

Biosafety Training for Research Labs

Please sign in

The George Washington University
Updated 11/10/11

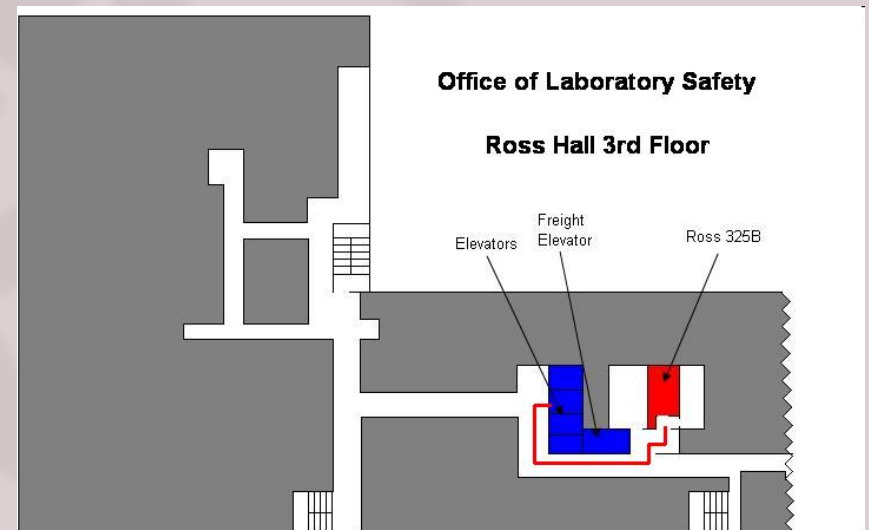
Biosafety Program

Biosafety is based on three primary documents

- CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th edition
- NIH guidelines for Recombinant DNA
- OSHA's Bloodborne Pathogen Standard (federal regulation)

GWU program elements

- Biosafety officer
- Biosafety committee to oversee
Administered by OLS
- Manual & Training (*covers manual*)



Ross Hall 325B

Biosafety at GWU

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** – periodic inspections of laboratories (mandatory for IBC registered labs); resource to campus on biosafety compliance issues; proper reporting to NIH/OBA for rDNA, administrator of the IBC; provide training; provide and update program documents.
- **Principal Investigators** –
 - All must comply with the biosafety manual
 - Establish specific procedures that are available to workers
 - Ensure that all workers are aware of the hazards and precautions to be taken
 - Lab workers are trained and proficient at procedures (*individual training form*)
 - SOPs for pathogens
 - Covered research submitted to the IBC for review
 - supervise lab operations to ensure proper technique and containment
 - Entry requirements are achieved

Biosafety at GWU

Responsibilities

- **Principal Investigators cont.** – must immediately report the following to the BSO:
 - Breach of containment for rDNA (e.g. escaped animals or microorganisms, spill outside of containment not easily cleaned by one person, any spill in BSL3 facility outside of containment)
 - Any worker exposure of rDNA, any potential exposure at BSL3
 - Any illness likely caused by rDNA exposure
 - Willfully violation of protocols or work conducted without IBC approval.
- **Lab workers** –
 - Comply with the manual and all lab procedures
 - Report all major spills and incidents (listed above) to PI or BSO
 - Consult with their physician if they have a condition that places them at increased risk of disease
 - Attend all required training.

OLS Website

Biosafety
Radiation Safety
Training
Resources
Quick links
IBC Registration page
Ross Biowaste
Bioshipping page
AU Toolbox
Other links
Health Research Home
Human Research Home
Animal Research Home

Biosafety

At GWU much research is conducted with microorganisms or the nucleic acids of these organisms. The National Institutes of Health (NIH) has published "[Guidelines for Research Involving Recombinant DNA Molecules](#)" and the Centers for Disease Control (CDC) has published "[Biosafety in Microbiological and Biomedical Laboratories \(BMBL\)](#)". These documents and other resources provide the basis for GWU's biosafety requirements. To comply with these guidelines, OLS has developed the following elements. Click on each link to find out more:

