Biosafety in Research Laboratories

This training module will introduce the general biosafety practices and policies in effect for laboratory work with biological materials.

Biosafety Training is recommended for work with BSL-1 and required for BSL-2 materials. Initial and annual training requirements can be fulfilled by:

- Completing this module and passing the Post-Test; or
- Attending department biosafety safety training seminar; or
- Completing PI/Lab Manager led training, utilizing the Lab-Specific Biosafety training form.
Biosafety in Research Laboratories

Topics:
- Policy Overview
- Risk Assessment
- Controls
- Waste Disposal, Spills & Decontamination
- BBP
- Post-Test
What is Biosafety?

- Biosafety is defined as the procedures and control mechanisms implemented to ensure that biohazardous materials are handled safely. Biosafety policies are established to provide standard levels of protection for...
  - Self, Co-workers, Lab support personnel
  - Emergency Responders
  - Experiment and Materials
  - Environment (ecosystem & community)
Biohazardous Materials

- Biohazardous materials are any part of a living organism that could affect the health of humans, plants, or animals.
- Bacteria
- Viruses
- Parasites
- Fungi
- Human and Non-Human Primate Material
  - Blood, bodily fluids, cells
- Animals
- Recombinant DNA
- Biological Toxins
Guidelines and Regulations

- Biosafety in Microbiological and Biomedical Laboratories (CDC)
- NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH)
- OSHA Bloodborne Pathogens Standard (OSHA)
- NC Regulated Medical Waste Rules
- Institutional Biosafety Committee (IBC)
- UNCG Biosafety Policy
- UNCG Bloodborne Pathogens Exposure Control Plan
Institutional Biosafety Committee (IBC)

• The Institutional Biosafety Committee (IBC) ensures that research involving biological materials and recombinant DNA are conducted safely, responsibly, and in accordance with applicable regulations and guidelines, by establishing the Biosafety Policy Manual and reviewing rDNA activities.

• Work with recombinant DNA is divided into two categories with regard to review by the IBC.
  – Registration with Exempt form
  – Full review and approval of protocol required
Institutional Biosafety Committee (IBC)

- Activities requiring the IBC Exempt form
- Some recombinant or synthetic nucleic acid molecules may be introduced, propagated and maintained in cells in tissue culture, without submission of a full IBC Protocol. This “Exempt” form may be used if the genetic constructs involved:
  - Contain NO toxin gene or eukaryotic viral genetic material, or less than one-half of the genome of any eukaryotic virus from RG1 or RG2.
  - Are derived entirely from non-viral sources and propagated in S. cervisiae or an E. coli strain with no conjugation proficient plasmid or generalized transducing phage, with fermentation volumes not exceeding 10 liters at any one time.
Institutional Biosafety Committee (IBC)

Recombinant DNA activities requiring a full IBC Protocol Form:

- Anything involving live biohazardous toxins or organisms of RG2 or higher
- Activities involving rDNA containing greater than 50% of the genome of a RG2 eukaryotic virus or any amount of genetic material from RG3 or RG4 organisms.
- Dual use agents
Institutional Biosafety Committee (IBC)

Activities NOT requiring IBC review:

- Activities involving in vitro use of non-self-replicating forms of nucleic acids (e.g. siRNA, PCR, DNA sequencing, chemical/analytical experiments) do not require disclosure to IBC.
Risk Assessment

- The establishment of biosafety policies is based on a Risk Assessment, which takes into account...
  - Biological risk
  - Potential for Exposure
  - Risk Management Controls
Risk Assessment

Biological Risk:

- Risk Groups (RG1-4): Biohazardous materials are classified into Risk Groups, based on the relative risk of the agent/material, considering the following factors:
  - Pathogenicity
  - Mode of transmission and host range
  - Availability of effective preventive measures (vaccines)
  - Availability of effective treatment (antibiotics)
  - Biosafety Levels (BSL1-4): Ascending levels of containment and precautionary procedures to be used when working with biohazardous materials, based on the following risk criteria:
    - Infectivity
    - Severity of Disease
    - Transmissibility
    - Nature of work being conducted
Risk Assessment

Risk groups and Biosafety levels are similarly divided, but are not exactly aligned. Thus, some materials may be classified as RG1 and BSL-2. It is generally more important to know the appropriate BSL for a material, so the proper containment and handling procedures are implemented.
Biosafety Levels

BSL-1: agents not known to cause disease (in healthy adults)
BSL-2: agents associated with disease
BSL-3: indigenous/exotic agents associated with human disease and with potential for aerosol transmission
BSL-4: dangerous/exotic agents of life threatening nature
Biosafety Levels

