

# GW Biosafety & Exposure Control Manual

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**The Office of Laboratory Safety  
Ross Hall B-05-F  
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This document is subject to change. The version on the web will always be the most current version. Please refer to the website periodically to ensure you are using the latest version.

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## INTRODUCTION

### Purpose

The purpose of this manual is to provide policies and procedures for the safe handling of infectious agents and potentially infectious material in order to protect lab workers, the GW and DC communities and the environment from harm by infectious agents and recombinant DNA. These policies are primarily based on the following resources:

- *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) published by The Centers for Disease Control and Prevention(CDC)
- Bloodborne Pathogen Standard from the Occupational Safety and Health Administration (OSHA)
- The Laboratory Biosafety Manual from the World Health Organization (WHO)
- The Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules from the National Institutes of Health (NIH).

### Scope / BBP compliance

The George Washington University (GW) has an exposure control plan to comply with the OSHA bloodborne pathogen standard which applies to all on campus whose job requires potential contact with blood or other potentially infectious material. Those who work in laboratories, however, have special considerations due to the non-routine nature of research and the variety of agents used including agents that are not bloodborne. As a result all laboratory workers, including students, staff, faculty or visitors, who work in labs maintained by GW must comply with this manual. *The content of this manual complies with all aspects of the Bloodborne Pathogen Standard and for lab workers satisfies the requirements of the exposure control plan.* The manual will be reviewed and updated periodically.

### Definitions

*Biological agent* – are microscopic organisms such as bacteria, viruses, fungus, yeast, mold, protozoa and prions that have the ability to adversely affect human health. In this context, potentially infectious higher eukaryotes such as hookworms, flukes etc. are also considered.

*Infectious agent (pathogens)* – are biological agents that can cause disease in healthy human adults and are assigned biosafety level 2 or higher. While infection does not necessarily lead to disease symptoms, the term is generally used to describe disease causing agents.

*Potentially infectious materials* – are human blood or other body fluids and mammalian cells or tissues that may potentially carry bloodborne pathogens or other infectious agents.

*Recombinant DNA (rDNA)* – are 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 the molecules that result from the replication of those cells.

*Select Agents* – are those infectious agents and toxins listed by the Centers for Disease control (CDC) and the United States Department of Agriculture (USDA) that could be used for purposes of terrorism against humans, animals, or plants.

### Organization

GW has appointed a Biosafety Officer (BSO) to direct the biosafety program for the GW campuses as well as research areas in the Medical Faculty Associates and the George Washington University Hospital. The BSO is in the Office of Laboratory Safety (OLS) in B-05 Ross Hall. The university has also established an Institutional Biosafety Committee (IBC). The purpose of the IBC is to review proposed work involving recombinant DNA, pathogens or select agents (see IBC charter online) and also to advise the BSO on biosafety program policy.

## Responsibilities

To protect workers from biohazards including recombinant DNA as well as to comply with applicable regulations and guidelines, the following responsibilities apply.

**Biosafety Officer** – It is the responsibility of the BSO to do the following: conduct periodic inspections of laboratories whose work is subject to IBC review; serve as a resource to campus on biosafety compliance issues; ensure proper reporting is done when required by the NIH/OBA with regard to recombinant DNA, Serve as administrator of the IBC; provide required training; ensure manuals and other program documents are updated as needed.

**Authority** – The BSO has been approved by the Associate Vice President for Research to administer the biosafety program for GW. The BSO may enter any space at any time to ensure compliance.

**Principal Investigators** – It is the responsibility of the Principal Investigator to do the following: comply with this manual; ensure that all those working in their lab comply with this manual; establish specific procedures for your lab and make sure all workers have access to procedures; ensure that all workers are aware of the hazards present in the lab and the precautions to be taken; ensure that all those working in the lab are trained for the procedures they perform and are proficient at those procedures (this involves completion of a *training documentation sheet* for each worker); prepare any required SOPs or protocols as required by this document or the IBC; timely submission of all covered research to the IBC for review; supervise lab operations to ensure proper technique and containment are achieved; inform workers of any entry requirements that exist for that lab and ensure that they are achieved.

Report the following to the BSO immediately:

- Breach of containment for rDNA such as escaped animals or microorganisms, or a spill, outside of containment (ie: BSC) that cannot be easily and quickly cleaned up by one person. Any spill in a BSL3 facility, which is outside of containment, must be reported.
- Any worker exposure of rDNA to mucus membranes or open skin or inhalation of aerosols and any potential exposure at BSL2 enhanced laboratories.
- Any illness likely caused by rDNA exposure
- Workers or PIs that willfully violate protocols or conduct work without prior IBC approval.

**Lab workers** – It is the responsibility of all those who work in a biological research laboratory to do the following: comply with this manual, follow the procedures and requirements established by their Principal Investigator; report all major spills and incidents (listed above) to their Principal Investigator or the BSO; consult with their physician if they have a condition that places them at increased risk in the lab; attend all required training.

## 1 HAZARDS OF INFECTIOUS AGENTS

### 1.1 Risk factors

There are several factors that influence how and to what extent an infectious agent can cause disease.

- **Host range** – Refers to which species can be infected by the organism. Those that affect humans as well as animals are considered zoonotic. Live animals as well as other species of cells can harbor pathogens that are infective to humans.
- **Virulence** – Refers to the severity of the disease caused by the agent and how likely those infected are to recover.
- **Infective dose** – Refers to how many organisms are required to initiate infection. Some agents require a few organisms to cause infection; *Giardia lamblia* has been reported from ingestion of one cyst. Other organisms, such as anthrax, require thousands of infective forms to cause infection.
- **Mode of transmission** – Following are the four modes of transmission:
  - Ingestion – Eating or drinking the infective form (many times inadvertently from poor hygiene)
  - Inhalation – Breathing the infective form in an aerosol

- Injections – Puncture of the skin
- Contact – Splash, spray or contact with infected hands or other objects to open skin or mucous membranes
- Communicability – How likely is the agent to spread between hosts. This is very contingent on other factors such as mode, stability and infective dose.
- Stability in the environment – Refers to how well the infective form can survive in the environment. Some forms, such as spores, cysts and prions, can be very resilient while other forms are easily deactivated.

## 1.2 Symptoms

Exposures can happen with out anyone’s knowledge so it is important to be aware of symptoms. The symptoms from an infection can be almost anything including: headache, dizziness, jaundice, fever, sweat, pain, stomach trouble, swelling of lymph nodes, etc

## 2 RISK ASSESSMENT

In order to determine how to safely handle an agent, a risk assessment must be performed. The risk of an agent is how likely it is to cause infection and if infection occurs how likely it is to cause serious harm or death. Potential harm to the community or environment is a major consideration as well.

### 2.1 Determining the Initial Risk of Agent

The NIH guidelines for research with recombinant DNA as well as the World Health Organization’s biosafety manual have very similar systems for assessing risk by placing agents into one of four “risk groups”. The table below from the NIH summarizes these groups. Each increasing risk group indicates increasing danger to individuals and the community based on the factors listed in section 1. Agents in Risk Group 2 or higher are considered pathogens.

Table 2.1 – Risk groups

Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are <i>often</i> available
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions <i>may be</i> available (high individual risk but low community risk).
Risk Group 4 (RG4)	Not permitted at GWU

From NIH guidelines Appendix B - Table 1 - Basis for the Classification of Biohazardous Agents by Risk Group (RG)

This table is for general application but many common agents are listed by name according to risk group in appendix B of the NIH guidelines.

With regard to recombinant DNA, when DNA from a pathogenic agent is inserted into a non-pathogenic agent the newly produced agent must be initially considered the same risk as the source until the risk assessment is complete which may raise or lower the risk. If DNA for an insertion is completely fabricated from raw materials and not taken from an organism, it must be considered the same risk as the agent with the most similar code. Viral DNA that comprises equal to or greater than two thirds of the genome of the wild type must be regarded as the same as the wild type agent. If the viral DNA is less than two thirds then it is usually considered defective and can possible be handled as if it were Risk group 1, however, the final decision is with the IBC.

Note: The risk of an agent is to be reassessed when there are substantial changes to research. See the IBC website and the IBC charter for more information.

## **2.2 Other Contributing factors that affect risk**

It is important to consider other information when assessing the risk of an agent as well as the particular work being considered. A determination of effective treatment for the agent and if it is available locally needs to be done. Is there a method of prophylaxis (i.e.: vaccine) available? With any work, consideration should also be given to whether there is the potential for aerosols to be generated or if work will involve large volumes or high concentrations. Will animals be involved; will sharps be used or will materials be transported down halls or room to room.

When recombinant DNA is involved there are special concerns: Will the new DNA insertion increase or decrease virulence, pathogenicity, infectious dose, environmental stability, host range, cell cycle or replication capacity? Will the insertion encode for an oncogene, integrate into the host genome or generate replication-competent viruses? Are there biological barrier options available (i.e.: attenuation) that would limit any of these characteristics and thus reduce risk? Options that would reduce risk should be considered and used if feasible and if there are processes that will increase risk then it will be determined if these are absolutely necessary. Once these other factors have been considered, the appropriate containment can be selected for that risk which may be lower, higher or the same.

Since risk to pathogens is “based on the potential effect of a biological agent on a healthy human adult” worker attributes must be considered. People can be at higher risk of disease and the severity of disease due to their circumstances such as preexisting diseases, medications, compromised immunity, pregnancy or breast feeding (which may increase exposure of infants to some agents). This is handled by providing adequate communication of hazards to workers (covered in section 3.2.3 below).

## **2.3 Containment:**

Section III of the BMBL defines four biosafety Levels (BSL) for working with biological agents; only 1-3 could be potentially used at GW. Risk groups (RG), covered in 2.1, are for the classification of agents, while biosafety levels are for classifying practices, equipment and facilities. In general (but not always), a BSL is used to contain the same RG (i.e.: BSL2 = RG2). This is similar to inmates and prisons, namely, a high risk inmate is kept in a high security prison.

Considering the original risk group and the risk assessment, you can now decide what corresponding containment level is appropriate for an agent. For instance, if you are using a RG2 pathogen but the agent is attenuated so that it cannot cause disease, then it may be appropriate to reduce the risk to RG1 and thus use BSL1 as containment. The biosafety levels are summarized in section III of the BMBL as shown in table 2.2.

As seen in Table 2.2, each biosafety level has criteria for physical containment (equipment and facilities) and work practices. Variation may be appropriate depending on circumstances. For instance, after the hazard assessment one may conclude that, for a particular agent, it is appropriate to conduct work at BL2 facilities but with the practices of BL3. The appropriate physical containment and practices to protect workers must be selected, including when this requires special requirements or procedures. It should be noted that BSL1 is not the same as non-biological labs since standard microbiological practices must be observed even at this low risk level. Standard microbiological practices are listed in section III of the BMBL and are covered in detail in this manual. All labs that handle biological agents must be designated a biosafety level that is appropriate to the agents or materials used and follow the appropriate guidelines. For assistance with the assignment of a biosafety level please contact OLS or the BSO.

There are also agent summaries given in section VII of the BMBL which give the appropriate containment level for agents with regard to varying circumstances. Since Risk Groups and Biosafety Levels are many times the same it may be instructive to look at the agent summaries when determining the initial Risk Group of an agent.

