INDIANA UNIVERSITY
Institutional Biological Safety Manual for
BLOOMINGTON, EAST, KOKOMO, NORTHWEST, SOUTH BEND, SOUTHEAST

Prepared June 2011
Adapted from the Indiana University Purdue University Indianapolis (IUPUI) Biosafety Manual, May 2002
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## IMPORTANT TELEPHONE NUMBERS

**Assistance Telephone Numbers, all campuses**
- Biological Safety Office: 812-856-5360
- Chemical Hygiene Officer: 812-855-5454
- Laboratory Safety Manager: 812-855-5454
- Radiation Safety Officer: 812-855-3230

**IU Bloomington Emergency Telephone Numbers**

- *Emergencies 911*
  - Biosafety Emergencies: 812-856-5360
  - Chemical Emergencies: 812-855-6311
  - Radiation Emergencies: 812-855-3230
  - Facilities & Maintenance: 812-855-8728
  - Hazardous Waste Pick-up: 812-855-6311
  - Non-Emergencies (IUPD): 812-855-4111

*All on-campus 911 calls in on the Bloomington campus are received by Indiana University Police Department (IUPD). IUPD will call additional emergency responders as needed. The 911 system is enhanced; calls from campus locations will display the location of the caller. 911 calls from cell phone or off campus locations are received by the City of Bloomington. Bloomington dispatchers will route Indiana University Bloomington campus emergency calls to IUPD.*

## EMERGENCY TELEPHONE NUMBERS, REGIONAL CAMPUSES

**IU Southeast**

- Emergencies: 911
- Biosafety Emergencies: 812-941-2636
- Chemical Emergencies: 812-941-2636
- Radiation Emergencies: 812-941-2636
- Non-Emergencies (Police): 812-941-2400
- Facility & Maintenance: 812-941-2330

**IU North**

- Emergencies: 219-980-6501
- Biosafety Emergencies: 219-981-4230
- Chemical Emergencies: 219-981-4230
- Radiation Emergencies: 219-980-6520
- Non-Emergencies (Police): 219-980-6501
- Facility & Maintenance: 219-980-6710
- EH&S: 219-981-4230

**IU South Bend**

- Emergencies: 911
- Biosafety Emergencies: 812-856-5360
- Chemical Emergencies: 812-855-6311
- Radiation Emergencies: 812-855-3230
- Non-Emergencies (Police): 574-520-4239
- Facility & Maintenance: 574-520-4386
- EH&S: 574-520-4575

**IU East**

- Emergencies: 911
- Biosafety Emergencies: 812-856-5360
- Chemical Emergencies: 812-855-6311
- Radiation Emergencies: 812-855-3230
- Non-Emergencies (Police): 765-973-8429
- Facility & Maintenance: 765-973-8254
- EH&S: 765-973-8254

**IU Kokomo**

- Emergencies: 911
- Biosafety Emergencies: 765-455-9371, 765-455-9281
- Chemical Emergencies: 765-455-9313
- Radiation Emergencies: 765-455-9305
- Non-Emergencies (Police): 765-455-9363
- Facility & Maintenance: 765-455-9273
- EH&S: 765-455-9313
SECTION 1 POLICY STATEMENT

Purpose

This is a statement of official Indiana University policy for Bloomington and Regional campuses to establish the process for compliance with the following documents available on the publications page of the Office of Research Administration Bloomington Institutional Biosafety Committee web site at http://www.researchadmin.iu.edu/Biosafety/IUB/bio_home.html. Related documents, such as the Exposure Control Plan and Waste Disposal Guidelines, are available on the Environmental Health and Safety web site at http://ehs.iu.edu/.

This manual was adapted from the IUPUI Biosafety Manual and the following documents:

National Institutes of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), March 2013 edition
• Recombinant and synthetic nucleic acid molecules

Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition.
• Guidelines for biological safety principles, practices and containment
• http://www.cdc.gov/biosafety/publications/bmbl5/

Occupational Health and Safety Administration Bloodborne Pathogens Standard 1910.1030
• Employee health and safety, federal law
• See IU Bloomington Exposure Control Plan for details

Policy

Indiana University is actively committed to preserving the health and safety of its students, staff, and faculty, and to protecting the environment and the community. It is recognized that use of potentially pathogenic microorganisms and organisms containing recombinant or synthetic nucleic acid molecules is necessary in many University research and teaching laboratories. To ensure the safe handling of these organisms, the University requires compliance with the NIH Guidelines and with the recommendations in the BMBL. Compliance with other applicable federal, state, and local regulations is also required.
SECTION 2 RESPONSIBILITIES

The Principal Investigator (PI) is directly and primarily responsible for the safe operation of the research or teaching laboratory, full compliance with *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed. (BMBL), the *National Institutes of Health NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)* and adherence to the policies and procedures of the IU Bloomington Institutional Biosafety Committee (IBC), the Institutional Animal Care and Use Committee (IACUC), and the Institutional Review Board (IRB). The Bloomington IBC oversees recombinant or synthetic nucleic acid molecule research, Bloodborne Pathogens, and biohazardous research as described in the IBC Standard Operating Procedure (IBC SOP) on the Bloomington campus and Regional Indiana University campuses Northwest, South Bend, Kokomo, East, and Southeast. Each campus with an active IACUC and/or IRB should adhere to the policies and procedures set forth by the oversight committee at their location. The IU Bloomington IBC does not oversee research conducted by Indiana University School of Medicine faculty located at these Regional campuses.

All laboratory personnel, IU staff and visitors are to be informed of the hazards associated with the work and proper safety precautions. It is a continuing process that begins before a new employee starts laboratory work and requires regular supervision and emphasis.

The PI must register all research or teaching activities involving recombinant or synthetic nucleic acid molecules, human, and animal pathogens, human tissues, fluids or cell lines, and animal tissues known or suspected to be contaminated with infectious agents with the IBC. The IU Institutional Biological Safety Manual is designed to assist investigators in the risk assessment process by describing safety practices, containment equipment, and facilities for the agent(s) used.

2.1 Laboratory Specific Biosafety Manual for BSL-2 Laboratories

When biohazardous materials are used in research (Biosafety Level 2, Animal Biosafety Level 2, toxins and attenuated pathogens manipulated in Biosafety Level 1 laboratories) the PI is also responsible for preparing a laboratory specific biosafety manual. Completion of biohazards sections of the IBC Protocol Registration form fulfills this requirement. The Biosafety Office is available to assist PI’s in developing laboratory specific biosafety manuals.


The Public Health Agency of Canada “Pathogen Safety Data Sheet (PSDS) for Infectious Substances” corresponding to each organism under study provides much of this information. PSDSs can be accessed through the Public Health Agency of Canada website at [http://www.phac-aspc.gc.ca/msds-ftss/index-eng.php](http://www.phac-aspc.gc.ca/msds-ftss/index-eng.php).

Laboratories research that utilizes human or nonhuman primate tissues, fluids, or cell lines, including commercially available cell lines, must complete the Exposure Determination as required by OSHA and the Environmental Health and Safety Exposure Control Plan (ECP). Completion of Form I of the IBC
Protocol Registration form fulfills this requirement. For informational purposes, the questions asked on the IBC protocol submission form that fulfill the requirement for a laboratory specific biosafety manual are provided at the end of this section. The Exposure Control Plan is available at http://www.ehs.indiana.edu/em/BBP_ECP_12_2010.pdf.

Safety should be a regular topic in laboratory personnel meetings. Written information and reference material should be made available to laboratory personnel. PI knowledge and judgment are critical in assessing risks and appropriately applying the recommendations in this manual. However, safety is a shared responsibility among all of the laboratory personnel. Many resources exist to assist the PI with these responsibilities, including the Biosafety Office, Environmental Health and Safety, the Institutional Biosafety Committee, and the Office of Research Administration.

