The packaging must be of good quality, strong enough to withstand shocks normally encountered during transport. A triple-packaging system must be utilized. The following must be met.

- Liquid is placed in a **leak-proof primary container**
- **Absorbent material** must be placed around the primary container (sufficient amount to absorb entire contents of primary container)
- Primary container(s) and absorbent material(s) are placed into **leak-proof secondary container**.
- Inner packages (primary and secondary container) are placed into a sturdy outer container (i.e. cardboard box). Cushioning material is added between secondary container and outer shipper if deemed necessary.

Laboratory Biosecurity

Laboratory biosecurity protects the materials used in the lab from loss, theft, or intentional misuse.

Responsibilities:

Principal investigators should take reasonable steps to ensure that their labs are secure by:

- Providing for physical security
 - Lock the laboratory door whenever the lab is left unattended.
 - Determine what materials should be subject to inventory accountability measures and what records should be maintained.
 - Store materials with the highest hazard potential in locked cabinets, refrigerators, etc.
 - Storage equipment (i.e. refrigerator, freezer) that is/are not contained within a space or lab that has restricted access (e.g. hallway) should be fashioned with a lockable device to prevent access by the general public.
- Integrating laboratory security measures into lab specific policies and procedures (i.e. standard operating procedures (SOPs)).
- Personnel Management
 - Identify the roles and responsibilities for employees who handle, use, store, and transport hazardous materials during the process of selection and hiring lab staff.
 - Develop policies for personnel and visitor identification, visitor management, and access procedures.
- Reporting Security Incidents
 - Report incidents or possible incidents, such as undocumented visitors, missing hazardous materials, and unusual or threatening phone calls or behavior to Duke Police 919-684-2444.

The Occupational and Environmental Safety Office (OESO) will:

- Assist in evaluating security risks or developing security measures for the laboratory.
- Assist with the development of SOPs.

REFERENCES

Biological Safety: Principles and Practices; Fleming D, Hunt D, 4th ed., ASM, 2006
Biosafety in Microbiological and Biomedical Laboratories; 5th ed., CDC/NIH, 2007
Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinet
North Carolina Administrative Code 10G.1201-.1207, General Statute 130A-309.26, 1990
Virginia Regulated Medical Waste Department of Environmental Quality, Waste Management Board Regulation: Title 9 VAC 20-120, Regulated Medical Waste Management Regulations; Effective June 19, 2002.
Occupational Exposure to Bloodborne Pathogens, Final Rule; 29CFR 1910.1030, OSHA, 1991
Occupational Health and Safety in the Care and Use of Research Animals
Dangerous Good Regulations; International Air Transport Association
Possession, Use, and Transfer of Select Agents and Toxins; USDHHS, 42 CFR Part 73
North Carolina Administrative Code G.S. 130A-149, Biological Agent Registry

APPENDICES

[OSHA Occupational Exposure to Bloodborne Pathogens Standard](#)
[Duke University Bloodborne Pathogens Exposure Control Plan](#)
[OESO Training Program](#)
[Duke University Institutional Biosafety Committee \(IBC\) Policies and Procedures](#)
[Recombinant DNA FAQs](#)
[Duke University's Select Agent Policy](#)
[Duke University Medical Waste Management Policy](#)
[National Select Agent Registry](#)