Table 2.2 – CDC biosafety levels

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults	Standard Microbiological Practices	None required	Open bench top, sink required
2	Associated with human disease, hazard = percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: <ul style="list-style-type: none"> <li>• Limited access</li> <li>• Biohazard warning signs</li> <li>• "Sharps" precautions</li> <li>• Biosafety manual defining any needed waste decontamination or medical surveillance policies</li> </ul>	Primary barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPEs: laboratory coats; gloves; face protection as needed	BSL-1 plus: <ul style="list-style-type: none"> <li>• Autoclave available</li> </ul>
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences (BSL2 enhanced is currently the highest level at GWU)	BSL-2 practice plus: <ul style="list-style-type: none"> <li>• Controlled access</li> <li>• Decontamination of all waste</li> <li>• Decontamination of lab clothing before laundering</li> <li>• Baseline serum</li> </ul>	Primary barriers = Class I or II BSCs or other physical containment devices used for all open manipulations of agents; PPEs: protective lab clothing; gloves; respiratory protection as needed	BSL-2 plus: <ul style="list-style-type: none"> <li>• Physical separation from access corridors</li> <li>• Self-closing, double-door access</li> <li>• Exhausted air not recirculated</li> <li>• Negative airflow into laboratory</li> </ul>
4	Not permitted at GWU			

From BMBL section III, table 1

General guide for Biosafety Level designation:

**BSL1:** For all biological labs using materials or agents that are not infectious such as non-pathogenic agents. (plants or blood, tissue or bodily fluids from non-mammals)

*Note: If using exotic plants or agents that are plant pathogens please contact OLS before procuring these items as there may be federal regulations pertaining to them.*

**BSL2:** For biological labs that use infectious agents such as pathogens or substances known to be infected with pathogens. Including labs that use potentially infectious agents such as human or other mammalian blood, tissues or body fluids or any substance known to be infected with these items.

**BSL2 enhanced:** This is a term used for higher containment that uses the facilities of BSL2 but incorporates many of the practices and equipment of BSL3 (See section 3.5 for more information)

**BSL3:** For work performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure.

#### 2.4 Institutional Biosafety committee (IBC)

The IBC was established to review all work involving the following (these are defined in the introduction section):

- recombinant DNA
- human, animal and plant pathogens (BSL-2 or higher)
- select agents and biological toxins
- human blood, tissues, organs, cell lines and other potentially infectious materials

ANY WORK WITH THESE TYPES OF AGENTS, REGARDLESS OF FUNDING, MUST BE APPROVED BY THE IBC BEFORE WORK CAN BEGIN. To initiate IBC review of research, a PI must submit a completed IBC registration form along with a research description form. All documents must be submitted electronically. Other documents may be required as well depending on the type of work. The IBC will conduct a full risk assessment and assign containment and class of research if conducting rDNA work. The IBC may prescribe special requirements if deemed necessary. More information about the IBC and the submission process can be accessed on the IBC website at: <https://labsafety.gwu.edu/institutional-biosafety-committee>

**Table 2.3** - Research categories for rDNA research and the approvals needed.

Level	Approval/Review	Requirements
III-A	NIH Dir, RAC, IBC <sup>†</sup>	A drug resistant gene transferred into a (new) microorganism.
III-B	NIH/OBA, IBC <sup>†</sup>	The cloning of toxin molecules with LD <sub>50</sub> < 100 ng/kg of body weight.
III-C	IRB, IBC <sup>†</sup>	rDNA (or DNA or rDNA derived from rDNA) transferred into humans.
III-D	IBC <sup>†</sup>	rDNA transferred to or from: whole animals, whole plants (high risk work) and associated small animals, experiments involving >10 Liters of culture, agents listed in Risk Groups 2, 3, or 4, or infective eukaryotic viruses in cell culture.
III-E	IBC <sup>§</sup> - most common	rDNA involving: eukaryotic viruses (not more than 2/3 genome) in cell culture, whole plants (low risk work) and associated small animals, arthropods, or generation of transgenic rodents (BSL1), any work not covered in the other categories (most non-pathogenic rDNA work)
III-F	IBC <sup>§</sup> - may not need full committee review	rDNA: not in organism or virus, entirely from a single viral source, from single prokaryotic host (including indigenous plasmids & viruses) used only in that host, from single eukaryotic host (excluding viruses) used only in that host, natural exchangers (appendix A), does not pose a significant threat to health or environment (appendix C), breeding of transgenic rodents at BSL1.

<sup>†</sup> Approval required before initiation.

<sup>§</sup> Notify IBC (register) when project is initiated. IBC approval is still required.

*Note: for work to qualify as category III-F it must satisfy the specific criteria in the standard for this special status and must still be submitted to the IBC using the registration form.*

### 3 CONTROLLING HAZARDS

Hazards can be controlled by four main layers of protection: universal precautions, administrative controls, safe practices, primary and secondary barriers.

#### 3.1 Universal Precautions

The practice of *Universal precautions* is the approach where human blood and other body fluids are treated as if infectious since there is the possibility that they could be. **Universal precautions are to be followed at all biosafety levels.** Other human body fluids that could possibly be infective are called **potentially infectious materials (PIM)**. Examples are:

- Cerebrospinal fluid
- Amniotic fluid
- Semen
- Synovial fluid
- Pleural fluid
- Pericardial fluid

At GW all mammalian cells and tissues are also considered potentially infectious due to the possibility of zoonotic disease. Also, all patient specimens are considered PIM since even fluids such as saliva may have blood in them. **As a result all blood and PIM (which includes mammalian cells and patient specimens) are to be handled at BSL2 on the GW campus.** In this manual PIM will refer to any material that is potentially infectious or known to be infectious.

#### 3.2 Administrative controls



It is very important for management at all levels to encourage and enforce safe practices. Investigators must promote a positive safety culture in their labs so that what is written is carried out in practice and safety is an expectation along with quality.

### **3.2.1 Inspections**

Self-inspections – Each lab is encouraged to periodically use a checklist to ensure safety is being practiced and that the appropriate materials and controls are in place.

Office of Laboratory Safety (OLS) inspections – Rooms will be inspected by OLS periodically to ensure that work is being done in compliance with the determined biosafety level as well as to ensure other requirements are being kept such as the proper use of protective equipment and proper disposal of biohazardous waste. Inspections may be unannounced. All labs subject to IBC review will be inspected at least annually but may be inspected more frequently if risk is higher. The inspection form is available in appendix F.

### **3.2.2 Standard Operating procedures (SOPs)**

SOPs must be written for all procedures involving pathogens. Viruses containing more than two thirds of the genome are considered pathogens, including viral vectors. While all work must be done according to standard practices in this manual, it is important to establish handling requirements that are specific to the agent and the lab. The SOP should be based on the risk assessment performed when the work was submitted to the IBC for review. SOPs do not have a required length but need to address PPE, waste, primary containment, disinfection, storage and procurement at a minimum. Anyone who works with pathogens must read and be familiar with the SOP.

### **3.2.3 Communication of hazards**

It is the responsibility of the principal investigator to communicate to workers the hazards present in the lab and for the work they will be performing. If a pathogen is present in the lab, all workers must be aware of: the route of exposure, the nature of the disease and symptoms of the disease. Those who work with human blood or body fluids or mammalian cells must be aware of the potential for zoonotic diseases. Those who work with rDNA should be aware of any potential hazards associated with that research. Each person must consider their particular situation and if they are in a condition that puts them at higher risk such as compromised immunity, pregnancy, etc., they should talk to their doctor. It may be appropriate for those at higher risk to wear additional protective equipment or to even refrain from doing some tasks all together; they should discuss these concerns with their PI, supervisor, or BSO. Those who believe that they are, or will be, at increased risk of infections can also receive confidential advice from the BSO (Ross B-05, 4-8258). For those who work in research and research support free consultations are provided, please contact OLS for details.

### **3.2.4 Controls priority**

When attempting to control hazards PIs and supervisors will have to make administrative decisions to best accomplish this. Once safe practices are instituted it is important to choose the best primary barriers and ensure appropriate secondary barriers are in place. Engineering controls (e.g. directional ventilation, biosafety cabinets, centrifuges with safety caps, and disinfectant traps) must be used first to control hazards. After these controls are in place, personal protective equipment and work practices are used to minimize hazards in conjunction with the engineering controls.

To comply with the BBP standard from OSHA, the IBC, with input from non-managerial employees, will consider commercially available safer medical devices and may implement new devices if deemed appropriate. This evaluation will take place at least annually.

### **3.2.5 Building transport**

Biological samples must be transported according to the requirements of section 3.3.5 anytime samples are carried in a non-lab environment, including hallways, stairs, and elevators. Since the outside container is not contaminated, no gloves are to be worn. In this way those transporting will not contaminate public use items such as elevator buttons, door handles, stair rails, phones, etc.

### 3.3 BSL1 standard microbiological practices

Microbiological standard procedures are to be followed at all biosafety levels.

#### 3.3.1 Access Control

- Access is limited to those who work in the lab or those who have a need to be there. Access must be limited when working with viable organisms containing rDNA or viable organisms.
- Children are not allowed in lab areas. Anyone under the age of 18 must go through biosafety training prior to entering an active lab space.

#### 3.3.2 Sharps handling

Safety handling of sharps is always important to avoid injury.

- Sharps containers must be easily accessible to those using them, kept upright and not overfilled.
- Sharps containers must be OSHA compliant: closeable, puncture resistant and leak proof on sides and bottom and have a biohazard label on it.
- As soon as possible after use, contaminated sharps must be placed directly in a sharps container for disposal.
- Do not alter a needle in any way such as bending or cutting. Do not remove a needle from a syringe but place the entire assembly in a sharps container.
- Where feasible, use needle-less systems or needles with engineered injury protection.
- DO NOT RECAP NEEDLES. In the rare event that recapping is necessary; it must be approved by the BSO and be performed with a one handed method such as placing the cap in a holder or scooping the cap off of a flat surface. When removing the cap again for reuse, loosen cap with hands overlapping to avoid a rebound needle-stick then remove the cap. Please see OLS for more details.
- Use plastic materials instead of glass when possible (i.e.: pipettes)
- Sharps can be anything that can puncture the skin such as: hypodermic needles, scalpels, glass slides, razor blades, Pasteur pipettes, etc. Non-contaminated broken glass can go in a regular sturdy box marked broken glass for the dumpster.

*Note: see appendix D for waste handling procedures*

#### 3.3.3 Hygiene

Developing good hygiene habits is important regardless of risk since the dynamic nature of research means the type of work can change quickly and increase risk while habits take longer to change.

- Decontaminate equipment and surfaces at a frequency appropriate for the biological material involved and immediately after spills
- Wash hands after handling biological material and after removing gloves or other PPE. If a sink is not available in a biohazard room then hand sanitizer must be used until a sink can be accessed.
- Do not wear protective equipment in non-lab areas
- Do not eat, drink, chew gum, smoke, apply cosmetics or lip balm or handle contact lenses in the lab.
- Fridges, freezers, microwaves, or anything else to be used with food that are located in break areas in close proximity to the lab must be labeled "for food only" and similar devices in labs must be labeled "no food".
- It is recommended to use bench paper for bench or BSC work for easy cleanup.
- Only mechanical pipetting is permitted.
- To avoid contamination, long hair must be tied up and large dangling jewelry or draping clothes cannot be worn.

#### 3.3.4 Containers and Labeling

- All primary containers with biological materials (including rDNA) such as test tubes, petri dishes, flasks, etc., must be labeled with contents.
- When biological samples are transported according to section 3.2.4, they must be carried in a container that is leak proof on bottom and sides such as a plastic tray or bin and which is free of outside contamination.
- When sharps are stored or transported the container must be puncture resistant as well. (If it is waste then they must be put in a sharps container according to section 3.3.2)

### 3.3.5 Containers and Labeling

- All primary containers with biological materials (including rDNA) such as test tubes, petri dishes, flasks, etc., must be labeled with contents.
- When biological samples are transported according to section 3.2.5, they must be carried in a container that is leak proof on bottom and sides such as a plastic tray or bin and which is free of outside contamination.
- When sharps are stored or transported the container must be puncture resistant as well. (If it is waste then they must be put in a sharps container according to section 3.3.2)

### 3.3.6 Protective equipment and primary containment

Primary containment is the same as primary barrier devices.