2.2 Roles and Responsibilities

The Biosafety Office (BSO) shall:

- Assist and advise the IBC in preparing the Institutional Biosafety Manual, with revisions as necessary;
- Make the Manual available to each laboratory performing work with biological materials;
- One or more BSO representatives serve as voting Members of the Institutional Biosafety Committee;
- Develop and implement a comprehensive Biosafety program for the Indiana University Bloomington and Regional campuses;
- Together with the IBC, oversee review of research projects utilizing Biosafety Levels 1-3, Animal Biosafety Levels 1-3, and Plant (recombinant or synthetic nucleic acid research only) Biosafety Levels 1 and 2 on Indiana University and Regional campuses and conduct all relevant biosafety training and inspections for these facilities;
- Conduct periodic laboratory inspections to ensure that appropriate laboratory standards as determined by the IBC are rigorously followed;
- Report to the IBC and the institution any significant problems, violations of the NIH Guidelines, and any significant research-related accidents or illnesses of which the Biosafety Office becomes aware unless the Biosafety Office determines that a report has already been filed by the PI;
- Develop emergency plans for handling accidental spills and personnel contamination and investigating laboratory accidents involving recombinant or synthetic nucleic acid or biohazards research;
- Provide advice on laboratory security, providing technical advice to PIs and the IBC on research safety procedures;
- Conduct biosafety risk assessments and training to ensure all investigators at the University are in compliance with all applicable federal biosafety laws and regulations;
- Collaborate with investigators and staff in all matters related to biosafety;
- Provide expertise for the design and management of containment facilities; and
- Serve the University as a resource in all aspects of education and training in biosafety.
The Principal Investigators shall:

- Make the initial determination of the required levels of physical and biological containment in accordance with the NIH Guidelines and the most recent edition of the Biosafety in Microbiological and Biomedical Laboratories (BMBL) and perform a risk assessment;
- Select the appropriate microbiological practices and laboratory techniques to be used for the research;
- Ensure that all laboratory personnel listed on the protocol have access to the currently approved version of the PI’s IU IBC protocol, the IU Institutional Biological Safety Manual, and the laboratory specific biosafety manual, and have thoroughly read them;
- Ensure that the laboratory personnel listed on the IBC protocol have sufficient knowledge and are sufficiently trained to safely perform the responsibilities to which they have been assigned;
- Ensure that the laboratory personnel fully understand the steps necessary following any spills or potential exposures with the agents described in the protocol;
- In accordance with the Indiana University Bloomington Incident, Accident, and Exposure Reporting policy, immediately report any one or more of the following events:
  - Any incident which results in the release of recombinant DNA to the environment (including escape of a transgenic animal).
  - Any significant spill of recombinant DNA-containing material outside of a biological safety cabinet. A significant spill is defined as a spill of recombinant or synthetic nucleic acid containing material which requires emergency spill response or other environmental remediation.
  - Any significant problems at any biosafety level pertaining to the operation and implementation of containment practices and procedures, violations of the NIH Guidelines. Such incidents are reported to NIH within 30 days.
  - Any significant research-related incidents and illnesses (including needle sticks, and bites from transgenic or infected animals) whether or not exposure leads to illness. Such incidents are reported to NIH within 30 days.
  - Spills and accidents in Biosafety Level 2 involving wild type infectious organisms, organisms containing recombinant or synthetic nucleic acid molecules, or potentially infectious material which result in overt personnel exposure. Such incidents are reported to NIH immediately with follow up report within 30 days. When incidents take place in ABSL-1 or ABSL-2 the Animal Facility Director must also be notified.
  - Spills and accidents in Biosafety Level 3 involving wild type infectious organisms, organisms containing recombinant or synthetic nucleic acid molecules, or potentially infectious material which result in potential or overt personnel exposure. Such incidents are reported to NIH immediately with follow up report within 30 days. When incidents take place in ABSL-3 the Animal Facility Director must also be notified.
  - Spills and accidents in any NIH nonexempt animal laboratory that result in environmental release or exposures of animals or laboratory workers to organisms containing recombinant or synthetic nucleic acid molecules. These incidents must also be reported to the Animal Facility Director. Such incidents are reported to NIH immediately with follow up report within 30 days.
  - Any Biosafety Level 2-Plant greenhouse or laboratory accident involving the inadvertent release or spill of microorganisms. When incidents take place in the greenhouse the
Greenhouse Director must also be notified. Such incidents are reported to NIH immediately with follow up report within 30 days.

- Comply with this Manual, the IU Exposure Control Plan, the Laboratory Chemical Safety Plan, the University Radiation Safety Manual, and all other applicable University Operating Procedures relating to safety and health;
- Fill out and submit all relevant forms pertaining to the following experiments with the Institutional Biosafety Committee, Institutional Review Board, and/or the Institutional Animal Care and Use Committee, as required:
  - recombinant or synthetic nucleic acid activities;
  - work with infectious agents;
  - experiments that involve human subjects;
  - experiments involving the use of human blood or other potentially infectious materials, such as unfixed human tissues, primary human cell lines, and certain body fluids; and
  - experiments involving vertebrate animals
- Comply with all applicable state and federal regulations and guidelines.

The IU Bloomington – Office of Research Administration Incident, Accident, and Exposure Reporting policy is available on the Bloomington and Regionals Biological Safety webpage under Policies at http://researchadmin.iu.edu/Biosafety/IUB/bio_policies.html.

The Institutional Biosafety Committee shall:
- Periodically review recombinant or synthetic nucleic acids research conducted at the institution to ensure compliance with the NIH Guidelines;
- Make an independent assessment of the containment levels required by the NIH Guidelines;
- Review research and teaching conducted at the IU Bloomington and Regional campuses involving biohazards or recombinant or synthetic nucleic acids for compliance with the NIH Guidelines, recommendations in the BMBL and the policies of the IU Bloomington IBC;
- Lower containment levels for certain experiments as specified in Section III-D-2-a Experiments in which natural or synthetic DNA from Risk Group 2, Risk Group 3, or Restricted Agents are cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems;
- Set containment levels as specified in Sections III-D-4-b, Experiments Involving Whole Animals, and III-D-5, Experiments Involving Whole Plants;
- Work with the NIH to establish containment requirements for those experiments that are not explicitly covered by the NIH Guidelines;
- Notify the Principal Investigator of the results of the IBC’s review and approval;
- Report any significant problems with or violation of the NIH Guidelines and any significant research-related accidents or illnesses to the appropriate institutional official and the NIH Office of Biotechnology Activities within 30 days, unless the IBC determines that a report has already been filed by the PI;
- Immediately report any suspected BSL-3 or confirmed BSL-2 research-related illnesses, and any accidents or, significant problems with or violations of the NIH Guidelines to the appropriate institutional officials and the NIH Office of Biotechnology Activities;
- Adopt emergency plans covering accidental spills and personnel contamination resulting from research involving recombinant or synthetic nucleic acids or biohazards;
- Follow the guidelines for IBC committee composition as defined by the NIH.
• On behalf of the institute, assure that laboratory personnel, Laboratory Animal Resources (LAR) and IU staff safety and health concerns are addressed as part of an animal protocol review.

2.3 Laboratory Specific Biosafety Manual

Information that may be asked for on IBC protocol submission that fulfills the requirement for a laboratory specific biosafety manual:

• A list of all disease-causing microorganisms that will be cultured (bacteria, viruses, fungi, parasites, protozoans), including attenuated strains, including Risk Group of wild type organism.
• The natural route of transmission/exposure
• The laboratory route of transmission or exposure
• The host range
• A description of lab specific procedures for accidents, exposures and/or emergencies, if they are different from the Indiana University Institutional Biological Safety Manual
• A list of potentially aerosol generating procedures and a description of aerosol containment
• Personal protective equipment and safety equipment in use

Medical Surveillance All Laboratories:

• IU employees who receive their paycheck through IU Payroll are eligible for Workers Compensation benefits if injured while performing a task for which they are being paid by Payroll.
• IU employees and student who are unpaid, or who receive a paycheck through the Bursar’s Office are not eligible for Worker’s Compensation benefits.

2.4 Exposure Determination

Under the OSHA 1910.1030 Bloodborne Pathogen Standard, an Exposure Determination is required in areas where materials covered by the Bloodborne Pathogen Standard are manipulated. An Exposure Determination is an evaluation of an employee’s tasks in order to determine which tasks have the potential to expose the employee to Bloodborne Pathogens.

When all personnel working in an area have the same potential for exposure, a list of names and position titles is sufficient. When a limited number of employees working in an area have potential for exposure, a list of names, position titles, and responsibilities that may lead to exposure must be provided. At IU Bloomington and Regional campuses, materials covered by the Bloodborne Pathogens Standard are manipulated in biosafety level 2 laboratories, all personnel working in the laboratory are considered to have potential for exposure. Therefore, IBC Form I, Protocol Registration Form, asks for a list of personnel and position titles.
SECTION 3 EMERGENCY PROCEDURES

Section 3.1 Biological Spills

A spill kit should be kept in each biosafety level 2 laboratory where work with recombinant or synthetic nucleic acid, infectious microorganisms or other biohazards are conducted. Biological spill kits will be combined with chemical spill kits whenever possible. Biological spill kits will be provided for Biosafety Level 2 labs where needed. Basic equipment includes:

- 1 bucket
- 1 instruction sheet with contents
- 1-24oz bottle of commercial bleach
- 1 spray bottle for making 20% bleach solution
- Paper towels
- 1 pair of forceps
- broom/dust pan set
- pairs of Nitrile glove sets (2-Med and 2-Lg) each size in a Ziploc bag
- clear autoclave bags
- 2 laminated biological spill signs (orange)
- 2 pair of disposable scrubs (1-Lrg and 1-XXL), for use when personal clothing has been chemically or biologically contaminated

Biological spill kit supplies may be shared with other labs. The Biosafety Office will provide replacements supplies as needed, provided the BSO has a record of biological or chemical incidents requiring use of items in the kit.