Resources
Risk Group and/or BSL classification of specific materials may be found in:

• Safety Data Sheet (SDS) - from supplier

• ABSA Risk Group Database

• NIH Guidelines:
  – Appendix B - Risk Group classification of etiologic agents

• Public Health Agency of Canada
  – Pathogen Data Safety Sheets and Risk Assessment
Biosafety Level 1

Immunocompromised workers are at risk

• Examples:
  – Adeno associated virus
  – Murine leukemia virus
  – Non pathogenic E. coli
  – Animal tissue and cells
Biosafety Level 2

- Agents associated with human disease
- Infectious dose will vary based on:
  - Agent
  - Exposure time
  - Exposure route
- Immunization or antibiotic treatment may be available
- Examples:
  - Hepatitis B Virus
  - Influenza
  - Salmonella
  - Human material
Biosafety Level 2

Human material

- At UNCG, any material capable of transmitting an infectious agent is classified as BSL-2, even if listed as BSL-1 by the supplier. This includes human...
  - Cells & Cell Lines
  - Unfixed tissue samples
  - Blood
  - Other potentially infectious materials (OPIM)
Other potentially infectious materials (OPIM) include bodily fluids capable of transmitting an infectious agent:

- Semen
- Vaginal secretions
- Cerebrospinal fluid (fluid surrounding the brain and spinal cord)
- Synovial fluid (fluid surrounding bone joints)
- Pleural fluid
- Pericardial fluid
- Peritoneal fluid
- Amniotic fluid
- Saliva in dental procedures
- Any body fluid that is visibly contaminated with blood
- Any body fluids in situations where it is difficult or impossible to differentiate between body fluids
Biosafety Level 3 & 4

Not currently approved at UNCG!

BSL-3: Agents with a potential for respiratory transmission and which may cause serious and potentially lethal infection.

BSL-4: Dangerous and exotic agents that pose a high risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy.
Routes of Exposure

**People** (Lab personnel, Coworkers, Emergency Responders, Community)

- **Inhalation**: 80% of lab acquired infections (LAI)
  - Improper containment barriers for aerosols or splatters
- **Percutaneous**
  - Improper handling of sharps
  - Contamination of broken skin
Routes of Exposure

People (Continued)

• Mucous Membrane (eyes, nose and mouth)
  – Contamination by direct contact (ie. Hands to face) or via aerosols and splatters
  – Inadequate disinfection of work area
  – Improper handling of PPE and waste
  – Poor hand hygiene

• Ingestion
  – Entry via the mouth or nose by direct contact or via aerosols and splatters
Routes of Exposure

Environment (local plant and animal life)

- Inadequate filtration of aerosols
- Inadequate treatment of waste
- Contaminated personal items or body
How Can You Protect Yourself?

• Exposure Prevention Strategies
  – Training
  – Work Practices/Standard Precautions (Administrative Controls)
  – Protective equipment: PPE/Safety Equipment (Engineering Controls)
  – Vaccination
  – Exposure response
How Can You Protect Yourself?

• Administrative Controls
  – Use attenuated or non-virulent strains
  – Limit the use of sharps and glassware
  – Standardized decontamination procedures
  – Limited access
  – Require restraint during animal injection
  – Training
How Can You Protect Yourself?

• Engineering Controls (Safety Equipment and PPE)
  – Gloves
  – Lab Coat/Gown
  – Safety Glasses/goggles
  – Facial protection
  – Close toed shoes
  – Biosafety Cabinets
  – Vacuum line HEPA Filters
  – Sealed Rotors/Safety Cups
  – Pipette Aids
  – Safety Enclosures for Cell Sorters
Biological Safety Cabinets

- Protection against particulates only
- No gas or vapor protection
- 70% return 30% exhausted
- Air flow is easily disrupted
- Don’t cover the grill
- Move arms slowly in and out of cabinet
- Walk slowly
Biological Safety Cabinets

• Must be Certified Annually
• BSC Provides:
  – Product Protection
  – Personnel Protection
  – Environmental Protection
• Use biosafety cabinets (class II) for work with infectious agents involving:
  – Aerosols and splashes
  – Large volumes
  – High concentrations
  – BSL-2 materials
Centrifuges

Safety Cups and Rotors

• Load and unload safety cups inside the BSC
Vacuum Line HEPA Filter Protection

- Placed in between the vacuum system and the aspiration flasks
Biosafety Level 1