- Work with cultures or rDNA, that may generate aerosols, must be performed in a BSC (see section 3.6.2) or other primary containment. Such activities include: sonicating, vortexing, grinding, blending and shaking.
- Work with cultures or rDNA at high volumes (> 10 liters) must be conducted in a BSC or other primary containment or in a closed system.
- Protective laboratory coats, gloves and eye protection (see section 3.6.3) are required to prevent contamination and exposure.
- When centrifuging comply with section 3.6.4 for safe use.

### 3.3.7 Minimizing aerosols

The inhalation of airborne contaminants is one of the most difficult routes of exposure to protect against, and as such, prevention of aerosols is paramount. Even if agents are not inhaled deep into the lungs they can contact mucus membranes in the upper respiratory tract and may lead to infection there. Aerosols with large droplets can also leave residue contamination on lab surfaces and objects and pose a contact hazard.

- Avoid mixing with pipette suction and expulsion and avoid expelling the last drop from pipettes
- Pipette liquid down the side of a tube or beaker to avoid splashing
- Use disposable transfer loops or a microincinerator
- Wrap tubes with tin foil for transport to reduce spatter if dropped
- Use gauze to extract needles from bottles
- Avoid over-pressurizing a bottle when extracting liquid

### 3.3.8 Disinfection

- Surfaces must be disinfected after work is complete or at the end of the day and tools, equipment, glassware, etc. must be disinfected after use. Overt spills must be cleaned up and surfaces disinfected immediately.
- Equipment must be disinfected before servicing or shipping. If disinfection is not feasible, the portions of the equipment that are contaminated must be labeled and this must be communicated to those who will be encountering the equipment.
- Protective coverings such as foil, bench paper, plastic etc. must be removed when overt contamination occurs and bins, pails and other containers must be periodically disinfected.
- The disinfectant must have appropriate action for the agent used and have sufficient contact time to kill agents. If you are unsure about the right contact time for liquid waste than use one hour in 10% bleach.

Table 3.1 - Disinfectants

Disinfectant	Positives	Negatives	comments
<b>Chlorine</b> (bleach, Clidox)	Broad activity, kills hardy organisms, inexpensive, quick kill	Inactivated with organic matter, corrodes metals	1:10 dilution most common. Make fresh before use. Irritant, corrosive
<b>70% ethanol</b>	Wide activity, inexpensive, noncorrosive	Evaporates quickly, poor contact time, not sporicidal	Flammable
<b>Iodophors</b> (Wescodyne, Betadyne)	Broad activity, low toxicity	Staining, limited activity in organic matter	Corrosive, irritant
<b>Phenolics</b> (Lysol, Metar)	Broad activity, maintain activity in organics	Not sporicidal	Corrosive, irritant
<b>Quaternary Ammonium</b> (Roccal Plus, Novalsan)	Contains detergent, low toxicity	Not sporicidal, limited activity in hard water, organic matter	"User friendly"

*Note: see appendix D for waste handling procedures*

The most effective way to disinfect materials or kill agents is autoclaving.

### Autoclave Use

Autoclaves are the best way to decontaminate materials and can kill even very resilient forms such as spores with correct run time and temperature. Any waste with known pathogenic material that can be transmitted by the contact or aerosol route must be autoclaved immediately and then put in regulated medical waste boxes. The following rules apply to autoclaves:

- Be sure to set correct run time and temperature for the agent in use.
- Half fill liquid containers, loosen caps.
- Loosely close bags and add water to dry loads.
- Leave space for steam to circulate and trays to catch moisture.
- Do not autoclave hazardous materials such as corrosives or flammables.
- Allow heat to dissipate when opening and use insulated gloves and face shield for removal.
- Use indicators such as autoclave tape.
- Make sure units are certified and in date.

*Note: see appendix D for waste handling procedures*

## 3.4 BSL2 practices

These practices are in addition to the practices of BSL1 and supersede them if indicated.

### 3.4.1 Access Control

- Animals or plants not involved with the work being performed are not permitted in the lab
- If a lab works with a pathogen and a vaccine is available, the vaccine must be made available to those who work in the lab free of charge. The IBC or the PI may require workers to have the vaccine to work in the lab.
- If a lab has special entry requirements these requirements must be posted on the entrance to the lab.

### 3.4.2 Containers and Labeling

- Containers with PIM, when not in use, must be kept in secondary containment, such as a tray, cabinet, fridge or freezer which is free of outside contamination and has a biohazard symbol attached (see section 5 for label requirements)
- Equipment that contains or is used with PIM must be labeled with a biohazard symbol. This may include: centrifuges, incubators, shakers, etc.

**3.4.3 Protective equipment and primary containment**

Primary containment is the same as primary barrier devices (see section 3.6 for more information)

- All work with pathogens must be conducted in a BSC or other primary containment. If BSC work is not feasible, the risk must be reduced to an acceptable level with additional protective equipment and approved by the BSO.
- Protective coats or gowns must always be worn in the lab
- Protective gloves and eye protection must be worn when working with PIM, even if using a BSC or other primary containment.
- When centrifuging pathogens (this includes human viruses with greater than 2/3 of the genome), use high quality plastic tubes with screw caps. Tubes must be placed in safety cups with screw caps or a sealed rotor

**3.4.4 Proficiency**

- Workers must be proficient before working with any pathogen; this determination is made by the principal investigator over the lab and must be documented on the workers training documentation sheet (see appendix C).

**3.5 BSL2 Enhanced practices**

BSL2 conditions are for agents and materials not associated with an aerosol route of exposure, however, when these agents are used at research quantities and/or concentrations they may pose an aerosol risk. As a result BSL2 containment may have some BSL3 practices and equipment employed to offset this risk. BSL2 enhanced containment has been divided into three categories and certain types of work may be assigned to a category depending on the level of risk (A is for highest risk).

**Table 3.2** - Types of work that require BSL2 enhanced containment and which category they belong to. Work may belong to more than one category in which case the highest containment must be used. Final determination of the risk of an agent or rDNA insert is made by the IBC:

Work with patient samples known to be infected with HIV, HBV, HCV or a similar bloodborne pathogen	C
Work with research concentrations of wild type HIV, HBV, HCV or a similar bloodborne pathogen	A
Work with research concentrations HIV, HBV, HCV or a similar bloodborne pathogen which has been attenuated but is greater than 2/3 of the genome.	B
Work with <u>high</u> risk viral vectors containing greater than 2/3 of the genome	B
Work with <u>moderate</u> risk viral vectors containing greater than 2/3 of the genome (this would include third-generation lentiviral vectors and most adenoviral vectors.	C
Work with a viral vector, infectious to humans (even if stripped of its pathogenicity), containing a high risk insert	C

*Note: those who work with HIV or HBV must read the informational sheets in appendix A or B.*

**Table 3.3 - BSL2 Enhanced Containment categories**

Practices	A	B	C
BSC use required for all manipulations involving potentially infectious material (this includes any mammalian blood or cells)	x	x	x
BSC not congested and no storage of disposable items	x	x	x
No sharps unless approved by the IBC.	x	x	x
Vacuum lines protected with traps and HEPA filters	x	x	x
Tips and pipettes disinfected or bagged in cabinet	x	x	x
Written Standard Operating Procedures must be available (workers must be trained according to the SOP)	x	x	x
An autoclave must be available.	x	x	
Doors must be closed when work is in progress	x	x	
Workers must be authorized by the PI using the training documentation sheet in appendix C. The form must be up to date and made available to safety if requested.	x	x	
Only plastic screw-cap vials are to be used	x	x	
Only centrifuges with sealed rotors or capped buckets are to be used	x	x	
Only what is needed for experiment in BSC. All items are to be disinfected and removed upon completion.	x		
Only a disposable smock that fastens in the back with cotton cuffs is to be used	x		
Workers must double glove with outer glove pulled over cuff. Outer glove must be removed before removing arms from the cabinet	x		
All items must be wiped with disinfectant before removal from the cabinet	x		

### 3.6 Primary Barriers

Primary barriers provide physical protection from biological substances.

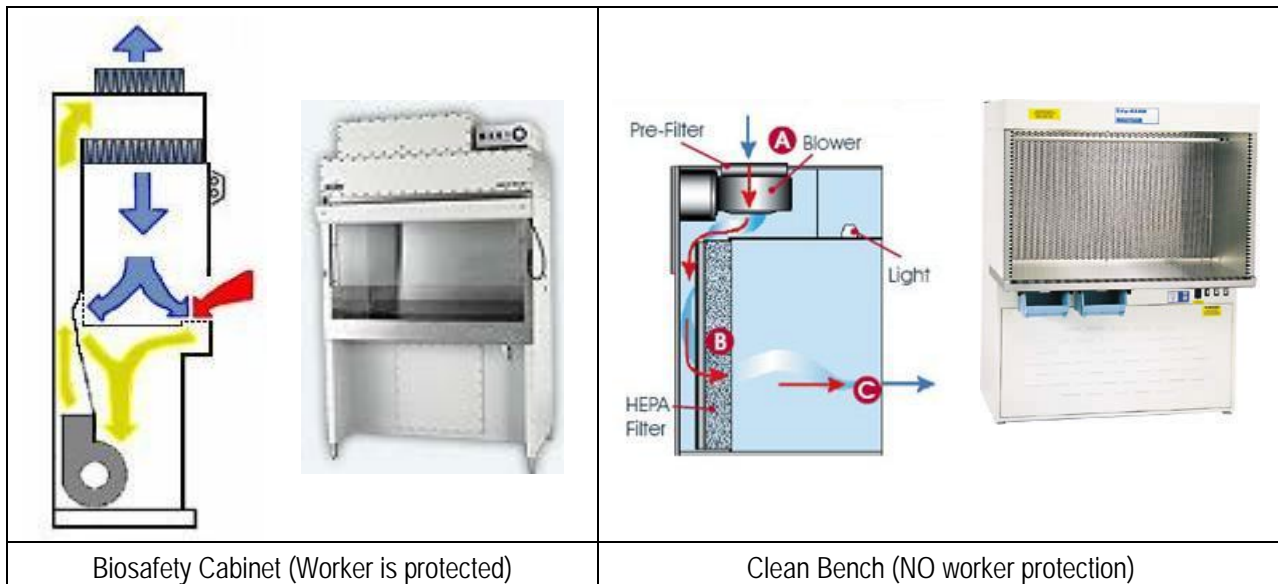
#### 3.6.1 Introduction to hoods & cabinets

Biosafety cabinets (BSCs) are devices engineered to protect the person from exposure as well as the environment. One of the most common types of BSCs is the Class II, A2. This cabinet draws air from the room through the sash and recirculates a portion of it while expelling part of the air back into the room both through HEPA filtration. The air that is recirculated flows down onto the work surface from above in a laminar fashion to reduce turbulence, and is then captured again by splitting between two intakes, the rear intake and the front grill. In this way the cabinet protects the worker from exposure; it protects the work from contamination and by filtering the exhaust it protects the environment.

Clean benches are devices designed only to protect the work from contamination and not to protect the worker. It passes room air through a HEPA filter and then in a laminar flow passing it over the work and past the person. It is important to never use hazardous or potentially infectious materials in these devices since they are not safety devices and in fact direct the flow of air directly into the worker's breathing zone.

Fume hoods are also common in labs and are designed to protect the worker but not the work and have no filtration. Fumes hoods are only to be used with hazardous chemicals.