General Spill Cleanup Guidelines
Disinfectants are only effective when appropriate concentration and contact time are used. The disinfectant used should effectively inactivate the recombinant or synthetic nucleic acid, organism, or material spilled. Review Section 4 Decontamination, for details regarding appropriate dilutions and effectiveness of disinfectants, or contact the Biosafety Office for assistance.

Specific Spill Cleanup Guidelines

Spill of Laboratory, Animal, or Plant material in BSL-1 (recombinant or synthetic nucleic acid, genetically modified material unlikely to pose a risk of infection)
- Inform Principal Investigator (PI) as soon as possible.
- Wear gloves, a lab coat, and eye protection.
- Place paper towels over the spill. Pour disinfectant over the paper towels.
- Allow a 15 minute contact time for disinfection. Add more disinfectant if evaporation occurs.
- Use forceps to pick up broken glass and discard into biohazard sharps.
- Discard paper towels and contaminated waste materials into biohazard waste container.
- Remove and bag lab coat and all contaminated clothing. If a disposable lab coat is used, discard the lab coat.
- Wash hands and exposed skin for 30 seconds or more with soap and running water.
- Autoclave all disposable towels, gloves, and other materials worn or used to clean up the spill. Autoclave or discard lab coat and all contaminated clothing. Do not autoclave bleach or items soaked in bleach.
- If spilled material contains recombinant or synthetic nucleic acid, or genetically modified material and the spill is such that it requires emergency response or environmental remediation, report the spill to the Biosafety Office.

Spill of Human Blood

- Inform Principal Investigator (PI) as soon as possible. Call the Biological Safety Office for assistance, if necessary.
- Wear gloves, a lab coat and eye protection.
- Dilute commercial bleach such that the final concentration of bleach in the total volume of spill plus water is 1:5, or 20%.
- Place paper towels over the spill. Pour 20% bleach over the paper towels.
- Allow a 15 minute contact time for disinfection. Add more disinfectant if evaporation occurs.
- Use forceps to pick up broken glass and discard into sharps container for contaminated sharps.
- Discard paper towels and contaminated waste materials into biohazard waste container.
- Wipe the spill site with paper towels soaked in 20% bleach.
- Follow with water. Use 70% ethanol if spill occurred on a metal surface to prevent the bleach from corroding the metal surface.
- Discard all contaminated materials into biohazard waste container.
- Remove and bag lab coat and all contaminated clothing. If a disposable lab coat is used, discard the lab coat.
- Wash hands and exposed skin for 30 seconds or more with soap and running water.
- Autoclave or discard lab coat and all contaminated clothing. Do not autoclave bleach or items soaked in bleach.
- Report the spill to the Biosafety Office.

Spill of Laboratory, Animal, or Plant material in BSL-2 (recombinant or synthetic nucleic acid, genetically modified material that may pose a risk to human health)

- Inform Principal Investigator (PI) as soon as possible. Call the Biological Safety Office for assistance, if necessary.
- Keep other workers out of the area to prevent spreading spilled material.
- Wear gloves and a lab coat. If needed, include face protection such as a face shield or mask.
- Assemble clean-up materials (Bio Spill Kit). Post a warning sign, if needed. Ask for assistance to avoid spreading biohazardous material around the lab.
- Cover the spill with paper towels and add appropriately diluted disinfectant.
- After at least 15 minutes contact time (30 minutes if agent is a spore forming organism), pick up the paper towels.
- Pick up any broken glass with forceps and dispose into sharps container for contaminated sharps.
- Re-wipe the spill area with diluted disinfectant.
- Decontaminate bottom of shoes prior to walking away from the spill.
- Collect all contaminated materials into biohazard waste container and autoclave.
Remove and bag lab coat and all contaminated clothing. If a disposable lab coat is used, discard the lab coat.

Wash hands and exposed skin for 30 seconds or more with soap and running water.

Autoclave or discard lab coat and all contaminated clothing. Do not autoclave bleach or items soaked in bleach.

Report the spill to the Biosafety Office.

**Spill in a Biological Safety Cabinet**

- Inform Principal Investigator (PI) as soon as possible. The Biosafety Office should be notified as soon as possible if the spill overflows into the grate in the interior of the cabinet. It may be necessary to do a more extensive decontamination of the cabinet.
- Wear gloves and a lab coat. Leave the cabinet turned on. Wait 5 minutes for aerosols to clear.
- Surface disinfect all items before removing them from the cabinet with disinfectant appropriate for the material in use.
- Cover the spill with paper towels. Pour disinfectant over paper towels. If spill overflowed the grate, pour disinfectant into the grate.

Note: If bleach is used, do NOT spray bleach in the biosafety cabinet. Spraying may cause aerosolized bleach to corrode surfaces and inaccessible portions of the biosafety cabinet.

- Allow 15 minutes contact time (30 minutes for spore forming organism). Continue adding disinfectant if evaporation occurs.
- Wipe cabinet walls, work surfaces, and equipment.
- Drain catch basin into a container. Lift front exhaust grille and tray, soak up excess spill and disinfectant with paper towels, and wipe all surfaces below the grille and tray with disinfectant.
- Ensure that no paper towels or solid debris are blown into area below the grille.
- Wipe down surfaces with 70% ethanol to remove any corrosive residues.
- Discard all clean-up materials into biohazard waste container.
- Wash hands and exposed skin areas with soap and running water.
- Report the spill to the Biosafety Office.

**Section 3.2 Spill of Biological Radioactive Material**

A spill involving both radioactive and biological materials requires emergency procedures that are different from the procedures used for either material alone. Do not use bleach solutions as a disinfectant on materials that contain iodinated compounds, because radioactive iodine gas may be released. Consult the Radiation Safety Manual for detailed radio-isotope spill clean-up procedures and for procedures to protect yourself from the radionuclide while you disinfect the biological material, or contact your applicable Radiation Safety Officer.

**Section 3.3 Illness or Injury Involving Biological Materials**

Refer to the Injury Procedure for All Campuses found at [http://hr.iu.edu/workers/index.html](http://hr.iu.edu/workers/index.html)

Accidents, exposures, potential exposures, clinical illness, and sero-conversions are to be reported to the Biosafety Office. Exposures include inoculation through cutaneous penetration, ingestion, contact with
mucus membranes (eyes, nose, mouth, etc.), probable inhalation following gross aerosolization, or any incident causing serious exposure to personnel or danger of environmental contamination.

For a Splash to the Eye
Use an emergency eyewash to immediately flush the eye with a gentle stream of clean, temperate water for 15 minutes. Hold the eyelid open. Be careful not to wash the contaminant into the other eye. Contact the most convenient local emergency room to obtain care. Report the accident to the PI immediately, and seek additional medical assistance if necessary. Contact the Biosafety Office as soon as possible.

For Contamination to the Body
Immediately remove contaminated clothing and drench skin with water. Wash with soap and running water, and flush the area for 15 minutes. Report the injury to the PI immediately and seek additional medical assistance if necessary. Contact the Biosafety Office as soon as possible.

Mild to Moderate Injuries
Wash the injured area with soap and water, pour antiseptic over the wound. Notify the PI immediately. Proceed to the appropriate location for medical treatment, if needed or required (all Bloodborne Pathogens injuries are required to be evaluated by the healthcare provider). Contact the Biosafety Office as soon as possible.

Severe Injuries (those that require immediate medical care by a trained medical professional/emergency responders)
Call 911 for assistance and transportation to the nearest emergency room. If possible, a second person should accompany the injured person to the medical facility and provide information to personnel about the accident/exposure. Report the accident to the PI as soon as possible. Contact the Biosafety Office.

Regional campuses: Follow instructions and obtain care from providers specific to the campus.

Employees (all persons who receive their paycheck thru IU Payroll):
- Submit the Bloomington Employer Authorization for Treatment form (http://hr.iu.edu/workers/index.html) before going to IU Occupational Health or the Bloomington Emergency Department. If this form is not filled out, you will be charged for medical care and will need to submit your receipts to IU Workers Compensation Services.
- Within 24 hours your supervisor must submit the Occupational/Injury Illness form (http://hr.iu.edu/workers/index.html)

Non-employees (unpaid students, visitors, persons who receive their paycheck from any IU unit other than Payroll):
- You or your supervisor must submit the Incident Reporting form (http://rmweb.indiana.edu/orm/Forms/Incident.cfm) to Risk Management within 24 hours.
Section 3.4 Fires Involving Biological Materials

- **If possible without placing yourself in danger**, put biological materials in a secure location, such as an incubator or freezer.
- Activate the building fire alarm.
- Leave the building at once.
- Call 911 from a safe location.
- Meet the fire department outside and direct them to the fire.

In the event of a fire, firefighting personnel should fight the fire as they would normally fight a laboratory fire. Personal protective gear normally worn by firefighters, including a self-contained breathing apparatus (SCBA) is appropriate for protection of firefighters from exposure to biological agents. The agent could be present in refrigerators, freezers, incubators, and biological safety cabinets, which should all be clearly marked with "biohazard" labels. If any of these locations are disrupted by firefighting activities, protective gear and other equipment can be decontaminated with a 10% solution of bleach or Lysol spray. The Biosafety Office will be on hand to assist and provide consultation regarding other questions on containment and decontamination of the area.