Standard Microbiological Practices

• Wash hands after handling viable materials, after removing gloves, and before leaving the laboratory.
• Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the laboratory.
• Perform procedures carefully to minimize the creation of splashes or aerosols.
• Lab equipment and work surfaces must be decontaminated with an effective disinfectant on a daily basis after work with potentially infectious materials is finished and after any spill or splash of viable material.
• A biohazard sign must be present outside all lab entryways, identifying the Biosafety Level and PI and safety supervisor contact information.
• An insect and rodent control program must be in place.
Biosafety Level 1

Standard Microbiological Practices (continued)

• Sharps:
  – Needles and syringes or other sharp instruments should be restricted to use only when there is no alternative.
  – Only needle-locking syringes or disposable syringe-needle units are used for injection or aspiration of potentially infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
  – Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
  – Broken glassware is not handled directly by hand, but is removed by mechanical means, such as a brush and dustpan, tongs, or forceps.
Biosafety Level 1

Engineering Controls

- Hand washing sink and disinfecting soap available in the lab.
- Non-porous, easily cleanable surfaces (benchtops & chairs)
- Face protection (goggles, mask, face shield/splatter guard) must be used for anticipated splashes or sprays of potentially infectious or other hazardous materials to the face when the materials must be manipulated outside the Biological Safety Cabinet.
- Gloves must be worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated and removed when the integrity of the glove is compromised.
Biosafety Level 2

Standard Microbiological Practices (BSL-1), plus…

• Immunizations or tests must be made available to lab personnel (at no cost) for the agents handled or potentially present in the lab (e.g. hepatitis B vaccine or TB skin testing).

• Access to the laboratory will be limited or restricted when work with infectious agents is in progress.

• Infectious material and equipment used to store or handle such material (e.g.: freezer, incubator, BSC) must be labeled with the universal biohazard symbol.

• Infectious materials must be secured in locked locations when authorized personnel are not present.
Engineering Controls

All BSL-1 controls plus:

- Properly maintained Biological Safety Cabinet (BSC), preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever.
  - Procedures with a potential for creating infectious aerosols or splashes are conducted. (may include centrifuging, grinding, blending, vigorous shaking or mixing, sonication, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonated eggs).
  - High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open lab if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.
Engineering Controls

• Protective laboratory coats, gowns, or smocks designated for laboratory use must be worn. This protective clothing must be removed and left in the laboratory before leaving for non-laboratory areas. All protective clothing must be either disposed of in the laboratory or laundered by the institution. Protective clothing known to be contaminated with infectious material should be autoclaved prior to laundering.

• An eyewash station must be readily available.
Engineering Controls
The materials required for the Biohazard Spill Kit shall be available (though not necessarily consolidated in one location) in areas where work is being conducted with biohazardous materials. The supplies available in a Biohazard Spill Kit should include, but are not limited to:

- Nitrile disposable gloves
- Face masks, lab coats (or gowns), and disposable shoe covers (booties)
- Goggles or safety glasses with side shields
- Absorbent material, such as absorbent paper towels, granular absorbent material, etc.
- All-purpose disinfectant, such as normal household bleach (freshly diluted 1:10)
- Autoclavable bucket for diluting disinfectant (can be used to store the kit contents)
- Something disposable or easily disinfected such as tongs, forceps, manila folders, etc. for picking up broken glass, other contaminated sharps, or absorbent materials
- Biohazard sharps waste container(s)
- Autoclavable biohazard waste bags
Extreme precaution with contaminated needles or sharp instruments

- Limit use of sharps to procedures where they are absolutely necessary.
- Never recap needles.
- Place contaminated sharps into a puncture resistant sharps waste container immediately after use.
- Do not attempt to retrieve items from a sharps waste container.
Biohazardous waste includes all biohazardous materials previously defined and any items potentially contaminated, including:

- Culture dishes, flasks, storage containers
- Gloves, gowns, masks
- Pipette tips, sharps
- Any liquid contacting biohazardous materials (culture media)
- Animal carcasses and bedding
All biohazardous waste must be collected in an appropriate waste container and decontaminated before disposal, using an effective method.

Biohazardous waste containers must be...

- Rigid and puncture resistant
- Lined with a biohazard bag
- Labeled with the Universal Biohazard Symbol
- Covered with a lid when not in use
- Leak proof when in the upright position
- Closed prior to transport out of the immediate lab area

Glass containers (cell culture media) on the floor must have secondary containment in place.
Infectious agents (RG2/BSL-2) and some bodily fluids fall under the NCDENR Regulated Medical Waste Rules and require specific treatment procedures, including weekly validation of the autoclave cycle parameters or pre-approval of chemical disinfection method, as described in the UNCG Regulated Medical Waste Policy. Chemical disinfection of infectious waste must be approved by NCDEQ.