Figure 3.1 – BSC versus laminar bench



### 3.6.2 Biosafety Cabinet Use

For proper worker protection the following apply:

- Disinfect the cabinet and purge the cabinet for at least 3 minutes before use if it was not previously running. It is; however, better to leave the hood running if possible. Ensure that it is working properly and certified.
- Introduce all needed materials into cabinet before working.
- Set a work flow, usually left to right and from clean to dirty, and observe all standard microbiological practices.
- Keep waste in the hood and do not constantly removal it while working. The waste can then be removed later at one time in a container to the main waste container. For pipettes and similar items there should be a container such as a tray for soaking in disinfectant.
- Do not block the rear or front air intake vents as this will compromise the cabinet's ability to capture impurities.
- Use slow, direct movements to avoid disturbing airflow and also avoid disturbing the air around the cabinet while others are working by walking past them or opening and closing doors.
- Make sure diffusers in the ceiling are not blowing directly into the cabinet's opening. If this is the case call OLS.
- Avoid the use of open flames.
- Decontaminate and remove all items and decontaminate the BSC when finished, usually with 70% alcohol.

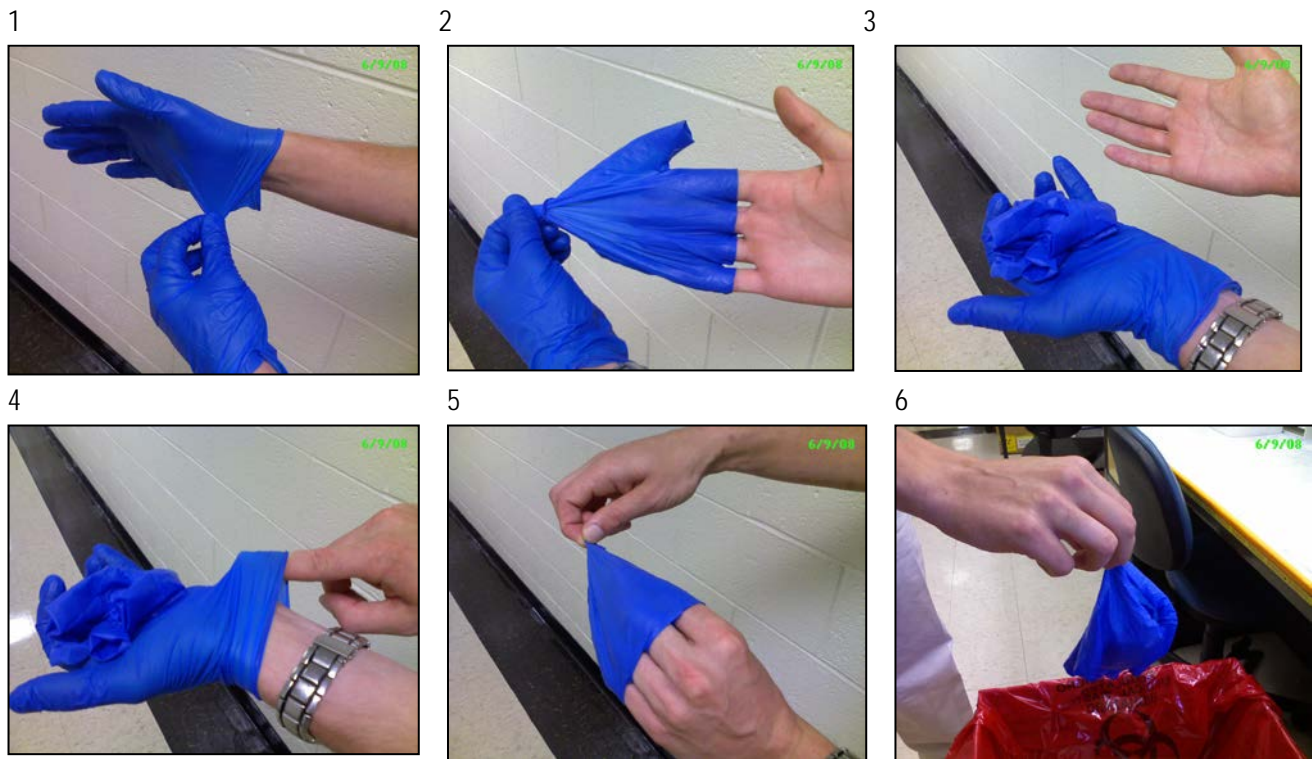
### 3.6.3 Personal Protective Equipment (PPE)

Once the hazards of a process are determined (see SOPs above) you must select the appropriate protective equipment to minimize the hazards. PPE is to be furnished at no cost to the employee and to be worn when contamination is reasonably anticipated. When conducting any work in a BSL2 lab (including rDNA), the minimum protective equipment includes: lab coat, eye protection and gloves. PPE must be worn even if using primary containment, such as a BSC. Minimum PPE is highly recommended for BSL1 and eye protection is required when the potential for splashes or aerosols exist.

- Lab coats – lab coats or gowns protect the worker from splashes. Lab coats must not be worn in non-lab areas such as offices or break areas. If they are contaminated, they must be decontaminated and they must be periodically laundered at no cost to the employee (see section 13).
- Eye protection –to protect from sprays and splashes and should have side shields. When using larger amounts of material where splashes are more likely, the added protection of goggles or a face shield is required.
- Gloves – Disposable gloves such as exam gloves must be discarded if contaminated or damaged. Exam gloves should be changed frequently and never reused. If hazardous chemicals such as phenol or formaldehyde are involved then nitrile gloves can be used for chemical protection since latex and vinyl provide poor chemical resistance. If using heavier, non-disposable gloves, they must be thoroughly decontaminated before re-use. Latex gloves can cause allergies for some people and can be replaced with vinyl or nitrile. Double gloving is extra

protection and also allows the worker to discard the outer glove in time sensitive situations and keep working. Gloves are to be removed properly as shown below.

Figure 3.2 – Proper removal of exam gloves



- Respirators – It should be determined if respirator use is required or voluntary for an operation (OLS can help with this determination). If someone uses a respirator they must be in the respiratory protection program managed by The Office of Health & Safety. Voluntary use of dust masks only requires the person to be provided appendix D from the OSHA respiratory protection standard. Training and fit testing is recommended.
- Other PPE may be needed such as shoe covers, head covers, boots, arm covering or even whole body suits when gross contamination is reasonably anticipated.
- PPE cannot be taken home and must be disinfected periodically and immediately when contaminated.
- Only close toe shoes are to be worn in labs and no draping clothes or dangling jewelry.

### 3.6.4 Centrifuge Use

- Ensure tubes are not flawed or cracked and match and balance tubes
- Only use rotors designated by the manufacturer and use according to the manufacturer specifications. Keep a log of rotor use and “retire” rotors according to manufacturer recommendation.
- If a tube breaks, close the lid and wait 30 minutes allowing aerosols to settle, then disinfect and clean.
- Ensure rotors are not defective or cracked and keep them clean, dry and disinfected.

## 3.7 Secondary Barriers

### 3.7.1 Basic Lab design

- Rooms should be high quality labs where bench tops are impervious to water and resistant to hazardous chemicals such as acids.
- Lab furniture must be sturdy and allow access for cleaning around and behind.
- Labs should be planned in a way so that workers do not have to travel through non-lab areas such as classrooms or break areas. All rooms in a lab suite are to be for lab support (offices are OK).



- Labs must be easily cleanable with no absorbent items such as rugs or fabric covered chairs.
- Biosafety cabinets should be located away from high traffic areas and where room ventilation is not disrupting the capturing ability of the cabinet.
- An American National Standards Institute (ANSI) compliant eyewash station is required for BSL2 and up and is recommended for BSL1.
- The ventilation should put the room under negative pressure causing directional air flow so that air flows into the room from the adjacent halls and offices. If people in adjacent rooms complain of odors, this is an indication that the lab may not be under negative pressure. OLS can help determine if there is a problem.
- Each main room in a suite should have a sink for hand-washing and preferably located near the door.
- Wall penetrations should be sealed around fixtures.
- In BSL2 labs and higher, vacuum lines must be protected with liquid disinfectant traps and High Efficiency Particulate Aerosol (HEPA) filters.
- Rooms need to be well illuminated.
- In BSL2 labs and higher, labs must have lockable doors for security.
- All rooms or suites that use biologicals must have the biohazard symbol checked on the hallway placard.
- All individual rooms that use pathogens must have a biohazard posting on the door.

## 4 REGULATED MEDICAL WASTE (BIOWASTE)

### 4.1 Red bag waste

The following items must be disposed of in Red bag medical waste according to appendix D.

- All blood or blood soaked items and any other PIM. Large quantities of blood must be solidified or completely absorbed into a non-hazardous absorbent such as paper towels or vermiculite before disposal. Small quantities of sealed glass vials with blood can go directly into red bags but substantial quantities should go in a sharps container.
- Mammalian cells or tissues, microbiological cultures or recombinant DNA.
- Items that appear to be biological or medical in nature such as used gauze, bench paper, gloves, stained towels or Petri dishes..
- Animal carcasses. If they have traces of formaldehyde from preservation they can go into red bags but no liquid formaldehyde can be present or dripping . Animal carcasses should be deposited in ARF freezers for packaging by ARF facility staff. Please contact OLS for details.
- All dry waste that is known to contain a pathogen that is transmitted by the aerosol route must be autoclaved before being picked up as biowaste.

### 4.2 Sharps disposal

- All contaminated sharp items (ie: needles, broken glass, scalpels, razor blades, Pasteur pipettes) go into an OSHA compliant sharps container and never directly into a red bag. All needles go into a sharps container even if unused or capped. Once the sharps container is ready for disposal it must be closed and put into a red bag. When disposing of many glass vials that are likely to break please use a sharps container.

*Note: Refer to appendix D of this manual for instructions for disposal of biowaste.*

### 4.3 Liquid waste

- Liquid contaminated with PIM, cultures or rDNA must be disinfected with an appropriate disinfectant for sufficient contact time to kill agents then drain disposed if non-hazardous.
- Use large amounts of water before, during and after disposal and pour near drain to prevent splattering.

### 4.4 Not biowaste

- No regular waste (cold trash) such as uncontaminated notebook paper or packaging but only biological waste.
- No hazardous waste such as mercury thermometers, phenol, formaldehyde, benzene, etc.

- Broken glass or large sharp items that are NOT contaminated or potentially contaminated should be put in a sturdy box, sealed and taken to the dumpster.

## 5. BIOHAZARD SYMBOL

The biohazard symbol must have a red-orange field with symbol and lettering in a contrasting color.



Figure 5.1 – Biohazard Symbol

## 6 EMERGENCY

Emergency procedures must be posted prominently in the lab area

### 6.1 Spills and Exposures

For spills or exposures to biological agents or rDNA, follow the instructions in appendix E. This appendix must be posted in all biological laboratories. Become knowledgeable about what to do in your circumstances depending on your location. Emergencies must always be reported to OLS.

All exposures to recombinant DNA must be reported to OLS even if in a BSL1 lab. This is important for reporting requirements to the NIH/OBA.

Symptoms (especially different than a common infection) should not be ignored and if they persist, the person should get medical attention. Anyone who works with a pathogen must be aware of the symptoms of that particular agent and get medical treatment at the earliest indication. Also, if you observe symptoms of disease in a fellow worker, confirm this with the individual and encourage medical attention and report it to the PI and OLS.

### 6.2 Eyewashes

All BSL2 or higher labs must have an eyewash station immediately available to areas where hazards are used; a suite must have one in the main room. Eyewash stations must be ANSI approved, in working order, inspected annually and tested monthly. All units must be attached to the building's potable water source. Squeeze bottle types are not acceptable and are not ANSI approved. The following gooseneck sink mount units are acceptable at GW (the units below are available at [www.labsafety.com](http://www.labsafety.com))

- 109424 Bradley® Faucet-Mount Eye Wash, Gooseneck 1 lb.
- 98240 GUARDIAN EQUIPMENT EyeSafe™ A Gooseneck Faucet-Mount Eye Wash

To use – Immediately turn on the faucet and place eyes in the stream while adjusting the temperature to be warm but not hot. Hold your eyelids open and slightly lift your eyelids to allow water to get underneath. Roll your eyeballs around to give maximum irrigation. Continue rinsing for 10 – 15 minutes then get medical attention.