Section 3.5 Laboratory Security and Emergency Response Planning

Laboratory security is related to, but different from laboratory safety. Traditional laboratory biosafety guidelines emphasize the use of good work practices, appropriate containment equipment, well designed facilities, and administrative controls to minimize risks of accidental infection or injury for laboratory workers and to prevent contamination of the environment outside the laboratory.

The “Security guide for select agent or toxin facilities” has been developed by the Center for Disease Control and Prevention (CDC) Division of Select Agents and Toxins (DSAT) and the Animal Plant Health Inspection Service (APHIS) Agriculture Select Agent Program in response to concerns about the possible use of biological, chemical, and radioactive materials as agents for terrorism to address laboratory security issues (e.g., preventing unauthorized entry to laboratory areas and preventing unauthorized removal of dangerous biological agents from the laboratory) for laboratories using biological agents or toxins capable of causing serious or fatal illness.

The document was developed for select agent facilities, but may serve as a resource for Principal Investigators concerned about possible security incidents (e.g., undocumented visitors, missing chemicals, unusual or threatening phone calls). The document is available on the internet at: [http://www.selectagents.gov/SecurityRelatedInformation.html](http://www.selectagents.gov/SecurityRelatedInformation.html).

Reports of security incidents are to be sent to the local campus police department.
SECTION 4 DECONTAMINATION AND DISPOSAL

Section 4.1 Introduction

Chemical disinfectants are used to render a contaminated material safe for further handling, whether it is a material to be disposed of as waste, or a laboratory bench on which a spill has occurred. It is important to choose a disinfectant that has been proven effective against the organism being used. The following definitions are based on the book *Disinfection, Sterilization, and Preservation*, Seymour S. Block, 5th ed.¹

- Decontamination – disinfection or sterilization of contaminated articles to make them suitable for use
- Sterilizer – physical processor agent, usually chemical, that destroys or eliminates all forms of life, especially spores.
- Disinfectant – usually a chemical agent, will inactivate viruses or kills vegetative microbes, but not resistant forms such as spores.
- Hospital Disinfectant - agent shown to be effective against *S. aureus*, *S. choleresis* and *P. aeruginosa*. It may be effective against *M. tuberculosis*, pathogenic fungi or specifically named viruses.
- Antiseptic – prevents or arrests growth or action of microbes, either by inhibiting their activity or by destroying them. An agent formulated to be used on skin or tissue - not a disinfectant.
- Sanitizer – for the purposes of disinfection and decontamination, the term organic is used to describe materials that contain carbon or that are carbon based. Examples: Blood, media, proteins, lipids, bedding, feces, and organic matter also builds up in dust, dirt, and grime.

Section 4.2 Disinfectants Commonly Used in the Laboratory

Table 1 Important Characteristics Of Disinfectants

<table>
<thead>
<tr>
<th>Property</th>
<th>Hypochlorites “Household Bleach”</th>
<th>Ethyl Alcohol</th>
<th>Iodophor “Wescodyne”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shelf-life &gt; 1 week</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Corrosive</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Residue</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Inactivation by organic matter</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin Irritant</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Respiratory Irritant</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eye Irritant</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Toxic</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
Hypochlorites (commercial bleach, Sodium Hypochlorite)
The use concentration is dependent on the organic load of the material to be decontaminated. Use a 10% bleach or 0.5% sodium hypochlorite solution to disinfect clean surfaces. Use a final concentration of 20% bleach, or 1% sodium hypochlorite, to disinfect surfaces contaminated with a heavy organic load or liquid media.

- **Advantages**
  - Effective against vegetative bacteria, mycobacteria, fungi, most viruses.

- **Disadvantages**
  - Least effective against bacterial spores and prions. Higher concentration and longer contact times are necessary to inactivate spores and prions.
  - Very corrosive, rapidly inactivated by organic matter.
  - Solutions decompose rapidly; **fresh solutions should be made weekly**. If stored in tightly closed brown bottles, bleach solutions retain activity for 30 days.

Alcohols (ethanol, isopropanol)
The effective dilution is 70%. Higher concentrations evaporate too rapidly to achieve necessary contact times. Ethyl alcohol and isopropyl alcohol diluted to 70% in water are used for surface disinfection of materials that may be corroded by other chemical disinfectants. Contact time is critical; at least 15 minutes must be used. Additional 70% alcohol must be added to achieve sufficient contact time if evaporation occurs.

- **Advantages**
  - Effective against vegetative bacteria and enveloped viruses.
  - Fast acting and leaves no residue.
  - Non-corrosive.

- **Disadvantages**
  - Longer contact times required for activity against fungi and mycobacteria.
  - Variable effectiveness against non-enveloped viruses and Giardia cysts.
  - Not effective against bacterial spores, prions, or Cryptosporidium oocysts.
  - Longer contact times are difficult to achieve due to loss by evaporation.

Iodophors
Use full strength provided by the manufacturer, provided stock solution is 10%.

- **Advantages**
  - Effective against vegetative bacteria, fungi, and enveloped viruses.
  - Stable in storage if kept cool and tightly covered.
  - Built-in color indicator; if solution is brown or yellow, it is still active.
  - Relatively harmless to humans.

- **Disadvantages**
  - Variable effectiveness against mycobacteria, bacterial spores, and non-enveloped viruses.
  - Effectiveness reduced by organic matter (but not as much as with hypochlorites).
  - Staining of treated items, corrosive, neutralized by organic material

Quaternary Ammonium Compounds (QAC)
Dilute per manufacturer instructions.

- **Advantages**
Effectiveness against Gram positive bacteria.
- Limited effectiveness against enveloped viruses, fungi, and Gram negative bacteria.
- Odorless, colorless, non-irritating, deodorizing.

**Disadvantages**
- Not effective against non-enveloped viruses, mycobacteria, bacterial spores, Giardia cysts, Cryptosporidium oocysts, or prions.
- Innate detergent action, but may be inactivated in the presence of some soaps or soap residues.
- Inactivated by organic material.

**Phenolics**
Dilute per manufacturer instructions.

**Advantages**
- Effective against vegetative bacteria and enveloped viruses.
- Not easily neutralized by organic material
- Noncorrosive

**Disadvantages**
- Variable effectiveness against mycobacteria and fungi, depending on the product.
- Limited activity against non-enveloped viruses.
- No activity against bacterial spores.
- Toxic, neutralized by hard water, strong odor, leaves a residue.

**Section 4.3 Biological Waste Disposal Procedures**

The biological waste management program at IU Bloomington is administered by the Office of Environmental Health and Safety (EH&S) in accordance with state regulation 410 IAC 1-3, other applicable regulations, and University policies developed by the IUB Biosafety Office (BSO) and the Institutional Biosafety Committee (IBC).

Specific waste handling procedures may vary between campuses. However, these basic rules must be followed by all persons generating waste:

- No waste collected in red or orange sharps containers or red or orange autoclavable bags, or containers or bags of any color marked with a biohazard symbol may enter the normal waste stream on the Bloomington campus. The normal waste stream in Bloomington terminates at local landfills, waste collected in red containers is prohibited in the landfill.
  - Containers or bags with biohazard symbols may enter the normal waste stream after the biohazard symbol has been completely defaced (removed or marked out with black marker).
  - Contact Bloomington EH&S Waste Management for assistance, 812-855-6311.

- All waste that contains recombinant or synthetic nucleic acids or genetically modified materials from Biosafety Level 1 and 2 (BSL-1, BSL-2) and Animal Biosafety Level 1 and 2 (ABSL-1, ABSL-2) laboratories must be decontaminated prior to disposal.
- Waste from Plant Biosafety Level 1 and 2 (BSL1-P, BSL2-P) laboratories that contains recombinant or synthetic nucleic acid molecule or genetically modified materials must be
decontaminated, **transgenic plant material must be rendered biologically inactive prior to disposal.**

- All waste containing biohazards or materials covered by the Bloodborne Pathogen Standard must be decontaminated prior to disposal.
- Decontamination and disposal are the responsibility of the person/laboratory generating the waste.

**Biohazardous Sharps** are sharps that have been in contact with recombinant or synthetic nucleic acids or biohazardous materials. Materials that have been in contact with recombinant or synthetic nucleic acids or genetically modified materials in BSL-1 or BSL-2, ABSL-1 or ABSL-2, and BSL1-P or BSL2-P must be autoclaved prior to disposal.