- **Manual** - All lab workers that are working with living organisms, cells or fluids must be familiar with and comply with this manual. Non-lab workers should refer to the Bloodborne Pathogen section below.
 - [Biosafety & Exposure Control Manual](#)
 - [Appendix A](#) - HIV fact sheet
 - [Appendix B](#) - HBV fact sheet
 - [Appendix C](#) - HIV/HBV worker form
 - [Appendix D](#) - Ross Hall biowaste
 - [Appendix E](#) - Bio emergency posting
 - [Appendix F](#) - Bio inspection form
- **[Institutional Biosafety Committee](#)** – The IBC is a committee established to review and approve the use of recombinant DNA, pathogens and Select Agents. IBC approval must be received before these agents are acquired. Click the link above for detailed information and definitions.
- **[Training](#)** - All those who work in a biological research laboratory, even if only BSL1, must attend biosafety training. The training page has a list of all OLS provided training..
- **[Bloodborne pathogens](#)** – This program is for anyone except lab workers who, because of their job requirements, may come in contact with blood or other potentially infectious material. Examples of those covered are: housekeeping and EMTs.
- **[Shipping biologicals](#)** - Biological shipments must comply with the Department of Transportation (DOT) regulations as well as the International Air Transport Association (IATA).

Definitions

- Pathogen – Any biological agent that can cause disease in healthy human adults
- Recombinant DNA - 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 - Pathogens (including genetically altered forms) or toxins deemed by the CDC or US Department of Agriculture to pose a high risk to human, animal or plant health and could be utilized for terrorist activity. Also included are nucleic acids from these agents that could produce an infectious form or code for a toxin

Risk Factors

- How and to what extent does an agent cause disease?
- Host Range – Which species can be infected?
- Virulence – Severity of disease and odds of recovery
- Infective dose – How many organisms are needed to initiate infection?
- Mode of transmission
 - Ingestion
 - Inhalation
 - Injection
 - Contact
- Communicability – How easily does the agent spread between hosts?
- Stability – How well can the agent survive in the environment

BBP Epidemiology/symptoms

Human Immunodeficiency Virus (HIV)

- Virus that leads to AIDS
- HIV depletes the immune system
- HIV does not generally survive well outside the body
- No real threat of contracting HIV through casual contact
- Symptoms include: fever, headache, fatigue and enlarged lymph nodes
- No vaccine

Hepatitis B (HBV)

- 1—1.25 million Americans are chronically infected
- Symptoms include: jaundice, fatigue, abdominal pain, loss of appetite, intermittent nausea, vomiting
- May lead to chronic liver disease, liver cancer, and death
- can survive for one week in dried blood
- Vaccination available since 1982

Hepatitis C (HCV)

- Most common chronic blood borne infection in the United States
- Symptoms include: jaundice, fatigue, abdominal pain, loss of appetite, intermittent nausea, vomiting
- May lead to chronic liver disease and death.
- Spread by: needles, sex, sharing personal care items that may have blood
- No vaccine

• Others: Malaria, Brucellosis, Syphilis

• Transmission: contact with mucus membranes, non-intact skin or contaminated sharps

BBP Universal Precautions

Universal Precautions

- Handling all blood and other human body fluids and tissue as if they are infectious (BSL2). They are considered Potentially Infectious Material (PIM). Some examples are:
 - Synovial fluid
 - Pleural fluid
 - Pericardial fluid
 - Semen
 - Amniotic fluid
 - Cerebrospinal fluid
- In a medical research environment, urine and saliva are usually considered PIM
- Mammalian cells and tissues are considered PIM
- Consider patient history to decide if sample is a known pathogen

Institutional Biosafety Committee

- All work involving the following must be reviewed by the IBC regardless of funding:
 - recombinant DNA
 - pathogens (BSL2 or higher)
 - select agents
- To initiate IBC review of research, a PI must submit the following:
 - IBC Registration Form
 - Research Description Form
 - Viral Vector Form (if necessary)
 - Pathogen SOP (if necessary)
- Forms and other IBC information including the charter can be found online
- Within the next few months all IBC registrations will be put onto an online system. When the conversion occurs OLS will send an email with a final date for accepting paper submissions.