### 6.3 Post-exposure evaluation & follow-up

The exposed employee will have access to an evaluation and follow-up by a licensed healthcare professional, at no cost and may do this during work hours. The medical professional will: document the route and circumstances of the exposure and duties of the exposed employee. Also, the medical professional will identify, document and test the source individual, if feasible and legal, and the exposed employee will receive all test results obtained and be informed of all related laws and regulations concerning the identity of the source. Post-exposure prophylaxis may be indicated and if so will be offered. The employee will receive counseling and evaluation for any illnesses. The employer must provide to the medical professional any medical records they have that are relevant to the exposure. The medical professional must send a written opinion to

the employer stating that the exposed worker has received all information, counseling and services required while keeping all other information confidential. This report must be given to the exposed employee within 15 days.

All biological exposures on campus are to be reported to the BSO in a timely manner to allow for an investigation of exposures. Changes are made as needed to the program to better protect against potential exposures based on these investigations.

## **7 HEPATITIS B VACCINE**

For employees whose job requires them to come into contact with blood or other potentially infectious material, the Hepatitis B vaccine will be available for free at Student Health, during work hours by a licensed health care professional, after they have received training. The vaccine is not needed if: the person has already been successfully vaccinated for Hepatitis B, if testing shows they are immune, if it is contraindicated for medical reasons or if they decline. Those who wish to not have the vaccine may be exempt by signing the declination form and may still get the vaccine free of charge in the future if they change their mind for any reason and at any time. The Hepatitis B vaccination kit is available from OLS or from The Office of Health & Safety. OLS highly recommends getting the vaccine due to the possibility of contracting HBV in a medical research setting. Immunity received from successful vaccine administration dramatically reduces the risk of contracting the disease. The vaccine is not free to students but OLS highly recommends that students, who are at risk, receive the vaccine.

Note: information sheets for Human Immunodeficiency Virus (HIV) and Hepatitis B are in appendix A and B of this manual respectively.

## **8 TRAINING**

Those who work in biological laboratories must attend OLS biosafety training before they may work with infectious or potentially infectious material and within 2 months of entering a lab to work. Training is at no cost and during work hours and must be received at least annually thereafter. Please visit the OLS website for times and locations.

Those who work in the Ross 704 facility must attend high containment training before gaining access.

## **9 SECURITY**

Access to biohazard labs is to be limited only to those who have a need to be there such as research workers, visitors, safety personnel, regulators, emergency personnel, maintenance personnel, etc. When work involves pathogens, doors must be closed to limit traffic and to maintain the negative pressure airflow design of the ventilation system. If someone unknown enters the lab, the lab workers should politely engage them to ensure they are in the right place. All persons in Ross Hall must prominently wear a GWorld identification badge or a visitors badge and visitors must be escorted to the room. When pathogens are present and nobody is in the lab, lab doors must be locked or stocks of the agents must be locked such as in a cabinet or fridge.

## **10 LIVE ANIMALS**

Any use of live vertebrate animals must be approved by the Institutional Animal Care and Use Committee (IACUC) and procured by the Animal Research Facility (ARF). Contact the ARF for more details at 202-994-2871. Smaller animals, generally considered vectors, such as fleas, ticks or flies, must be approved by the IBC (see IBC webpage online). No animal may be brought into a lab without the proper approval in advance. When handling ARF approved animals in lab rooms, all procedures and requirements from the ARF must be followed. At a minimum, gloves and other PPE must be worn when working with any animals.

Those who handle animals and those who work in an animal facility must read and sign the Animal Exposure Registry Form (AER form). This form is designed to help those covered, determine if they may be at increased risk due to a particular condition and inform them of how to get free medical advice. Those labs not in the ARF but where animals are housed for 12 hours or more are considered animal facilities. All those who work in these labs must read and sign the AER form.

## 11 HUMAN RESEARCH

All research involving human subjects must be approved by the Institutional Review Board (IRB) before any work can begin. This includes work with recombinant DNA, administering drugs, drawing blood and administering questionnaire. Contact the Office of Human Research for more details at 202-994-2715.

## 12 SHIPPING BIOLOGICAL SUBSTANCES

Biological substances must be shipped in accordance with the International Air Transport Association (IATA) regulations. Those that ship biologicals must have IATA training and must ensure packages are compliant. Only a trained person can sign for an outgoing shipment; please contact OLS before shipping any biological substances if you are not trained. If biological substances are shipped into the country or out of the country there may be restrictions or a permit or license may be required depending on the substance and origin or destination. Please contact OLS before shipping out of the country or arranging to receive a shipment from outside of the country. Anyone transporting a package by vehicle, they must first complete courier training. Please contact OLS before shipping or receiving any exotic plants or any plant pathogens.

## 13. LAUNDRY

Lab coats and other protective garments that become contaminated must be cleaned by using a laundering company that provides services for biological contamination or by using a method such as autoclaving and at no cost to the employee. Contaminated garments must be kept in designated areas near where they are used and that is well marked and prevents spreading the contamination. Laundry must be handled with minimum agitation. It is the responsibility of each department to arrange for laundering. Lab wear must not be taken home.

## 14 ROSS 704 FACILITY

Ross 704 is a BSL3 facility that is currently operating at BSL2 enhanced, category A. All work with research concentrations of HIV, HBV, HCV or HTLV are currently conducted in Ross 704.

### 14.1 Access

The Ross Hall 704 facility is currently being used primarily for those who have research conducted at BSL2 enhanced category A. All proposed research must be reviewed by the Institutional Biosafety committee who will conduct a comprehensive risk assessment and determine if the work requires this level of containment. Any significant change to an approved protocol must be reviewed by the IBC so containment determinations can be reassessed and the protocol amended. Following are the requirements to gain access to a pod in the 704 facility:

- *Each worker must have read the biosafety manual and be familiar with the 704 requirements.*
- *Each worker must have attended high containment training.*
- Each worker must be authorized by their PI using the training documentation form in appendix C. By signing this form the PI certifies that the worker is proficient at the tasks to handle the agents either by previous experience or by training in the lab. This also certifies that the worker has read and knows the Standard Operation Procedure for the techniques they will perform.
- Once these requirements have been satisfied, the BSO will inform Health & Safety that the employee has completed all requirements and OLS will give the worker an access code for entry.

Note: If a person only wants to have access to the common areas of 704 (BSL2) to use equipment then high containment training is not required and that person can contact OLS for access.

### 14.2 Definitions:

**BSL3:** This biosafety level combines facilities, equipment and practices to attain containment for handling pathogens that have an aerosol route of exposure. This level is currently not used at GW.

**BSL2 enhanced:** This biosafety level is usually defined as working in BSL2 facilities while using some of the practices and/or equipment for BSL3. There is much variation in what practices and equipment are used and depends on the institution and the particular work being done. Requirements are found in section 3.5.

### **14.3 Facility description & containment**

**Entrance room [BSL1]** – This room is only for entry. Visitors must sign in and be escorted.

**Exit room [BSL1]** – For washing hands and receiving waste from the pass-through autoclave if applicable.

**Common area [BSL2]** – The common area is BSL2 and has the common use equipment. Lab coats are required here. This room contains the following equipment:

- chemical fume hood
- two -80C freezers
- super-speed centrifuge
- ultra-speed centrifuge in a biosafety cabinet enclosure
- pass through autoclave

**Cold room [BSL2]** – The cold room is BSL2 and is for short term cold storage of samples. The room temperature is set at 3.6C(38F) with a range of 0C to 4C.

**Warm room [BSL2]** – The warm room is BSL2 and is for short term warm storage of samples. The room temperature is set at 37C(98F) with a range of 30C to 40C.

**Pods [BSL2 enhanced category A]** – Each pod is assigned to a particular PI. The PI is solely responsible for maintaining their room and reporting facility issues to Facilities Management if repairs are needed to the facility. For issues with equipment please contact OLS.

Each pod contains the following equipment:

- Class II, Type A/B3 biosafety cabinet (with thimble connections)
- under-counter CO2 incubator
- under-counter refrigerator

Any other equipment than what is listed above must be purchased by the PI. All equipment in the pod, regardless of owner, must be kept clean and in good working order and must comply with the requirements of BSL3 enhanced, category A containment.

**Note:** Anyone with access to the common area and cold and warm rooms may use the equipment as well as the cold and warm rooms. These areas have BSL2 containment and practices and those using them must comply with this manual.

### **14.4 Ventilation**

The facility has directional air flow to ensure air flows from areas of least containment to areas of highest containment. The pods operate at 30 air changes per hour and the commons at 10. All air is 100% dedicated exhaust (no recirculation) and HEPA filtered.

### **14.5 Entrance / Exit**

To access the facility, use your code and biometrics to access the entrance room. Visitors must sign in, always be accompanied by someone with access and they must have a need to be there. When entering the common area a lab coat must be worn. Do not bring in items that are absorbent and minimize the amount of books or papers that are brought in as well.

To exit the facility you must use your code. In the exit room you must wash your hands before leaving regardless of what you have touched.

## **14.6 Emergency**

### **14.6.1 Spill response in pods**

In the event of a spill in containment such as a BSC or centrifuge, you may clean it up yourself if you feel comfortable doing so but you can always call OLS to get help with any cleanups. If the spill is outside of containment, immediately remove your outer gloves and exit the pod. Check yourself carefully to make sure you were not contaminated. If you were contaminated, follow the procedure below. If you were not contaminated use the telephone to notify OLS at 4-2630.

- Do not leave the common space and stay in a small area outside the door to minimize spreading contamination. Carefully remove your protective equipment and place it in a red biohazard bag. Use the telephone to call OLS for assistance.
- If there is no answer at the office number, call the mobile phone numbers on the list. In the unlikely event nobody can assist, leave a message for OLS, reporting what happened, then notify UPD at 4-6111.
- Remove any contaminated clothing and put it in the red bag. If your shoes are contaminated, disinfect them with chemical spray disinfectant before removal as well as the floor anywhere you have stepped.
- Disinfect your skin with wipes if you were exposed.
- Don a lab coat, gloves and eye protection and disinfect anything that may have been contaminated (in the common area) such as the doors, telephone, stools, counters, etc.
- Do not re-enter the room but contact Lab Safety as soon as available who will assist in the cleanup later. Also post a sign on the door of the pod barring access to others. Also, fill out an incident report.
- If you were exposed with potentially infectious material such as by any contact to skin or by inhalation go to Employee Health at the hospital for treatment. If you are injured, go immediately to the emergency room at the hospital.

### **14.6.2 Spill clean-up procedure**

If cleared by Lab Safety you can clean up the spill using the following method:

- Don a Tyvek suit, foot covers, gloves (double glove - outside gloves covering the cuffs) and eye protection.
- Enter the room and use spray disinfectant anywhere there is contamination and a sizable margin around it to ensure any splattering is covered.
- Clean from outside (less contaminated areas) first and work toward the center (most contaminated areas).
- Put all waste and cleaning materials in a red bag and keep applying more disinfectant until everything has been thoroughly disinfected and cleaned.
- Once the spill is cleaned, perform a general decontamination of all surfaces in the room.
- Remove your protective equipment in-side-out by first removing your shoe covers, then the outside gloves, then your suit and finally the inside gloves. Put all used protective equipment in a red bag for disposal.