• Exceptions to this policy are items contaminated with blood or bodily fluids contained on bandages and personal hygiene products, and cleanup materials not related to research activities. These items are not required to be treated prior to disposal.
Waste Disposal Procedures

• Solid:
  – Autoclave

• Liquid:
  – Autoclave
  – *Chemically treated prior to drain disposal
  – **10% (total volume) sodium hypochlorite solution (Bleach; Hg free) for at least 30 minutes

*Chemical treatment of infectious waste must be formally approved by NCDEQ.

**Liquids treated with bleach should NOT be autoclaved.
Waste Disposal Procedures

Autoclave procedures

Solids
• Mark an “X” with autoclave tape over the biohazard symbol on the waste bag (preferably prior to waste collection).
• Place waste bag in stainless steel or polypropylene tray or pan in case liquids leak out.
• Be sure to use autoclavable bags and pans that will not melt.
• Do not overfill waste bags or the autoclave.
• Add 50-100 ml of water to each bag of solid waste if there is no naturally occurring water in the waste.
• Keep waste bag slightly open to allow steam penetration into the bag.
• After cooling, seal bag and place in an opaque trash bag for disposal.
Waste Disposal Procedures

Autoclave procedures

Liquids

- Avoid using glass containers if possible.
- Containers should not be filled to more than 75% capacity.
- Leave containers unsealed
- Place containers in stainless steel or polypropylene tray or pan in case liquids overflow.
- Autoclaved liquid waste may be discharged directly into the sanitary sewer.
# BSL2 Waste Disposal

Appropriate treatment method for specific types of biological waste, which includes any other materials or equipment, including PPE, contaminated with the biological material.

<table>
<thead>
<tr>
<th>Waste Type</th>
<th>Treatment Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stocks and cultures of nonpathogenic materials and microorganisms (RG/BSL-1)</td>
<td>Autoclave or chemical treatment</td>
</tr>
<tr>
<td>Pathogenic microorganisms (RG/BSL ≥ 2)</td>
<td>³Validated autoclave or approved chemical treatment</td>
</tr>
<tr>
<td>Human blood, blood components and products, and OPIM², in individual</td>
<td>Autoclave or chemical treatment</td>
</tr>
<tr>
<td>containers less than 20mL</td>
<td></td>
</tr>
<tr>
<td>Human blood, blood components and products, and OPIM², in individual</td>
<td>³Validated autoclave or approved chemical treatment</td>
</tr>
<tr>
<td>containers greater than 20mL</td>
<td></td>
</tr>
<tr>
<td>Animal or Human primary cells, cell lines, and culture media</td>
<td>Autoclave or chemical treatment</td>
</tr>
<tr>
<td>rDNA waste, transgenic flies and plants</td>
<td>Autoclave or chemical treatment</td>
</tr>
<tr>
<td>¹BSL1 rDNA waste which is not in organisms or viruses</td>
<td>None required</td>
</tr>
<tr>
<td>Unfixed tissue, cells (and culture media), and fluids from humans or animals</td>
<td>³Validated autoclave or approved chemical treatment</td>
</tr>
<tr>
<td>known to have been infected with human pathogens.</td>
<td></td>
</tr>
<tr>
<td>Animal tissues, organs, parts</td>
<td>Autoclave or incineration</td>
</tr>
<tr>
<td>Animal carcasses</td>
<td>Incineration by commercial vendor</td>
</tr>
</tbody>
</table>

1BSL1 rDNA waste which is not in organisms or viruses (e.g., DNA, RNA, oligos waste, PCR waste, Microarrays, etc.). rDNA waste from experiments which use Escherichia coli K-12 host-vector systems that use only non-conjugative plasmids as vectors (e.g., pBR322, pBR313) and does not contain conjugation proficient plasmids or generalized transducing phages. rDNA waste from experiments involving Saccharomyces cerevisiae and Saccharomyces uvarum host-vector systems.

2Other Potentially Infectious Materials (OPIM) are defined as human semen; vaginal secretions; cerebrospinal, synovial, pleural, pericardial, peritoneal, and amniotic fluids; and body fluids visibly contaminated with blood or in situations where it is difficult to differentiate between body fluids.