<table>
<thead>
<tr>
<th>Item</th>
<th>Item</th>
<th>Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>Needles</td>
<td>Scalpel blades</td>
<td>Exacto knives</td>
</tr>
<tr>
<td>Syringes</td>
<td>Razor blades</td>
<td>Microtome blades</td>
</tr>
<tr>
<td>Lancets</td>
<td>Glass Pasteur pipettes</td>
<td>Any other sharp waste</td>
</tr>
</tbody>
</table>

Discard all contaminated needles, needle and syringe units, scalpels, and razor blades, directly into rigid, white or clear, labeled sharps containers with a biohazard symbol on it. The use of orange or red containers has been discontinued ON THE Bloomington campus, do not use red or orange sharps containers. Needles/syringes are never reused, they must be discarded. Do not recap, bend, remove or clip needles. Sharps containers should be discarded when they are 2/3 full. When full, close and allow the lid to lock, place autoclave tape over the lid, and autoclave the container. After autoclaving, the biohazard symbol on the sharps containers must be completely defaced or removed, then left for housekeeping staff pick-up.

Sharps that are contaminated with radioactive materials must be discarded into separate sharps containers labeled with the name of the isotope. Specify isotope content when requesting pick-up by Radiation Safety.

**Biohazardous Solids** include any solid materials that have been in contact with biohazards or recombinant or synthetic nucleic acids. This includes Petri plates, culture flasks or bottles, plastic, paper, gloves, pipette tips, etc.

**Biohazardous Liquids** include bacterial cultures, blood, blood products and other potentially infected materials, or liquids containing recombinant or synthetic nucleic acids in BSL-1 labs. All human blood and other potentially infectious materials should be handled using Universal Precautions. See the IU Exposure Control Plan (ECP) for details.

**Animal Carcasses and Tissues:** ALL research animals whether infectious, transgenic, domestic, or wild must be treated as biohazardous for disposal. Cages that housed experimentally infected animals in Animal BSL-2 containment must be autoclaved along with the components (bedding, food, water bottles, etc) prior to transport to Lab Animal Resources (LAR) Core Facilities, unless animal experiments result in chemical contamination of the cage, bedding and other contents. Contact EHS for guidance regarding cage handling and decontamination of cages containing chemical residues. See Section 4.6 for details regarding autoclave procedures for animal cages in ABSL-2 labs.

**NOTE:** Use of animals in research must be in accordance with protocols previously approved by the BIACUC. This includes field research and laboratory research.
Multi-hazard Waste (Mixed Waste): Avoid generating mixed waste if possible. Keep volume to a minimum. Do not autoclave mixed waste. When discarding waste containing an infectious agent and radioactive material, inactivate the infectious agent first, and then dispose of as radioactive waste. Seek advice from your applicable Radiation Safety Officer before beginning inactivation procedures. When discarding waste containing an infectious agent and a hazardous chemical, inactivate the infectious agent first, and then dispose of as chemical waste. Seek advice before beginning inactivation procedures.

Section 4.4 Autoclaving Procedures

Autoclaves use pressurized steam to destroy microorganisms, and are the most dependable system available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents. For efficient heat transfer, steam must flush the air out of the autoclave chamber. Before using the autoclave, check the drain screen at the bottom of the chamber and clean it if it is blocked. If the sieve is blocked with debris, a layer of air may form at the bottom of the autoclave, preventing efficient operation.

Autoclave Container Selection

Disposal Containers
Each laboratory is responsible for purchasing containers for the disposal of biological waste. The following types of containers are available:

- Sharps containers may be purchased from department stockrooms or various outside sources, such as from laboratory product distributors. They are available in various sizes, and must be puncture resistant, clear, white, or non-red, labeled as "Sharps," and have a tightly closing lid. Do not purchase "needle-cutter" devices, which may produce aerosols when used.
- Biohazard Autoclave Bags may be purchased from various laboratory product distributors, such as Fisher Scientific, VWR, Lab Safety Supply, and Baxter. Be sure to select bags that are able to withstand autoclaving.
- Polypropylene bags
Commonly called biohazard or autoclave bags, these bags are tear resistant, but can be punctured or burst in the autoclave. Therefore, place bags in a rigid container during autoclaving. Bags are available in a variety of sizes, and some are printed with an indicator that changes color when processed.
  - Polypropylene bags are impermeable to steam, and for this reason should not be twisted and taped shut, but gathered loosely at the top and secured with a large rubber band or autoclave tape. This will create an opening through which steam can penetrate. Only clear autoclavable bags may be used on the Bloomington campus in Laboratory, Animal, and Plant BSL-1 and BSL-2. If the clear bag is printed with a biohazard symbol, the biohazard symbol must be defaced after autoclaving and prior to pickup. Orange or red autoclavable bags are used for disposable materials in Animal BSL-1 and Animal BSL-2 and Laboratory and Animal BSL-3.

Polypropylene containers and pans
Polypropylene is a plastic capable of withstanding autoclaving, but resistant to heat transfer. Therefore, materials contained in a polypropylene pan will take longer to autoclave than the same materials in a
stainless steel pan. To decrease the time required to sterilize material in these containers, remove the lid or in the case of a vial insert a needle, turn the container on its side when possible, and select a container with the lowest sides and widest diameter possible for the autoclave.

**Stainless steel containers and pans**

Stainless steel is a good conductor of heat and is less likely to increase sterilizing time, but is more expensive than polypropylene.

**Preparation and Loading of Materials**

- Fill liquid containers only half full.
- Loosen caps, or use vented closures.
- Always put bags of biological waste into pans to catch spills.
- Position biohazard bags on their sides, with the bag neck closed loosely.
- Leave space between items to allow steam circulation.
- Household dishpans melt in the autoclave. Use autoclavable polypropylene or stainless steel pans.

**Cycle Selection**

- Use liquid cycle (slow exhaust) when autoclaving liquids to prevent contents from boiling over.
- Select fast exhaust cycle for glassware.

**Time Selection**

- Take into account the size of the articles to be autoclaved. A 2-liter flask containing 1 liter of liquid takes longer to sterilize than four 500-ml flasks each containing 250-ml of liquid.
- Material with a high insulating capacity (animal bedding, high-sided polyethylene containers) increases the time needed for the load to reach sterilizing temperatures.
- Biohazard autoclave bags should be autoclaved at a minimum of 121°C for 60 minutes under 15 pounds per square inch (PSI) of pressure to assure decontamination.

**Removing the Load**

- Check that the chamber pressure is zero.
- Wear lab coat, eye protection, heat insulating gloves, and closed-toe shoes.
- Stand behind door when opening it.
- Slowly open door only a crack. Beware of the rush of steam that may escape. Opening the door and removing liquids too quickly may result in flash boiling and personal injury.
- After the slow exhaust cycle, open autoclave door and allow liquids to cool for 20 minutes before removing.

**Autoclave Indicators**

Autoclave indicators are categorized into three fundamental groups; physical monitors, chemical indicators and biological indictors.

- **Physical monitors** are installed by the manufacturers to record the time, temperature, and pressure within the autoclave during the process cycle. Multiple physical monitors are installed to record the conditions in the chamber, jacket, and other locations within the equipment.
Chemical and biological indicators are used to verify conditions within the materials being sterilized and to verify the operation of the physical monitors.

- **Biological indicators** verify that all the parameters necessary for sterilization were present. They consist of a standardized population of bacterial spores known to be resistant to the mode of sterilization being utilized. Biological indicators are considered the most ideal monitors of the sterilization process because they measure the sterilization process directly by using the most resistant microorganisms (i.e., Bacillus spores), and not merely testing the physical and chemical conditions necessary for sterilization.

- **Chemical indicators** are intended to detect potential sterilization failures immediately that could result from incorrect packaging, incorrect loading of the sterilizer, or malfunctions of the sterilizer.

The Association for the Advancement of Medical Instrumentation has defined five classes of chemical indicators:

- **Class 1** (process indicators) include autoclave indicator tape and the indicators found on paper or plastic peel pouches. The color change on standard autoclave tape does not indicate the autoclave reached the appropriate time, temperature and pressure, and may not be used for autoclave validation testing.

- **Class 2** (Bowie Dick and Dart products) are used in steam sterilizers to test for the presence of air in the steam sterilizing chamber. A positive test would mean that air, which inhibits the conditions necessary for sterilization, has either not been removed during the cycle or has entered the chamber through leaks in the system.

- **Class 3** (single-parameter indicator) reacts to one of the critical process parameters of sterilization and indicates exposure to a sterilization cycle at stated values of the chosen parameter. Critical parameters typically chosen for steam sterilization processes are time or temperature.

- **Class 4** (multi-parameter indicator) indicators are more accurate by design than Class 3 indicators. They react to two or more critical parameters of the sterilization process and indicate exposure to the sterilization cycle at stated values of the chosen parameters. Time and temperature are examples of steam sterilization parameters, or time and concentration of ethylene oxide (EO) are chosen for EO sterilization.

- **Class 5** (integrating indicator) are designed to react to all critical parameters over a specified range of sterilization cycles. Their performance has been correlated to the performance of a biological indicator (BI) under its labeled conditions for use. **This class of indicator can be used in place of the BI.**

**Section 4.5 Autoclave Validation**

Autoclaves used to decontaminate laboratory waste should be tested periodically to assure effectiveness. Only Class 4 or 5 indicators may be used in autoclave validation testing. Both types of tests should be placed well down in the center of the bag or container of waste, at the point slowest to heat. If you need assistance, please contact the Biological Safety Office.