### **14.6.3 Alarm**

In the event of an alarm please evacuate the facility after securing hazardous materials according to the following procedure:

- Proceed in a brisk manner but remain calm
- Cap or cover all infectious agents
- Put all contaminated items such as tips, tubes, pipettes, etc. in a bleach bath and if not submerged, spray with disinfectant thoroughly.
- Take off outer gloves and close the sash on the BSC, exit the pod and remove smock and other protective equipment.
- Exit the facility washing your hands on the way (no need to dry them)

## HIV/AIDS: The Basics

### Key Points

- HIV is the virus that causes HIV infection. AIDS is the most advanced stage of HIV infection.
- HIV is spread through contact with the blood, semen, pre-seminal fluid, rectal fluids, vaginal fluids, or breast milk of a person with HIV. In the United States, HIV is spread mainly by having anal or vaginal sex or sharing injection drug equipment, such as needles, with a person who has HIV.
- Antiretroviral therapy (ART) is the use of HIV medicines to treat HIV infection. People on ART take a combination of HIV medicines (called an HIV regimen) every day.
- ART is recommended for everyone who has HIV. ART can't cure HIV infection, but HIV medicines help people with HIV live longer, healthier lives. HIV medicines can also reduce the risk of HIV transmission.

### What is HIV/AIDS?

HIV stands for human immunodeficiency virus, which is the virus that causes HIV infection. The abbreviation "HIV" can refer to the virus or to HIV infection.

AIDS stands for acquired immunodeficiency syndrome. AIDS is the most advanced stage of HIV infection.

HIV attacks and destroys the infection-fighting CD4 cells of the immune system. The loss of CD4 cells makes it difficult for the body to fight off infections and certain cancers. Without treatment, HIV can gradually destroy the immune system and advance to AIDS.

### How is HIV spread?

The spread of HIV from person to person is called HIV transmission. HIV is spread only in certain body fluids from a person who has HIV. These body fluids include:

- Blood
- Semen
- Pre-seminal fluid
- Vaginal fluids
- Rectal fluids
- Breast milk

HIV transmission is only possible through contact with HIV-infected body fluids. In the United States, HIV is spread mainly by:

- Having anal or vaginal sex with someone who has HIV without using a condom or taking medicines to prevent or treat HIV
- Sharing injection drug equipment (works), such as needles, with someone who has HIV

The spread of HIV from a woman with HIV to her child during pregnancy, childbirth, or breastfeeding is called mother-to-child transmission of HIV. For more information, read the *AIDSinfo* fact sheet on [Preventing Mother-to-Child Transmission of HIV](#).

You can't get HIV by shaking hands or hugging a person who has HIV. You also can't get HIV from contact with objects such as dishes, toilet seats, or doorknobs used by a person with HIV. HIV is not spread through the air or in water or by mosquitoes, ticks, or other blood-sucking insects.

### **How can I reduce my risk of getting HIV?**

To reduce your risk of HIV infection, use condoms correctly every time you have sex, limit your number of sexual partners, and never share injection drug equipment.

Also talk to your health care provider about pre-exposure prophylaxis (PrEP). PrEP is an HIV prevention option for people who don't have HIV but who are at high risk of becoming infected with HIV. PrEP involves taking a specific HIV medicine every day.

HIV medicines, given to women with HIV during pregnancy and childbirth and to their babies after birth, reduce the risk of mother-to-child transmission of HIV. In addition, because HIV can be transmitted in breast milk, women with HIV who live in the United States should not breastfeed their babies. Baby formula is a safe and healthy alternative to breast milk and is readily available in the United States.

### **What is the treatment for HIV?**

Antiretroviral therapy (ART) is the use of HIV medicines to treat HIV infection. People on ART take a combination of HIV medicines (called an HIV treatment regimen) every day.

ART is recommended for everyone who has HIV. ART prevents HIV from multiplying, which reduces the amount of HIV in the body (called the viral load). Having less HIV in the body protects the immune system and prevents HIV infection from advancing to AIDS. ART can't cure HIV, but HIV medicines help people with HIV live longer, healthier lives.

ART also reduces the risk of HIV transmission. A main goal of ART is to reduce a person's viral load to an undetectable level. An undetectable viral load means that the level of HIV in the blood is too low to be detected by a viral load test. People with HIV who maintain an undetectable viral load have effectively no risk of transmitting HIV to their HIV-negative partner through sex.

### **What are the symptoms of HIV/AIDS?**

Within 2 to 4 weeks after infection with HIV, some people may have flu-like symptoms, such as fever, chills, or rash. The symptoms may last for a few days to several weeks. During this earliest stage of HIV infection, the virus multiplies rapidly.

After the initial stage of infection, HIV continues to multiply but at very low levels. More severe symptoms of HIV infection, such as signs of opportunistic infections, generally don't appear for many years. (Opportunistic infections are infections and infection-related cancers that occur more frequently or are more severe in people with weakened immune systems than in people with healthy immune systems.)



Without treatment with HIV medicines, HIV infection usually advances to AIDS in 10 years or longer, though it may advance faster in some people.

**HIV transmission is possible at any stage of HIV infection—even if a person with HIV has no symptoms of HIV.**

### **How is AIDS diagnosed?**

Symptoms such as fever, weakness, and weight loss may be a sign that a person's HIV has advanced to AIDS. However, a diagnosis of AIDS is based on the following criteria:

- A drop in CD4 count to less than 200 cells/mm. A CD4 count measures the number of CD4 cells in a sample of blood.

OR

- The presence of certain opportunistic infections. Although an AIDS diagnosis indicates severe damage to the immune system, HIV medicines can still help people at this stage of HIV infection.

This fact sheet is based on information from the following sources:

- From CDC: [HIV Basics](#)
- From the Department of Health and Human Services (HHS): Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection: [Introduction](#)
- From the National Institute of Allergy and Infectious Diseases (NIAID): [HIV/AIDS](#)

# Hepatitis B Fact Sheet

## Key Points

- Hepatitis B is a liver infection caused by the hepatitis B virus (HBV).
- HBV is spread through contact with the blood, semen, or other body fluid of a person who has HBV.
- Among adults in the United States, HBV is spread mainly through sexual contact. According to the Centers for Disease Control and Prevention (CDC), approximately 10% of people with HIV in the United States also have HBV. Infection with both HIV and HBV is called HIV/HBV coinfection.
- People with HIV/HBV coinfection should be treated for both infections.

## What is hepatitis B?

Hepatitis B is a liver infection caused by the hepatitis B virus (HBV). The abbreviation HBV can stand for either the virus or the infection it causes. HBV can be a short-term (acute) or a long-term (chronic) illness:

- Acute HBV occurs within 6 months after a person is exposed to HBV. In some people, acute HBV can lead to chronic HBV.
- Chronic HBV is a lifelong disease. Without treatment, chronic HBV can cause liver cancer or liver damage that leads to liver failure.

HBV is a contagious infection that can spread from person to person.

## How does HBV spread from person to person?

HBV is spread through contact with the blood, semen, or other body fluid of a person who has HBV. Among adults in the United States, HBV is spread mainly through sexual contact. HBV can also spread from person to person in the following ways:

- By sharing needles or other injection drug equipment (works) with someone who has HBV
- By sharing razors, toothbrushes, or similar personal items with someone who has HBV
- From contact with the blood or open sores of a person who has HBV
- From an accidental prick or cut from an HBV-contaminated needle or other sharp object
- From a mother who has HBV to her child during childbirth

## Can HBV infection be prevented?

Yes. The best way to prevent HBV infection is to get the hepatitis B vaccine. This vaccine is offered at no charge in the GWU Hospital.

## What are the symptoms of HBV infection?

Some people with acute HBV don't have symptoms. But some people can have signs of HBV soon after becoming infected. Symptoms of acute HBV can include the following:

- Loss of appetite
- Tiredness

- Nausea
- Vomiting
- Fever
- Abdominal pain
- Dark urine
- Clay-colored bowel movements
- Joint pain
- Jaundice (yellowing of the skin or the whites of the eyes)

Most people with chronic HBV don't have any symptoms and may not have symptoms for many years. Abnormal results on liver function tests may be the first sign of chronic HBV infection.

### **What is the treatment for HBV?**

In general, HBV is treated with antiviral medicines. The medicines work to help limit damage to the liver.

People with HIV/HBV coinfection should be treated for both infections. Some HIV medicines are effective at treating both HIV and HBV.

The choice of medicines to treat HIV/HBV coinfection depends on the person. For example, some people may take HIV medicines that are also effective at treating HBV. Other people may take HIV medicines and an HBV antiviral medicine. If you have HIV/HBV coinfection, talk to your health care provider about the best medicines for you.

This fact sheet is based on information from the following sources: From CDC:

- [Hepatitis B Questions and Answers for the Public](#)
- [HIV and Viral Hepatitis](#)
- [Travelers' Health: Hepatitis B](#)



## Regulated Medical Waste procedures for GW

### Biological waste - YES

- All blood or blood soaked items (large quantities of blood must be solidified, sealed vials with blood are OK). Also, other human body fluids or patient specimens.
- Mammalian cells or tissues, microbiological cultures or recombinant DNA.
- Items that appear to be biological or medical in nature such as used gauze, bench paper, gloves, stained towels or Petri dishes.
- Animal carcasses. If they have traces of formaldehyde from preservation they are OK but no liquid formaldehyde can be present

### Bio waste - sharps

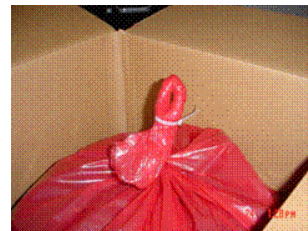
- All contaminated sharp items (ie: needles, broken glass, scalpels, razor blades) go into an OSHA compliant sharps container and never directly into a red bag. All needles go into a sharps container even if unused or capped. Once the sharps container is ready for disposal it must be closed and put in a red bag. When disposing of many glass vials that are likely to break please use a sharps container.

### Bio waste - NO

- No liquids: liquids should be disinfected and drain disposed (except blood). Liquid soaked items are OK but please include extra absorbent such as paper towels or vermiculite.
- No regular waste such as uncontaminated notebook paper or packaging but only biological waste
- No hazardous waste such as mercury thermometers, phenol, formaldehyde, or benzene
- Broken glass or large sharp items that are NOT contaminated or potentially contaminated should be put in a sturdy box and sealed for regular trash

### Bags must be:

- Only red bags provided by vendor
- Not leaking
- Closed (zip-tied/taped – see pictures)
- No punctures (if pipettes are causing leaks they must go in a sharps container)



### Boxes must be:

- Only boxes provided by vendor
- In good condition with no signs of leaking.
- 40 lbs or less (overweight will be repacked by lab)
- Marked with: a) room # b) date c) PI name
- Boxes will be taped shut on pickup

### Pick-ups

- If boxes or bags are not acceptable, they will not be picked up (A note telling the problems to correct will be left on the box)
- Repeat violations will be reported
- If not in Ross Hall, please contact [safety@gwu.edu](mailto:safety@gwu.edu) to schedule a pickup
- If in Ross Hall, place box outside lab with sealed bag. No boxes can be in the hallway unless the bag is sealed.

### Emergency and Other

- No red bags, sharps containers or bio-boxes are to go into regular trash even if they are empty.
- If there is a spill or exposure, follow emergency procedures and call the Office of Laboratory Safety at 4-2630 or Health & Safety at 4-4347. Also, contact OLS with any concerns with service or supplies.

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## Emergency Procedures

### Biological Spills

The Principal Investigator must establish detailed spill cleanup measures tailored to the biological agent(s), amounts, and procedures used in the lab. The proper spill response material must be immediately accessible. Basic equipment is concentrated bleach, paper towels, household rubber gloves and forceps to pick-up broken glass. The following procedures should be utilized as a basis for cleaning spills of biological materials including bloodborne pathogens, potentially infectious agents, and recombinant or synthetic nucleic acid molecules.