Risk Assessment

Risk Assessment

Initial Risk

- The NIH categorizes agents according to Risk Groups (RGs) based on risk factors previously discussed. The RGs are more of a continuous spectrum as opposed to obvious dividing lines.

RG1	Agents that are not associated with disease in healthy adult humans
RG2	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are <i>often</i> available
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)
RG4	Not allowed at GWU

- For rDNA consider the risk of the whole viable agent
- Appendix B of the NIH guidelines list many common agents according to risk group

Risk Assessment

Other factors

- Effective treatment of prophylaxis
- Aerosols, high concentrations or high volumes
- RDNA: will the new insertion:
 - change: virulence, infectious dose, host range, stability, cell cycle or replication capacity?
 - code for an oncogene?
 - integrate into the genome?
 - produce replication competent viruses?
 - Are biological barrier options being implemented (i.e. attenuation)
- Once all factors have been taken into account the risk may be the same, lower or higher and then assigned to the appropriate containment (4 categories from CDC BMBL)

Risk Assessment

Containment

- The CDC categorizes containment according to biosafety level (BSL)
- Each biosafety level has associated practices, equipment and facilities that are used together to accomplish containment
- Risk groups usually equal the Biosafety Level (RG2 = BSL2). Read agent summaries in the BMBL.
- BSL2 or higher is for pathogens
- BSL3 is aerosol route of transmission
- The IBC can always prescribe special practices (e.g. BSL2 enhanced)

Biosafety Levels

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

Risk Assessment

Worker Attributes

- Some workers may be at higher risk due to their circumstances
 - Pre-existing conditions
 - Compromised immunity
 - Pregnant or breastfeeding
- These are private issues but workers should consult their physician if they have concerns
- If the risk is too high they may need extra protective equipment or even refrain from certain tasks while in that condition

Symptoms

- Be alert for symptoms in you or others especially for a pathogen you are using

Institutional Biosafety Committee

Following are the research categories for rDNA research and the approvals needed

Level	Approval/Review	Requirements
III-A	NIH Dir, RAC, IBC [†]	A drug resistant gene transferred into a (new) microorganism.
III-B	NIH/OBA, IBC [†]	The cloning of toxin molecules with LD ₅₀ < 100 ng/kg of body weight.
III-C	RAC, IRB, IBC [†]	rDNA (or DNA or rDNA derived from rDNA) transferred into humans.
III-D	IBC [†]	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 [§] - 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 any work not covered in the other categories (most non-pathogenic rDNA work)
III-F	Exempt from NIH Guidelines	rDNA involving: not in organisms/viruses, recreating a single plasmid, indigenous plasmids/viruses propagated in original host, specific exemptions from NIH (including the creation of transgenic BSL1 mice)

† Approval required before initiation.

§ Notify IBC (register) when project is initiated. IBC approval is still required.

Note: category III-F is considered exempt from guidelines, however notification needs to be sent to the BSO in order to verify the work is III-F

Controlling Hazards

- Administrative Controls
- Standard Microbiological Practices
- Primary Barriers
- Secondary Barriers

Administrative controls

- Inspections – audits will be conducted at least annually (*Checklist of items – App F of Biosafety Manual*). Labs are encouraged to perform self inspections.
- Priority – engineering controls then protective equipment
- Building transport – When biological materials are transported between floors; through corridors; in non-lab areas, or between buildings they must be carried in a clean carry container **without** gloves.
- Standard Operating Procedures (SOPs) – SOPs must be written for any work that involves pathogens.
- Training – every worker must have a training documentation sheet on file. This form can vary from lab to lab depending on work done, but should show when each worker was trained on protocols. (See example on OLS website).