The Biosafety Office validates autoclave function of General Use autoclaves. General Use autoclaves include those located in common areas, and those used to sterilize classroom waste.
PI’s are responsible for validating Personal Use autoclaves. Personal Use autoclaves are those that are purchased and maintained by a PI, located within a laboratory. The Biosafety Office provides the Biological Indicators, instructions, and incubator for Personal Use autoclave validations.

Section 4.6 Standard Operating Procedure for Research Staff Autoclaving of Dirty ABSL-2 Animal Cages following the use of bacterial pathogens that do not form heat-stable spores

1. Using a paper clip, attach 3M Comply Sterigage Chemical Indicator (catalog #1243a or another brand of Class 4 or Class 5 chemical indicator strip) to a string. The string attached to the indicator should be threaded between the cage lid and cage, and the indicator placed in the used bedding of one cage per bag.
2. Leave all cage components as they are in the cage and close the lid.
3. Place the cages with the lids on into an autoclave bag. Loosely tape the bag shut. Do not make airtight. Do not stack the cages.
4. Add Autoclave Indicator Tape to each bag and write the PI’s underlined initials on each bag.
5. Used animal cages should not be stored in the animal facility; they should be autoclaved as soon as reasonably possible or at the end of each experiment. Do not leave them unattended in the autoclave room.
6. Before leaving the animal room with the cages, spray the outside of the bags with 70% ethanol, QAC, or 10% bleach or disinfectant specified by the IBC.
7. Place the bags in the autoclave. Do not stack the bags in the autoclave.
8. Attach an outside 3M Comply Sterigage Chemical Indicator strip on the outside of each bag.
9. Autoclave at 121°C for 60 minutes. Confirm that the autoclave reaches proper temperature and pressure before leaving the room.
10. Attach a sign that says “Biohazardous Waste – Do Not Remove” to the autoclave door.
11. When the run has finished, check that the external 3M Comply Sterigage Chemical Indicator show “accept” for temperature and steam.
12. Unseal the bags and check the internal indicators in each cage to make sure they pass. Each 3M Comply Sterigage Chemical Indicator should indicate “accept”.
13. Assuming that the load passes, reseal the bags so that the internal 3M Comply Sterigage Chemical Indicator is taped to the opening of the bag in such a way that LAR staff can read the indicator. Both/all indicators must read “accepted”.
   a. If any one indicator does not read “accepted”, LAR staff should call the supervisor and Director of Biosafety and NOT touch the bag.
   b. Every bag should have a minimum of 2 indicators, one run externally, one run internally. If the load fails, re-autoclave with new indicators in a different autoclave and inform the Building Manager that the autoclave needs service.
14. The passed bags can now be turned over to LAR personnel for disposal of the bedding and washing of the cages, bottles, and tops. LAR personnel should confirm that the internal indicators passed.
SECTION 5 RISK ASSESSMENTS of Infectious Agents

Section 5.1 Introduction

Risk assessment is a process used to identify the hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person’s exposure to an agent, the likelihood that such exposure will cause a Laboratory Acquired Infection (LAI), and the probable consequences of such an infection. Successful control of hazards in the laboratory also protects persons not directly associated with the laboratory, such as other occupants of the same building, and the public. When addressing laboratory activities involving infectious or potentially infectious material, risk assessment helps to assign the biosafety levels (facilities, equipment, and practices) that reduce the worker's and the environment's risk of exposure to an agent to as low as reasonably achievable. The intent of this section is to provide guidance and to establish a framework for selecting the appropriate biosafety level.

The primary factors to consider in risk assessment and selection of precautions fall into two broad categories: agent hazards and laboratory procedure hazards. In the presence of known hazards (e.g., concentration or volume of sample containing infectious material), quantitative assessments can be done. But in many cases, quantitative data will be incomplete or even absent (e.g., investigation of an unknown agent or receipt of an unlabeled sample). Types, subtypes, and variants of infectious agents involving different or unusual vectors, the difficulty of assays to measure an agent's amplification potential, and the unique considerations of natural or synthetic genetic recombinants are but a few of the challenges to the safe conduct of laboratory work. In the face of such complexity, meaningful quantitative sampling methods are frequently unavailable. Therefore, the process of doing a risk assessment for work with biohazardous materials cannot depend on a prescribed algorithm.

The Principal Investigator (PI) is responsible for conducting the initial risk assessment in order to determine the physical and biological containment for the work. The initial assessment should begin with the Risk Group of the wild type infectious agent. It may be appropriate to lower containment based on the physical containment (personal protective equipment and safety equipment), and biological containment (attenuation, vaccines, use of approved host vector systems) in use. The risk assessment should be done in close collaboration with the Biosafety Office and Institutional Biosafety Committee (IBC) to ensure compliance with established guidelines and regulations. The IBC is responsible for making an independent assessment of the containment levels for research involving recombinant or synthetic nucleic acid molecules and biohazards.

There is no standard approach for conducting a biological risk assessment, but following these five steps may be helpful in guiding the process.

First, identify agent hazards and perform an initial assessment of risk. Make a preliminary determination of the biosafety level that best correlates with the initial risk assessment based on the identification and evaluation of the agent hazards. Consider the principal hazardous characteristics of the agent, which include its capability to infect and cause disease in a susceptible human host, severity of disease, and the availability of preventive measures and effective treatments.
When assessing the hazards of a newly attenuated pathogen, experimental data should support a judgment that the attenuated pathogen is less hazardous than the wild type parent pathogen before making any reduction in the containment recommended for that pathogen.

**Second, identify laboratory procedure hazards.** The principal laboratory procedure hazards are agent concentration, suspension volume, equipment and procedures that generate small particle aerosols and larger airborne particles (droplets), and use of sharps. Procedures involving animals can present a number of hazards such as bites and scratches, exposure to zoonotic agents, and the handling of experimentally generated infectious aerosols. The complexity of a laboratory procedure can also present a hazard.

**Third, make a final determination of the appropriate biosafety level and select additional precautions indicated by the risk assessment.** The final selection of the appropriate biosafety level and the selection of any additional laboratory precautions require a comprehensive understanding of the practices, safety equipment, and facility safeguards.

**Fourth, evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment.** The protection of laboratory personnel, other persons associated with the laboratory, and the public will depend ultimately on the laboratory personnel themselves. In conducting a risk assessment, the PI should ensure that laboratory personnel have acquired the technical proficiency in the use of microbiological practices and safety equipment required for the safe handling of the agent, and have developed good habits that sustain excellence in the performance of those practices. The PI should also ensure that the necessary safety equipment is available and operating properly.

**Fifth, review the risk assessment with a biosafety professional, subject matter expert, and the IBC.**

**Agent Hazards**

- The *pathogenicity* of the infectious or suspected infectious agent, including disease incidence and severity (i.e., mild morbidity versus high mortality, acute versus chronic disease). The more severe the potentially acquired disease, the higher the risk.

- The *route of transmission* of the infectious agent. Routes of transmission include 1) parenteral inoculations, 2) spills and splashes onto skin and mucous membranes, 3) ingestion, 4) animal bites and scratches, and 5) inhalation exposures to infectious aerosols.

- The established *availability of an effective prophylaxis or therapeutic intervention* is another essential factor to be considered. The most common form of prophylaxis is immunization with an effective vaccine. Risk assessment includes determining the availability of effective immunizations. However important, immunization only serves as an additional layer of protection beyond engineering controls, proper practices and procedures, and the use of personal protective equipment.

- *Agent stability* is a consideration that involves not only aerosol infectivity (e.g., from spore-forming bacteria), but also the agent's ability to survive over time in the environment. Factors such as desiccation, exposure to sunlight or ultraviolet light, or exposure to chemical disinfectants must be considered.
The infectious dose of the agent is another factor to consider. Infectious dose can vary from one to hundreds of thousands of units. The complex nature of the interaction of microorganisms and the host presents a significant challenge even to the healthiest immunized laboratory worker, and may pose a serious risk to those with lesser resistance. The immune status of laboratory personnel is directly related to his/her susceptibility to disease when working with an infectious agent.

The concentration (number of infectious organisms per unit volume) will be important in determining the risk. Such a determination will include consideration of the milieu containing the organism (e.g., solid tissue, viscous blood or sputum, or liquid medium) and the laboratory activity planned (e.g., agent amplification, sonication, or centrifugation). The volume of concentrated material being handled is also important. In most instances, the risk factors increase as the working volume of high-titered microorganism increases, since additional handling of the materials is often required.

The origin of the potentially infectious material is also critical in doing a risk assessment. "Origin" may refer to geographic location (e.g., domestic or foreign); host (e.g., infected or uninfected human or animal); or nature of source (potential zoonotic or associated with a disease outbreak). From another perspective, this factor can also consider the potential of agents to endanger American livestock and poultry.