#### • Small Biological Spills (< 1 liter)

- If the spill occurred inside a biological safety cabinet, close the sash and allow the cabinet to operate for 15 minutes before continuing with the spill cleanup.
- If the spill has a pathogen with an aerosol route of exposure, leave the room immediately and allow the aerosols to dissipate for 15 minutes.
- Alert people in area of spill and limit the access.
- Remove any contaminated protective clothing and place it in the biohazard waste.
- Don the appropriate personal protective equipment (PPE), which include a lab coat, gloves, eyes and face protection.
- Cover the spill with paper towels or any other absorbent material.
- Carefully pour disinfectant (10-20%v/v bleach) on the spill working from the outside toward the center.
- Allow sufficient contact time with the disinfectant (usually 15-20 minutes).
- Pick up towels and discard into the biohazard waste. Do not use hands if glass or other sharps are involved in the spill. Use forceps.
- Pick up broken glass with forceps and dispose of it in a Sharps container.
- Re-wipe the spill area with disinfectant.
- Remove the PPE and dispose of in the biohazard waste.
- Wash your hands with soap and water.
- Notify your supervisor of the incident.

#### • Large Biological Spills

- Alert lab personnel in the laboratory to the spill and keep people out of the area to prevent spread of the contamination.
- Check if you have been contaminated or if any of your PPE has been breached. If so follow exposure procedures.
- Remove any contaminated clothing and place it the biohazard waste.
- Wash your hands and post a sign on the door.
- Notify your supervisor of the incident and call OLS (4-8258) for assistance.
- If the situation involves an imminently life-threatening injury or has catastrophic potential, call 911.

## Emergency Procedures

### Biological Exposures

To prevent exposures to blood or potentially infectious materials, always use Universal Precautions by wearing gloves, goggles, and other barrier devices. However, if your skin, mucous membranes or your eyes become exposed you should:

- **CUTS:** Immediately apply pressure with a clean paper towel directly on the laceration. Go to a nearby sink and flush the open wound with copious amounts of water. Apply additional direct pressure until all of the bleeding has stopped. Then, apply antiseptic and a sterile dressing.
- **PUNCTURES:** A puncture wound should instead be made to bleed immediately to better wash out the wound. Wash with copious amounts of water, apply antiseptic to the surface of the puncture and a sterile dressing.
- **SPLASHES OR INDIRECT CONTACT:** Immediately flush the affected area with water and then wash with soap and water. Eyes can be flushed at an eye wash station.

Report **ALL** injuries to the PI immediately and seek medical evaluation, treatment, and post exposure follow-up at the **Employee Health Office at GWU Hospital (900 23rd St., NW, Suite G-1090, Phone: 202-715-4275)**. Students should go to the **Students Health Office at Marvin Center**. After hours treatment can be received at the **GWU hospital emergency room**.

In addition, **ALL** injuries or potential hazard exposure (cuts, puncture, etc) should be reported to the Office of Risk Management at [risk@gwu.edu](mailto:risk@gwu.edu) IMMEDIATELY FOLLOWING THE OCCURRENCE. If the exposure involves a bloodborne-pathogen or recombinant DNA please submit a copy of the report to the Office of Laboratory Safety at [labsafety@gwu.edu](mailto:labsafety@gwu.edu).

## Biosafety Level One/Two (BSL-1/2) Inspection Checklist

GW's research and teaching laboratories that work with biohazards are required to have annual inspections by Office of Laboratory Safety. These inspections are used to evaluate the implementation of appropriate laboratory principles and practices, identify any deficiencies, and provide guidance to assist lab personnel with creating a safer laboratory environment.

This inspection checklist is designed to help reduce potential exposures to biohazards. Biohazards may include: Agents that can infect and/or cause disease in humans, animals, or plants; biohazardous waste; experimentally-infected animals and animals naturally harboring zoonotic infectious agents; genetically-modified organisms; human blood, tissue, organs, cell lines, or other materials of human origin; recombinant and synthetic nucleic acid molecules; select agents and toxins; and transgenic plants and animals.

Please use this checklist to perform a self-inspection of your own laboratory. If you have any questions, please contact [labsafety@gwu.edu](mailto:labsafety@gwu.edu).

### Lab Information

<b>PI:</b>	<b>Inspection Date:</b>	<b>Inspected By:</b>
<b>Lab Location (Bldg/Rm)</b>	<b>College/Department:</b>	<b>Lab Phone:</b>
<b>Lab Representative:</b>	<b>Biosafety Level :</b>	
<b>Lab Members:</b>		
<b>IBC Protocols:</b>		
<b>List of Agents that will be Used/Stored in Lab (List recombinant DNA, bacterial, viral, fungal, parasitic, prion, toxic, or other agents):</b>		

### Biosafety Manual

<b>Questions:</b>	<b>Yes</b>	<b>No</b>	<b>N/A</b>	<b>Reference(s)</b>
1. Does your Biosafety Manual contain a current copy of GW's <a href="#">Biosafety Manual</a> ?				GW's Biosafety Manual
2. Does it contain lab contact information?				GW's Biosafety Manual
3. Does it contain documented lab-specific training/manual?				GW's Biosafety Manual
4. Does it contain emergency procedures, spill procedures, and exposure procedures?				GW's Biosafety Manual



### Biosafety Manual (Cont.)

Questions:	Yes	No	N/A	Reference(s)
5. Does it contain a copy of approved IBC paperwork?				GW's IBC policy
6. Does it contain copies of training certificates for all lab members?				GW's IBC policy
7. Does it contain lab specific SOP's?				GW's Biosafety Manual
8. Is it accessible to all lab members?				GW's Biosafety Manual

### Required Trainings

Questions:	Yes	No	N/A	Reference(s)
9. Have all lab members completed the Biosafety & Bloodborne Pathogens Training? <b>*Required for all individuals working with biohazard agents, toxins, and recombinant and synthetic nucleic acid molecule experiments or materials and for all individuals having occupational exposure to human blood, OPIM of human origin (cells/cell lines, unfixed tissues) or human BBP. <u>Required annually by OSHA.</u>*</b>				29 CFR 1910.1030
10. Have all lab members completed the in-person <a href="#">Laboratory Safety Training</a> provided by HEMS? <b>*Required annually for all individuals working in a laboratory*</b>				GW requirement
11. Have all members completed the <a href="#">Biosafety Cabinet Training</a> provided by the CDC? <b>*Required for all individuals working in a Biological Safety Cabinet (BSC). Required once.*</b>				GW OLS requirement
12. If, you have an IBC protocol have all lab members completed the <a href="#">NIH/IBC Guidelines training</a> ? <b>*This training is required every 5 years for all lab members listed on an IBC protocol*</b>				NIH Guidelines
13. Biological Shipping Training? <b>*Required if shipping biological materials and/or dry ice. <u>Required for one lab member every two years by IATA.</u>*</b>				IATA 1.5.0.3
14. N-95/PAPR Fit Test? <b>*Required for all individuals assigned to projects requiring the use of respiratory protection. <u>Required annually by OSHA*</u> Training is provided by HEMS.</b>				29 CFR 1910.134 App A
15. Lentiviral Vector Training? <b>*Required for all individuals assigned to projects involving lentiviral vectors. <u>Required once.</u>*</b>				GW requirement

## STANDARD PRACTICES

Questions:	Yes	No	N/A	Reference(s)
16. Does the supervisor limit access to the room in accordance with institutional policies? ( <a href="#">Biosafety Manual</a> )				BMBL: BSL-1, A1, p. 30; BSL-2, A1, p. 33; NIH G-II-A-1-a; NIH G-II-B-1-a
17. Do personnel wash their hands after handling potentially biohazardous materials, after removing gloves, and before leaving the laboratory?				BMBL: BSL-1, A2, p. 30; BSL-2, A2, p. 33; NIH G-II-A-1-f; NIH G-II-B-1-f
18. Is eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption prohibited in the lab?				BMBL: BSL-1, A3, p. 30; BSL-2, A3, p. 33; NIH G-II-A-1-e; NIH G-II-B-1-e
19. Is mouth pipetting prohibited and are mechanical pipetting devices used?				BMBL: BSL-1, A4, p. 30; BSL-2, A4, p. 34; NIH G-II-A-1-d; NIH G-II-B-1-d
20. Are written policies for the safe handling of sharps (such as needles, scalpels, pipettes, and broken glassware) followed and included in the laboratory-specific biosafety manual? ( <a href="#">Sharps Safety</a> )				BMBL: BSL-1, A5, p. 30; BSL-2, A5, p. 34
21. Are needle-locking syringes or safety hypodermic needles used when working with biohazards?				BMBL: BSL-1, A5, p. 30; BSL-2, A5, p. 34; NIH G-II-B-2-j
22. Do personnel understand that used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated?				BMBL: BSL-1, A5a, p. 30; BSL-2, A5a, p. 34
23. Are all syringes/needles/sharps disposed of in rigid, puncture-resistant, leak-proof containers?				BMBL: BSL-1, A5b, p. 31; BSL-2, A5b, p. 34; 1910.1030(d)(2)(viii)(C)
24. Are re-usable sharps placed in a hard-walled container for transport to a processing area for decontamination?				BMBL: BSL-1, A5c, p. 31; BSL-2, A5c, p. 34; 1910.1030(d)(2)(xiii)
25. Do lab personnel use mechanical means, such as a brush and dustpan, tongs, or forceps to clean up broken glassware?				BMBL: BSL-1, A5d, p. 31; BSL-2, A5d, p. 34
26. Are all procedures performed carefully in a manner to minimize the creation of splashes or aerosols?				BMBL: BSL-1, A6, p. 31; BSL-2, A6, p. 34; NIH G-II-A-1-g; NIH G-II-B-1-g
27. Are work surfaces decontaminated with an effective disinfectant on completion of work or at the end of the day, and especially after overt spills or splashes of biohazardous materials?				BMBL: BSL-1, A7, p. 31; BSL-2, A7, p. 34; NIH G-II-A-1-b; NIH G-II-B-1-b
28. Are all wastes that are contaminated with biohazardous materials autoclaved or decontaminated with an effective disinfectant before they are scheduled for pick-up? ( <a href="#">Autoclave Procedures</a> )				BMBL: BSL-1, A8, p. 31; BSL-2, A8, p. 34; NIH G-II-A-1-c; NIH G-II-B-1-c; NIH-G-II-B-2-i; 29 CFR 1910.1030(d)(2)(xiv)

### STANDARD PRACTICES (CONTINUED)

Questions:	Yes	No	N/A	Reference(s)
29. Do all laboratory personnel receive training regarding their duties, safety policies, precautions and do they receive annual updates and additional training when changes in procedures or policies occur?				BMBL: BSL-1, p. 32; A11, BSL-2, A11, p. 35; 1910.1030(g)(2)
30. Have all personnel, and particularly women of childbearing age, been provided information regarding immune competence and conditions that may predispose them to infection? Are individuals encouraged to self-identify health conditions to their healthcare provider for appropriate counseling and guidance?				BMBL: BSL-1, A11, 32; BSL-2, A11, p. 35
31. Has everyone working in the laboratory completed a Lab-Specific Biosafety Training Checklist?				29 CFR 1910.1030