Standard Microbiological Practices

Access control

- Access limited to those with a need to be there. Children are not allow in labs.
- Access limited when work with viable organisms containing rDNA is in progress and any work at BSL2.
- No non-research animals or plants
- If indicated for agent in use, vaccines must be made available free
- Special entry requirements posted



Standard Microbiological Practices

Sharps handling

- Contaminated sharps must be discarded directly in a sharps container soon after use. Sharps include:
 - Razor blades
 - Scalpels
 - Pasteur pipettes
 - Hypodermic needles (all)
- Sharps containers must be, easily accessible, closable, upright and leak-proof and not allowed to overfill. Labs are responsible for purchase.
- Do not alter a needle or remove a contaminated needle from a syringe. Place entire assembly in the container and no recapping
- Use safety needles when high risk and use plastic instead of glass when feasible



Standard Microbiological Practices

Hygiene

- Decontaminate equipment and surfaces after work, at the end of day and immediately after spills
- Wash hands after contacting PIM and after removing gloves/PPE (use sanitizer if no sink)
- No PPE in non-lab areas
- Do not: drink, eat, chew gum, apply cosmetics, lip balm, handle contact lenses, or use tobacco
- Food fridges in break areas marked “Food only”
- Temporary bench paper for easy clean up
- Only mechanical pipetting
- Tie up loose hair and no draping clothes or dangling jewelry

Standard Microbiological Practices

Minimizing aerosols

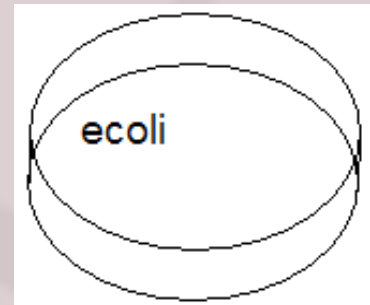
- Work with cultures, PIM or rDNA, that may generate aerosols (e.g. sonicating, vortexing, grinding, blending and shaking) in primary containment
- Work with cultures, PIM or rDNA at high volumes (> 10 liters) must be done in primary containment.
- Avoid expelling last drop from a pipette.
- Pipette down side of glass
- Cover opening of containers when agitating
- Use disposable loops or micro incinerator
- Covering samples with foil can reduce aerosols in the event of a spill
- Use gauze to extract needles from PIM containing diaphragm bottles and avoid over-pressurizing bottles



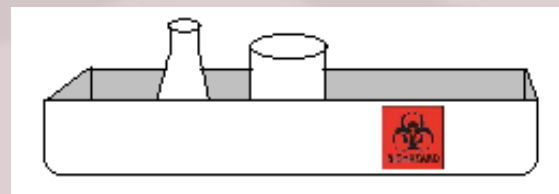
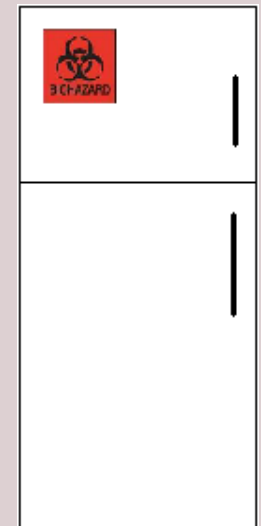
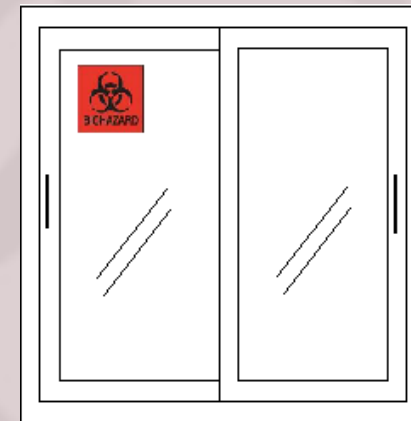
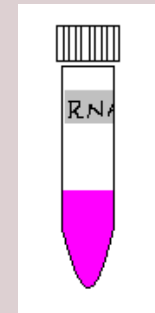
Standard Microbiological Practices