The availability of data from animal studies, in the absence of human data, may provide useful information in a risk assessment. Information about pathogenicity, infectivity, and route of transmission in animals may provide valuable clues. Caution must always be exercised, however, in translating infectivity data from one species of animal to another species. Experiments that demonstrate transmission of disease from an infected animal to a normal animal housed in the same cage are reliable indicators of hazard. Experiments that do not demonstrate transmission, however, do not rule out hazard.

Procedure Hazards
Investigations of LAIs have identified five principal routes of laboratory transmission. These are 1) parenteral inoculations with syringe needles or other contaminated sharps, 2) spills and splashes onto skin and mucous membranes, 3) ingestion through mouth pipetting, 4) animal bites and scratches, and 5) inhalation exposures to infectious aerosols.

Aerosols are a serious hazard because they are ubiquitous in laboratory procedures, are usually undetected, and are extremely pervasive, placing the laboratory worker carrying out the procedure and other persons in the laboratory at risk of infection. Procedures that impart energy to a microbial suspension will produce aerosols. Parameters that characterize aerosol hazards include an agent’s inhalation infective dose, its viability in an aerosol, aerosol concentration, and particle size. Procedures and equipment that generate respirable size particles also generate larger size droplets that can contain multiple copies of an infectious agent. The larger size droplets settle out of the air rapidly, contaminating the gloved hands and work surface and possibly the mucous membranes of the persons performing the procedure. The potential risk from exposure to droplet contamination requires as much attention in a risk assessment as the respirable component of aerosols.

Technique can significantly impact aerosol output and dose. The worker who is careful and proficient will minimize the generation of aerosols. A careless and hurried worker will substantially increase the aerosol
hazard. Experiments show that the aerosol burden with maximal aeration is approximately 200 times greater than aerosol burden with minimal aeration. Similar results were shown for pipetting with bubbles and with minimal bubbles. Containment and good laboratory practices also reduce this risk.

Laboratory Personnel Training
Risk assessment must also include an evaluation of the experience and skill level of at-risk personnel such as laboratory personnel and maintenance, housekeeping, and laboratory animal care personnel, and should identify any potential deficiencies in the practices of the laboratory personnel. Laboratory personnel are the first line of defense for protecting themselves, others in the laboratory, and the public from exposure to hazardous agents. Protection depends on the conscientious and proficient use of good microbiological practices and the correct use of safety equipment. Carelessness is the most serious concern, because it can compromise any safeguards of the laboratory and increase the risk for other laboratory personnel. Training, experience, knowledge of the agent and procedure hazards, good habits, caution, attentiveness, and concern for the health of coworkers are prerequisites for laboratory personnel in order to reduce the inherent risks that accompany work with hazardous agents. Additional education may be necessary to ensure the safety of persons working at each biosafety level.

Section 5.2 Bloodborne Pathogens

Bloodborne Pathogens are pathogenic microorganisms that are present in human blood and can cause disease in humans. These pathogens include, but are not limited to, hepatitis B virus (HBV) and human immunodeficiency virus (HIV).


Human blood, certain other body fluids, and unfixed tissue are considered potentially infectious for bloodborne pathogen. All research utilizing materials covered by the Bloodborne Pathogens Standard must be conducted in BSL-2 laboratories using Universal Precautions as described in the IU Exposure Control Plan (online at http://ehs.iu.edu/topics/occupational-safety/index.shtml).

All laboratory personnel working with human tissues, fluids or cell lines must be offered the Hepatitis B vaccine. Laboratory personnel who decline the vaccine must sign a waiver (Contact EH&S @ 855-6311 for BBP information). These personnel may choose to receive the vaccine at a future date. Investigators are responsible for notifying the Biosafety Office of their use of human clinical materials so that training and immunization (if applicable) can be provided as necessary by Environmental Health and Safety. A laboratory inspection, training, and an approved IBC protocol are required prior to initiating the work, and annually thereafter.

Biological materials covered by the OSHA Bloodborne Pathogen Standard:
Blood or blood products;
- Human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures;
- Any body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids;
• Any unfixed tissue or organ (other than intact skin) from a human (living or dead);
• HIV-containing cell or tissue cultures, organ cultures, and HIV- or HBV-containing culture medium or other solutions; and blood, organs, or other tissues from experimental animals infected with HIV or HBV;
• Primary and immortalized cell lines, commercially available cell lines, if untested and not documented to be free of Bloodborne pathogens.

Laboratory personnel who handle or manipulate human or animal cells and tissues are at risk for possible exposure to potentially infectious latent and adventitious agents that may be present in those cells and tissues. There also is evidence of accidental transplantation of human tumor cells to healthy recipients which indicates that these cells are potentially hazardous to laboratory personnel who handle them.16

Human derived materials that are not included in the Bloodborne Pathogens Standard: Saliva, urine, sweat, vomit, feces, when blood is not present. Gloves should be worn when handling these materials.

Section 5.3 Materials Containing Biohazards

Biohazards include recombinant or synthetic nucleic acids, infectious agents or hazardous biological materials that present a risk or potential risk to the health of humans, animals or the environment. The characteristics of most known infectious agents have been well identified. Information useful for assessing agent hazards can be obtained from laboratory investigations, disease surveillance, and epidemiological studies. A list of additional resources is provided at the end of this section.

Microorganisms: Parasites, viruses, bacteria, fungi, prions, etc. The IU Bloomington Institutional Biosafety Manual includes only agents that cause infectious disease in humans or vertebrate animals. Toxins, either cloned or in the purified state. Venom, or work with insects or animals that produce toxins or venom.

Human or animal tissues, fluids and cell lines known to be contaminated or infected with human or animal pathogens or zoonotic infectious agents, or collected from animals known to be sick. The challenge here is to establish the most appropriate biosafety level with the limited information available. Often these are clinical specimens. Some questions that may help in this risk assessment include:
• Why is an infectious agent suspected?
• What epidemiological data are available? What route of transmission is indicated?
• What is the morbidity or mortality rate associated with the agent?
• What medical data are available?

The responses to these questions may identify the agent or a surrogate agent whose existing agent summary statement can be used to determine a biosafety level. In the absence of hard data, a conservative approach is advisable. A conservative approach to risk assessment uses the professional judgment of the PI, Biosafety Office, and Institutional Biosafety Committee to assess the suspected hazards or potential severity of the outcome, and mitigates the risk through the use of personal protective equipment, practices, and engineering controls.
Section 5.4 Materials Containing Recombinant or Synthetic Nucleic Acid Molecules

This category of agents includes microorganisms that have been genetically modified with natural recombinant or synthetic nucleic acid molecules using recombinant DNA technologies. These technologies continue to evolve rapidly. Experimental procedures designed to derive novel recombinant viruses, bacteria, yeast, and other microorganisms have become commonplace in recent years. It is highly likely that future applications of recombinant and synthetic nucleic acid technology will produce new hybrid viruses. The NIH Guidelines are a key reference in establishing an appropriate biosafety level for work involving recombinant microorganisms. Compliance with the NIH Guidelines is mandatory for investigators conducting recombinant or synthetic nucleic acid molecule research funded by the NIH or performed at, or sponsored by, any public or private entity that receives any NIH funding for recombinant or synthetic nucleic acid research. The IU Bloomington IBC reviews and approves all nonexempt recombinant and synthetic nucleic acid research and teaching activities, and requires registration of all exempt recombinant and synthetic nucleic acid research and teaching activities using the IUB IBC protocol submission forms. The IUB IBC also requires submission of research involving materials covered by the Bloodborne Pathogen Standard and biohazards including functional toxins, and microorganisms that cause infectious disease in humans or vertebrate animals.

In selecting an appropriate biosafety level for such work, perhaps the greatest challenge is to evaluate the potential increase or decrease in the biohazard associated with a particular genetic modification (those that increase tissue tropism, affect the organism’s susceptibility to antibiotics or other effective treatments, or attenuate the pathogenicity of the organism). In most cases, the selection of an appropriate biosafety level begins by establishing the classification of the non-modified virus. It is important to remember that the nature and severity of disease caused by a laboratory infection and the probable laboratory route of transmission of the infectious agent may differ from the route of transmission and severity associated with the naturally-acquired disease.

Among the recombinant viruses now routinely developed as viral vectors are adenoviruses, alphaviruses, retroviruses, vaccinia viruses, herpesviruses, and others designed to express heterologous gene products. However, the nature of the genetic modification and the quantity of virus must be carefully considered when selecting the appropriate biosafety level for work with a recombinant virus. Among the points to consider in work with recombinant microorganisms are:

- Does the inserted gene encode a known toxin or a relatively uncharacterized toxin?
- Does the modification have the potential to alter the host range or cell tropism of the virus?
- Does the modification have the potential to increase the replication capacity of the virus?
- Does the inserted gene encode a known oncogene?
- Does the inserted gene have the potential for altering the cell cycle?
- Does the viral DNA integrate into the host genome?
- What is the probability of generating replication-competent viruses?