3See UNCG Regulated Medical Waste Policy for specific treatment requirements.
Sharps Disposal

All sharps should be disposed of directly into an approved sharps container immediately after use. Do not attempt to recap, bend or break needles. Sharps containers must be hard walled, spill-proof when upright, and labeled with the universal biohazard symbol.

Prepare for disposal when the sharps container is 2/3 full:

- Mark an “X” over the biohazard label with autoclave tape or place in an autoclave bag.
- Autoclave, following procedures for solid waste.
- Seal container and bag prior to disposal.
Biological Spills & Disinfection

Spill Kits

• Location
  – Inside Tissue Culture Rooms

• Contents
  – Gloves, Goggles/shield, N95 Respirator, Lab coat/gown
  – Absorbent material
  – Bleach or suitable disinfectant
  – Tongs and dust pan

• Spill Procedures
  – Place absorbent material over spill
  – Pour disinfectant around outer edges of absorbent ending in the middle
  – Wait for inactivation of bio-hazardous material
  – Properly dispose of all materials in biohazard waste container
OSHA Bloodborne Pathogens Standard

- Occupational Exposure Determination
- BBP Exposure Control Plan
- Training
- Hepatitis B Vaccine
- Exposure response
- Regulatory Text: OSHA 1910.1030

Bloodborne Pathogens

Occupational Exposure

- People at risk for skin, eye, mucous membrane, or parenteral/subcutaneous contact with blood or *OPIM that may result from the performance of an employee’s duties. Not to be confused with an exposure incident or event.

- *OPIM: Other Potentially Infectious Materials include human: primary cells, cell lines unfixed tissues, semen, vaginal secretions; cerebrospinal, synovial, pleural, pericardial, peritoneal, and amniotic fluids; and body fluids visibly contaminated with blood or in situations where it is difficult to differentiate between body fluids.
Bloodborne Pathogens

BBP Exposure Control Plan

• All individuals at UNCG who are deemed to have an occupational exposure (AT RISK for exposure, not an actual exposure incident) must enroll in the UNCG Bloodborne Pathogens Exposure Control Plan, receive annual training, and be offered the Hepatitis B vaccine.

• Links to the BBP Exposure Control Plan and the Enrollment Form are available on the EH&S Laboratory Safety Website: https://safety.uncg.edu/LaboratorySafety.html
Bloodborne Pathogens

Training

• Must be completed at the time of initial assignment to tasks where occupational exposure may take place, and annually thereafter.
Bloodborne Pathogens

Hepatitis B

• A contagious liver disease caused by the Hepatitis B virus, which ranges in severity from a mild illness lasting a few weeks to a serious, lifelong illness.

• In the United States, an estimated 700,000 to 1.5 million persons have chronic hepatitis B virus infection. (CDC)
Bloodborne Pathogens

Hepatitis B Vaccine

• Provides greater than 90% protection against the virus. (CDC)
• Administered by a series of three injections, the second administered one month after the first, and the third administered five months after the second injection.
• Provided at no cost to the employee.
• If you decline the HBV vaccine, you must sign a declination form.
Post Exposure Response

What is a BBP Exposure?
• Any contact of:
  – Human blood, blood products, unfixed tissue, or OPIM
To:
  – Mucous membrane (mouth, nose, eyes, trachea, lungs, stomach, intestine)
  – Broken Skin (cuts, abrasions, punctures)
By:
  – Inhalation
  – Ingestion
  – Needle stick
  – Contact to wounds
  – Animal Bites
Post Exposure Response

Immediate Response

• For needle sticks, cuts from contaminated objects, animal bites or scratches:
  – Remove contaminated gloves and clothing, if possible.
  – Allow the wound to bleed freely for a minute.
  – Wash with soap and water for 5 minutes and apply sterile gauze or a bandage if necessary.
  – Decontaminate and remove protective clothing and report to Gove Student Health Center.
  – If the injury requires immediate medical attention, go to the Emergency Room.

• For mucous membrane exposures (eye, nose, or mouth):
  – Rinse tissue with copious amounts of water.
  – Flush eyes for 5 minutes using the emergency eyewash station.
  – Decontaminate and remove protective lab clothing and report to Gove Student Health Center.
Post Exposure Response

Reporting:

• Immediately report the incident to your supervisor and/or immediately call or report to Gove Student Health Center.

• After initial medical care is provided, report the exposure to EH&S.

• Gove Student Health Center 336-334-5340
• Environmental Health & Safety 336-334-4357
Lab Specific Details

In addition to this training module, the Biological Hazards section of your Lab Safety Plan should be reviewed to identify the specific bloodborne pathogens risks and safety practices for your lab.
Post Test

Take the post-test