Containers & Labeling

- All containers must be labeled.
- PIM stored in cabinets, fridges, trays, etc. with biohazard label
- PIM transported in containers with biohazard label
- All waste must have biohazard label (use vendor supplies)



Bottom or side of dish



Standard Microbiological Practices

Additional safety practices for BSL2

- PPE must always be worn when working with PIM (even with a BSC)
- Pathogens work – must be in physical containment: BSCs, glove box, sealed cup centrifuge
- Proficiency must be demonstrated before workers are allowed to work with pathogens (A training sheet is recommended)



minimum: coat,
gloves, eyewear

Standard Microbiological Practices

Bloodborne Pathogen labs

HIV, HTLV, HBV or HCV have special use requirements from the OSHA bloodborne pathogen standard.

- Doors closed when working
- Authorization form for proficiency for all those who work with BBPs
- Needles must be approved and only for parenteral injection and aspiration from animals or diaphragm bottles
- Vacuum lines have traps and HEPA filtration
- Autoclave is available

* This work is usually done in Ross 704, but can be approved in other areas by the IBC

Standard Microbiological Practices

Decontamination

- Surfaces & equipment decontaminated daily, after work and immediately after spills.
- Disposables discarded when contaminated (e.g. bench paper)
- Equipment decontaminated before shipping
- Disinfectant – appropriate for agent



Disinfectants

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

Standard Microbiological Practices

Autoclave

- Correct run time and temp for agent
- Add water
- Good circulation
- No hazardous materials
- Allow steam to dissipate and use PPE
- Indicators & certification

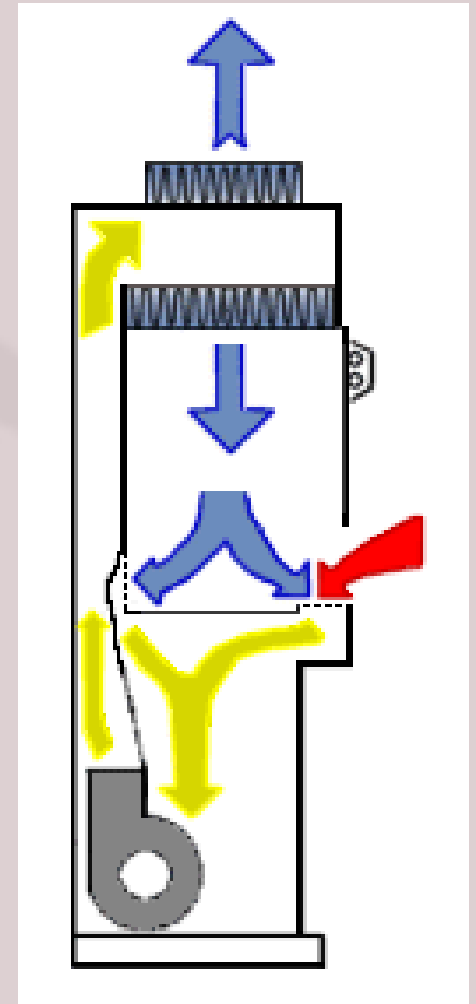


Primary Barriers

Biosafety cabinets (BSCs)

- protection from aerosols
- Class II BSCs protect work, worker and environment (most common)
- BSCs should be placed away from high traffic and vent diffusers
- No hazardous chemicals
- Avoid open flames

Do not use fume hoods or clean benches



Primary Barriers

NOT a safety device!