### SPECIAL PRACTICES

Questions:	Yes	No	N/A	Reference(s)
32. Does the PI/supervisor inform personnel who work in the laboratory about the potential hazards and specific entry requirements (e.g., immunization)?				BMBL: BSL-1, A11, 32; BSL-2, B1, p. 35; NIH G-II-B-2-c
33. Is a medical surveillance program in place for the laboratory?				BMBL: BSL-2, B2, p. 35
34. Are serum samples collected and stored from at-risk personnel?				BMBL: BSL-2, B3, p. 35; NIH G-II-B-2-I
35. Has the PI/supervisor developed lab-specific biosafety procedures and incorporated them into either a Biosafety Manual or Standard Operating Procedures?				BMBL: BSL-2, B4, p. 35
36. Have all laboratory personnel demonstrated proficiency in standard and special microbiological practices before working in the laboratory?				BMBL: BSL-2, B5, p. 35
37. Are cultures, tissues and other biohazardous materials placed in a container with a cover that prevents leakage during collection, handling, processing, storage, or transport?				BMBL: BSL-1, A8a, p.31; BSL-2, B6, p. 35; NIH G-II-A-2-a; G-II-B-2-a
38. Is laboratory equipment routinely decontaminated, as well as after spills, splashes, and before repair, maintenance or removal from laboratory?				BMBL: BSL-1, A8, p.31; BSL-2, B7, p. 35
39. Are spills involving infectious materials contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material?				BMBL: BSL-1, A8, p.31; BSL-2, B7a, p. 36
40. Are incidents that may result in exposure to infectious materials immediately evaluated and treated according to procedures described in the laboratory-specific safety manual?				BSL-2, B8, p. 36
41. Is the PI/supervisor immediately notified if there are spills and accidents that result in exposures to biohazardous materials?				BSL-2, B8, p. 36
42. Are there written procedures for responding to exposure incidents?				BSL-2, B8, p. 36
43. Is medical follow-up obtained after spills, accidents, and potential exposures?				BSL-2, B8, p. 36
44. Are animals and plants not associated with the work prohibited from the laboratory?				BSL-2, B9, p. 36

## CONTAINMENT

Questions:	Yes	No	N/A	Reference(s)
45. If there is a biological safety cabinet in the lab, has it been certified within the past year? ( <a href="#">Biological Safety Cabinets SOP</a> )				BSL-2, C1, p. 36; NSF 49
46. Is the biological safety cabinet free of equipment or supplies that can block the air grills and disrupt proper airflow?				p. 308; NSF 49
47. Is a biological safety cabinet used for all procedures with a potential for creating biohazardous aerosols or splashes? These may include: grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of biohazardous materials (especially whose internal pressures may differ from ambient pressures), inoculating animals intra-nasally, and harvesting infected tissues from animals or embryonated eggs. ( <a href="#">Preventing Aerosol Production</a> )				BSL-2, C2a, p. 36
48. Is a biological safety cabinet used when high concentrations or large volumes of biohazardous materials are handled?				BSL-2, C1b, p. 36
49. Is equipment (e.g., refrigerator, freezers) for use or storage of biohazardous materials labeled with a biohazard symbol?				29 CFR 1910.1030(g)(1)(i)(A)
50. If centrifuges are used, are sealed rotor heads or safety cups used and only opened in an approved biological safety cabinet or other ventilated containment device?				
51. If an autoclave is used, are procedures posted? ( <a href="#">Proper Use of Autoclave</a> )				CFR 1910.132

## PERSONAL PROTECTIVE EQUIPMENT

Questions:	Yes	No	N/A	Reference(s)
52. Do personnel wear lab coats whenever they are in the lab and remove them before leaving the lab? ( <a href="#">Biosafety PPE</a> )				BMBL: BSL-1, C2, p. 32; BSL-2, C2, p. 36; 29 CFR 1910.132; NIH G-II-A-1-h; NIH G-II-B-2-f
53. Are personnel prohibited from taking their lab coats home for laundering?				BMBL: BSL-2, C2, p. 36; NIH G-II-B-2-f
54. Do personnel remove gloves before touching "clean" surfaces (keyboards, telephones, elevators, etc.) and before leaving the lab?				BMBL: BSL-2, C2, p. 36
55. Do personnel wear protective eyewear when performing procedures that have the potential to create splashes or microorganisms or other hazardous materials?				BMBL: BSL-1, C3, p. 32; BSL-2, C3, p. 36
56. When biohazardous materials must be manipulated outside a biological safety cabinet, do personnel use eye and face protection?				BMBL: BSL-2, C3, p. 36
57. Do personnel wear gloves to prevent contact with biohazardous materials?				BMBL: BSL-1, C4, p. 32; BSL-2, C4, p. 37; NIH G-2-B-2-h; 29 CFR 1910.132

### PERSONAL PROTECTIVE EQUIPMENT

Questions:	Yes	No	N/A	Reference(s)
58. Are alternatives to latex gloves available for personnel with latex sensitivity?				BMBL: BSL-1, C4, p. 32; BSL-2, C4, p. 37; 29 CFR 1910.132
59. Are gloves changed when contaminated, when glove integrity is compromised, or when otherwise necessary?				BMBL: BSL-1, C4a, p. 32; BSL-2, C4a, p. 37; 29 CFR 1910.132
60. Are hands washed after removing gloves?				BMBL: BSL-1, C4b, p. 32; BSL-2, C4b, p. 37; 29 CFR 1910.132
61. Are disposable gloves prohibited from being washed or reused?				BMBL: BSL-1, C4c, p. 32; BSL-2, C4c, p. 37; 29 CFR 1910.132
62. Are contaminated gloves disposed properly?				BMBL: BSL-1, C4c, p. 32; BSL-2, C4c, p. 37; 29 CFR 1910.132
63. Are eye, face, and respiratory protection worn in rooms containing infected animals?				BMBL: BSL-2, C5, p. 37; 29 CFR 1910.132

### LABORATORY FACILITIES

Questions:	Yes	No	N/A	Reference(s)
64. Is a BIOHAZARD sign posted on the lab entrance door, which includes the biosafety level, any required immunizations, emergency contact numbers, and any personal protective equipment that must be worn in the lab?				BMBL: BSL-1, A9 p. 31; BSL-2, A9, p. 34; NIH G-II-B-2-
65. Do laboratories have doors for access control?				BMBL: BSL-1, D1, p. 33; BSL-2, D1, p. 37
66. Do laboratories have doors that are self-closing?				BMBL: BSL2, D1, p. 37
67. Does the lab have a sink for hand washing?				BMBL: BSL-1, D2, p. 33; BSL-2, D2, p. 37; NIH G-II-A-4-d; NIH G-II-B-4-d
68. Are carpets and rugs prohibited in the laboratory?				BMBL: BSL-1, D3, p. 33; 4-d-2, D3, p. 37
69. Is furniture in the laboratory capable of supporting anticipated loads and uses?				BMBL: BSL-1, D4, p. 33; BSL-2, D4, p. 37; NIH G-II-A-4-c
70. Is the room clean and are spaces between benches, cabinets and equipment accessible for cleaning?				BMBL: BSL-1, D4, p. 33; BSL-2, D4, p. 37; NIH G-II-A-4-c; NIH G-II-B-4-c

### LABORATORY FACILITIES (CONTINUED)

71. Are bench tops impervious to water and resistant to moderate heat and the chemicals used to decontaminate the work surfaces and equipment?				BMBL: BSL-1, D4a, p. 33; BSL-2, D4a, p. 37; NIH G-II-A-4-b; NIH G-II-B-4-b
72. Are chairs and other furniture used in the lab covered with a non-fabric material that can be easily decontaminated?				BMBL: BSL-1, D4b, p. 33; BSL-2, D4b, p. 37
73. Are windows that open to the exterior fitted with screens?				BMBL: BSL-1, D5, p. 33; BSL-2, D5, p. 37; NIH G-II-A-4-e; NIH G-II-B-4-e
74. Are biological safety cabinets located away from doors, windows that can be opened, heavily traveled lab areas, and other potentially disruptive equipment?				BMBL: BSL-2, D6, p. 38
75. If vacuum lines are used, are they protected with High Efficiency Particulate Air (HEPA) filters or liquid disinfection traps?				BMBL: BSL-2, D7, p. 38
76. Is there an eyewash station readily available in the lab?				BMBL: BSL-2, D8, p. 38
77. Does the room provide an inward flow of air without recirculation to spaces outside the room?				BMBL: BSL-2, D9, p. 38
78. Is there a method for decontaminating waste available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method)?				BMBL: BSL-2, D11, p. 38; NIH G-II-B-4-f

### REQUIREMENTS FOR OSHA BLOODBORNE PATHOGENS

Questions:	Yes	No	N/A	Reference(s)
79. Have personnel been offered and received appropriate immunizations for the agents potentially present in the lab (e.g., hepatitis B)? Or declined in writing? ( <a href="#">Bloodborne Pathogen Exposure Control Plan</a> )				29 CFR 1910.1030(f)(1)(i)
80. Do personnel have access to the GW <i>Bloodborne Pathogens Exposure Control Plan</i> ? ( <a href="#">Bloodborne Pathogen Exposure Control Plan</a> )				29 CFR 1910.1030(g)(2)(vii)(D)
81. Have personnel with the potential for exposure to bloodborne pathogens or other potentially infectious materials completed OLS's Biosafety & Bloodborne Pathogens Training?				29 CFR 1910.1030(g)(2)(i)

### REQUIREMENTS FOR RECOMBINANT AND SYNTHETIC NUCLEIC ACID MOLECULES

Questions:	Yes	No	N/A	Reference(s)
82. Has the PI's recombinant/synthetic nucleic acid research been reviewed and approved by the <a href="#">Institutional Biosafety Committee</a> ?				B068; NIH Section III
83. Does the laboratory have 10 or more liters of culture present?				B069; NIH III-D-6
84. Is the PI familiar with which section of the <a href="#">NIH Guidelines</a> their research falls under?				B070; NIH IV-B-7

## REQUIREMENTS FOR RECOMBINANT AND SYNTHETIC NUCLEIC ACID MOLECULES

Questions:	Yes	No	N/A	Reference(s)
85. Does the laboratory have an emergency response plan for dealing with accidents, spills, or other incidents involving recombinant/synthetic nucleic acid molecules?				B071; NIH IV-B-2-b-(6)
87. Are personnel familiar with the emergency response procedures for spills or exposures involving recombinant/synthetic nucleic acid molecules?				NIH IV-B-2-b-(6)
88. Do lab personnel have access to the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules? ( <a href="#">NIH Guidelines</a> )				NIH G-I
89. Do personnel have access to copies of procedures (e.g., SOPs) for recombinant/synthetic nucleic acid molecules?				NIH G-I
90. Are animals not involved in the research prohibited in the room?				NIH G-II-B-2-g
91. Are hypodermic needles and syringes used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles?				NIH G-II-B-2-j
92. Are only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) used for the injection or aspiration of fluids containing organisms that contain recombinant or synthetic nucleic acid				NIH G-II-B-2-j
93. Is extreme caution used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal?				NIH G-II-B-2-j
94. Are spills and accidents, which result in overt exposures to organisms containing recombinant or synthetic nucleic acid molecules immediately, reported to the Biological Safety Officer and Institutional Biosafety Committee?				NIH G-II-B-2-k
95. Is an insect and rodent control program in effect?				NIH G-II-A-2-b; NIH G-II-B-2-e
96. Has a laboratory-specific biosafety manual been adopted? ( <a href="#">Lab-Specific Manual</a> )				NIH G-II-B-2-m
97. Are personnel required to read and follow the laboratory-specific biosafety manual? ( <a href="#">Lab-Specific Manual</a> )				NIH G-II-B-2-m

## REQUIREMENTS FOR TOXINS

Questions:	Yes	No	N/A	Reference(s)
98. Does the research involving working with or generating any toxins of biological origin? If yes, which toxin(s) and how many milligrams?				42 CFR 73
99. Does the laboratory have any toxins listed on the Select Agent and Toxin list? ( <a href="#">Select Agents and Toxins</a> )				42 CFR 73
100. Is an inventory control system in place for the toxins?				BMBL, Appendix I

101. Is all work with toxins conducted within a certified chemical fume hood or Biological Safety Cabinet?				BMBL, Appendix I
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**Equipment Inventory**

Is there an autoclave present? (Y/N)					
If yes, please include the following information for each:					
	Model Number	Serial Number	Location	Last Certification Date	Certification Due Date
Autoclave (1)					
Autoclave (2)					
Autoclave (3)					

Is there a biological safety cabinet (BSC) present? (Y/N)					
If yes, please include the following information for each:					
	Model Number	Serial Number	Location	Last Certification Date	Certification Due Date
BSC (1)					
BSC (2)					
BSC (3)					
BSC (4)					
BSC (5)					