Laminar Bench

Primary Barriers

BSC Safe Practice

- Disinfect and purge (3 min) cabinet and ensure that it is working properly and certified
- Introduce all needed materials
- Work flow - usually left to right and from clean to dirty and observe all standard microbiological practices.
- Keep waste in the hood (pipettes in disinfectant).
- Do not block the front air vent and use slow, direct movements to
- Remove all items and decontaminate when finished



Primary Barriers

Personal Protective Equipment (PPE)

Minimum PPE for BSL2 is : lab coat, eye protection and gloves

- Eye protection protects from splashes & sprays. Goggles and face shields for higher hazard.
- Lab coats must always be worn in the lab and disinfected before washing (never laundered at home)
- Closed toe shoes only
- Respirators only used when in the respiratory protection program
- Gloves: re-glove often, for allergies use nitrile or vinyl, use nitrile for hazardous materials

Primary Barriers

Centrifuge Use

- Use sealed rotors or sealed tubes to reduce aerosols
- Plastic screw-cap vials for pathogens and bucket caps if concentrated
- Tubes not flawed or cracked, match and balance tubes
- Use rotors according to manufacturer recommendations.
- If tube breaks close lid and wait 30 minutes to clean
- Load and unload tubes in a BSC when aerosol hazards are present
- Rotors in good condition and clean



Secondary Barriers

Basic Lab Design

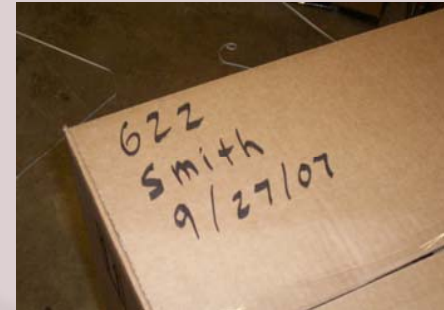
- easily cleanable, no absorbent items such as rugs or fabric chairs
- Ventilation - negative pressure so that air flows into the room FROM the adjacent halls and offices
- Immediate access to a sink, preferably located near the door
- No perforations in the walls, floor and ceiling
- Immediate access to an eyewash
- Well illuminated
- Lockable doors
- Appropriate hazard on placard



Regulated Medical Waste

Red Bag Waste “dry”

- Items contaminated with blood or other potentially infectious material (e.g. protective equipment, bench paper, gauze, patient specimens, cultures, items medical in nature, rDNA, etc.)
- Must go in vendor provided bags and boxes; not overfilled
- Full bags must **sealed with tape** (in the box) and placed in hall with room# and date on box, do not tape box shut
- Not punctured or leaking, **30lbs or less**. If pipettes puncture your bags they must be placed in sharps containers.
- No liquids, hazardous waste or regular trash
- Handout in appendix E of manual. Non-compliant boxes will not be picked up



NOT Regulated Medical Waste

Regular
Trash



Hazardous
material

Regulated Medical Waste

Sharps containers

- Sharps containers go in red bags. Non-contaminated broken glass should go in a sturdy box for the dumpster

Liquid waste

- Contaminated liquid must be disinfected then drain disposed (prevent splashing). Blood is not considered liquid

General

- Any red bag or sharps waste that contains pathogens transmitted by the aerosol route must be autoclaved before disposal.



Regulated Medical Waste

- All animal carcasses must be disposed of as regulated medical waste
- DO NOT simply throw carcasses into lab red boxes for disposal
- Special carcass freezers are available for any euthanized animals
- Contact the ARF for help in determining where the appropriate freezer is located



Emergency

- Workers must be aware of emergency procedures for spills and exposures and they must be posted in each laboratory

Eyewashes

- Immediate access
- Unobstructed
- Certified annually
- Tested monthly



Emergency

Spills – of PIM, cultures or rDNA

1. Leave room and wait 15 minutes, call OLS if spill is large 4-2630 (a large spill is one that cannot be easily and quickly cleaned up by one person)
2. If you were exposed, follow exposure procedures.
3. Limit access to area. Make sure those leaving are not contaminated.
4. Don gloves, coat and eye protection.
5. Cover spill with absorbent, pour disinfectant (10-20% bleach), wait
6. Broken glass - use tongs or a dustpan and sharps container.
7. Clean up gross contamination first, work inward from cleaner to dirty, keep applying disinfectant as needed.
8. For hazardous chemical call Health & Safety, 4-4347. For radioactive contamination call OLS, 4-2630.

Emergency

Exposures - to PIM, cultures or rDNA

1. Remove contaminated clothing, place in biohazard bag.

Note: if you believe you have inhaled an aerosol, get medical attention (#4)

2. Wash exposed area for 15 minutes. Use soap or a mild disinfectant.
3. Helpers wear PPE.
4. Get medical treatment. If serious go directly to the emergency room at GW Hospital. For non-serious incidents:
 - Main campus - Student Health at 2141 K St NW, Ste 501
 - Medical Center – Employee Health on ground level of hospital
 - Personal physician (must take appropriate paperwork).
5. Fill out an incident report, check recombinant DNA if applicable
6. Contaminated garments must be disinfected or discarded as biowaste.

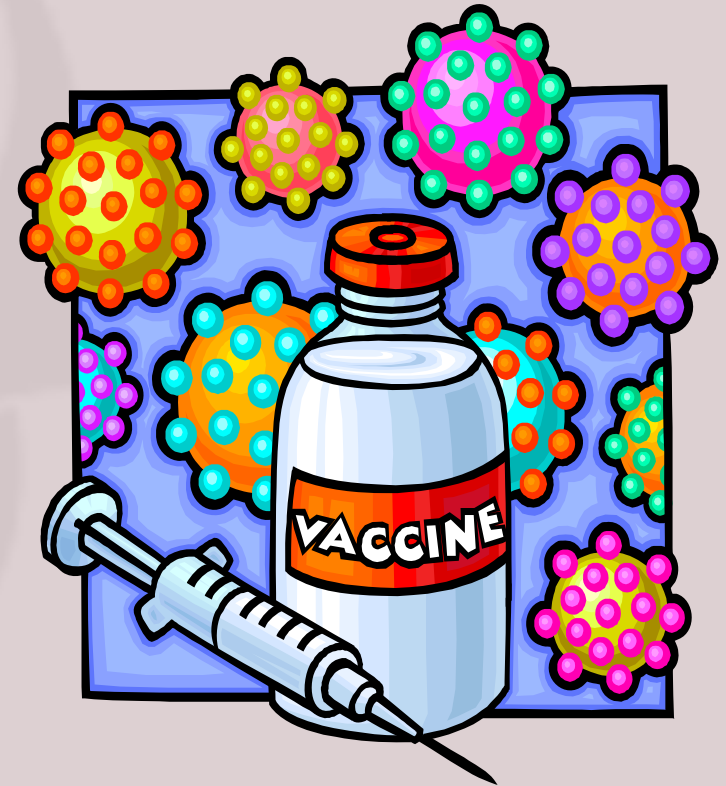
Medical Evaluation/Follow-up

- Confidential medical evaluation
- Healthcare professional will:
 - Document route of exposure and circumstances
 - Identify and test source individuals blood (with consent)
 - Provide results to exposed employee
 - Provides employer with written opinion
- Students should follow up with their physician



Hepatitis B Vaccination

- Safe
- Very effective (80-95% efficacy)
- Offered to all employees potentially exposed to human blood or PIM or cells at no cost
- form
- It is highly recommended that students get vaccinated, even if they must pay



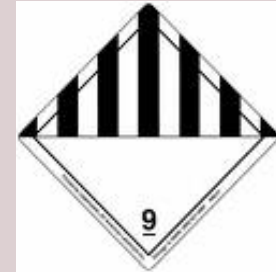
Biosecurity

- All workers must maintain security
- All people entering the lab should be wearing identification. Engage unknown individuals.
- Doors should be closed when working with pathogens and locked when unattended



Shipping Biologicals

- Only people trained in IATA/DOT requirements can sign to send a package
- The FAA has visited us recently
- Get help with category A



Other offices

Live Animals

- All live animals must be procured by the Animal Research Facility (ARF) and use must be approved by the Institutional Animal Care and Use Committee (IACUC)
- Individual hazard assessment – free consultation



Human Research

- All research involving human subjects must be approved the Institution Review Board (IRB)... even drawing blood or giving a questionnaire.





Thank You

Questions?