This list of questions is not meant to be inclusive. Rather, it serves as an example of the information needed to judge whether a higher biosafety level is needed in work with genetically modified microorganisms. Since in many cases the answers to the above questions will not be definitive, it is important that the organization have a properly constituted and informed IBC as outlined in the NIH Guidelines, to evaluate the risk assessment.
Section 5.5 Animal Studies

Laboratory studies involving animals may present many different kinds of physical, environmental, and biological hazards. The specific hazards present in any particular animal facility are unique, varying according to the species involved and the nature of the research activity. The risk assessment for biological hazard should particularly focus on the animal facility's potential for increased exposure to human pathogens, zoonotic agents, and animal pathogens.

The animals themselves can introduce new biological hazards to the facility. Latent infections are most common in field-captured animals or in animals coming from unscreened herds. However, risk assessment should also consider the potential biohazard associated with animals xenografted or transplanted with human cells.

The animal routes of transmission must also be considered in the risk assessment. Animals that shed virus through respiratory dissemination or dissemination in urine or feces are far more hazardous than those that do not. Animal handlers in research facilities working on infectious agents have a greater risk of exposure from the animals' aerosols, bites, and scratches. Section 8 describes the practices and facilities applicable to work on animals infected with agents assigned to corresponding Animal Biosafety Levels 1 and 2.

Section 5.6 Other Applications

The described risk assessment process is also applicable to laboratory operations other than those involving the use of primary agents of human or animal disease, such as plant pathogens. Risk assessments for plant pathogens should focus on the effect of the natural or genetically modified pathogen on natural or managed ecosystems and agricultural crops. It is true that microbiological studies of animal host-specific pathogens, soil, water, food, feeds, and other natural or manufactured materials, pose comparatively lower risks for the laboratory worker. Nonetheless, microbiologists and other scientists working with such materials may find the practices, containment equipment, and facility recommendations described in this publication of value in developing operational standards to meet their own assessed needs.

Useful resources for conducting risk assessments include:

- Center for Disease Control Biosafety in Microbiological and Biomedical Laboratories 5th ed. (BMBL), Section VIII, Agent Summary Statements
- American Biological Safety Association, Searchable database; Risk Groups: Bacteria [http://www.absa.org/riskgroups/Bacteria.html](http://www.absa.org/riskgroups/Bacteria.html)
- Center for Disease Control, [http://www.cdc.gov/](http://www.cdc.gov/)
• American Public Health Association *Control of Communicable Diseases*\textsuperscript{7}
• *NIH Guidelines*

Resources are also available on the Bloomington Biological Safety website under Publications, [http://ehs.iu.edu/topics/biosafety/index.shtml](http://ehs.iu.edu/topics/biosafety/index.shtml).

Literature reviews on laboratory acquired infections also may be helpful.\textsuperscript{8,9,10,11,12,13} In some cases, one must rely on other sources of information such as field data from subject matter experts. This information is interpreted for its tendency to raise or lower the risk of laboratory-acquired infection.\textsuperscript{14} The challenge of risk assessment lies in those cases where complete information on these factors is unavailable. A conservative approach is generally advisable when insufficient information forces subjective judgment. A conservative approach to risk assessment uses the professional judgment of the PI, Biosafety Office, Bloodborne Pathogens Training Coordinator, and Institutional Biosafety Committee to assess the suspected hazards or potential severity of the outcome, and mitigates the risk through the use of personal protective equipment, practices, and engineering controls. Universal Precautions are always advisable.
SECTION 6 RISK GROUPS, PRIMARY AND SECONDARY BARRIERS

Section 6.1 Introduction

Federal and state regulations and guidelines govern procedures and facilities involved in protecting laboratory personnel, the public, and the environment from laboratory biological hazards. Many granting agencies require that grant recipients certify that they adhere to both the guidelines and the regulations. A few of these are the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), Biosafety in Microbiological and Biomedical Laboratories, 5th ed. (BMBL), and all applicable OSHA Standards including Title 29 CFR 1910.1030 Bloodborne Pathogens. It is the policy of Indiana University that all Bloomington and Regional laboratories adhere to all applicable state and federal guidelines and regulations.

The Indiana University Bloomington Institutional Biosafety Committee (IBC) Standard Operating Procedure (SOP) requires that all teaching and research involving the use of recombinant or synthetic nucleic acid molecules or biohazards be submitted to the IBC for review and approval.

Biological Research Laboratories

Four biosafety levels are described for activities involving biohazards. These four combinations of practices, safety equipment, and facilities are designated Biosafety Levels 1, 2, 3, and 4, (BSL-1, BSL-2, BSL-3, BSL-4), and provide increasing levels of protection to personnel and the environment. BSL-1 and BSL-2 are described in Section 7. Compliance with the most current version of the Arthropod Containment Guidelines, is required when research utilizes experimentally infected arthropod vectors of disease, or arthropods collected from animals known to be sick or carrying agents of zoonotic infectious disease.

Indiana University does not have the facilities to support BSL-4 research. BSL-3 research is described in a separate BSL-3 program manual; persons seeking to begin BSL-3 research should contact the Biosafety Office.

Animal Facilities

Four biosafety levels are also described for activities involving infectious disease work with experimental animals. These four combinations of practices, safety equipment, and facilities are designated Animal Biosafety Levels 1, 2, 3, and 4, (ABSL-1, ABSL-2, ABSL-3, and ABSL-4). ABSLs parallel the biological safety containment levels, BSL 1-4. Indiana University does not have the facilities to support ABSL-4 research. ABSL-1 and ABSL-2 are described in Section 7. ABSL-3 research is described in a separate ABSL-3 program manual; persons seeking to begin ABSL-3 research should contact the Biosafety Office.

Plant Facilities

Four biosafety levels are described for activities involving recombinant or synthetic nucleic acid molecules and plants. These four combinations of practices, safety equipment, and facilities are designated Plant Biosafety Levels 1, 2, 3, and 4, (BSL1-P, BSL2-P, BSL3-P, and BSL4-P). Plant biosafety containment levels parallel the biological safety containment levels, BSL 1-4. Indiana
University does not have the facilities to support BSL3-P or BSL4-P. BSL1-P and BSL2-P are described in Section 7.

**Biohazards and recombinant or synthetic nucleic acids**

Biohazards are defined as infectious agents or hazardous biological materials that present a risk or potential risk to the health of humans, animals or the environment. All activities that involve biohazards must be approved by the IBC prior to initiating the work. A laboratory inspection and training must also be completed prior to initiation of work and annually thereafter. Biohazards include:

- Disease-causing microorganisms, e.g., parasites, viruses, bacteria, fungi, prions, etc;
- Human or animal tissues, fluids and cell lines known to be infected with human or animal pathogens or zoonotic infectious agents, or collected from animals known to be sick;
- Biological toxins, either cloned or in the purified state;
- Venom, or work with insects or animals that produce toxins or venom;

Genetically modified materials are pathogenic and nonpathogenic microorganisms, animals, plants, cell lines, etc., that have been genetically modified through the introduction of recombinant or synthetic nucleic acids, and considered biohazards by the National Institutes of Health.

According to the National Institutes of Health, for the purposes of recombinant or synthetic nucleic acid research, animals are defined as everything in the Kingdom Animalia. This includes invertebrate animals in addition to vertebrate animals. Under the *NIH Guidelines*, the following categories are considered transgenic:

- Knockout and knock-in animals in which recombinant DNA techniques were used to genetically modify the animal.
  - Offspring of breeding or cross breeding these animals
- DNA modifications to the somatic cells of non-transgenic animals
- Gene ablation, when recombinant DNA is used to knock out the gene

All activities that use recombinant or synthetic nucleic acid must be registered with the IBC, including NIH exempt teaching or research activities. All non-exempt work must be approved by the IBC. Whether IBC approval is required in advance of initiating the research or upon submission of the IBC protocol may vary according to the risk group of the infectious agent in use, or the use of whole animals or plants.

**Section 6.2 Risk Group Classification of Potentially Infectious Agents**

The *NIH Guidelines* provide risk group classification criteria for four general risk groups, and a list of commonly encountered wild type genera and species of microorganisms that are known to be pathogenic to humans. The *NIH Guidelines* do not provide a comprehensive list of all genera and species known to cause disease in humans, or information on animal or plant pathogens. A risk assessment should be conducted to determine agent hazards and containment for infectious agents for which a Risk Group classification is unavailable. Organisms for which the Risk Group classification is available may be used as a reference for determining containment.
The World Health Organization (WHO) also provides risk group classification criteria for laboratory use for four risk groups based on the principal agent characteristics and the route of transmission of the natural disease.\textsuperscript{15} The WHO risk groups address the risk to both the laboratory worker and the community. The descriptions of the WHO and NIH risk group classifications are based on natural routes of transmission and correlate with, but do not equate to, biosafety levels. The nature and severity of disease and route of transmission in a Lab Acquired Infection (LAI) may differ from that of a naturally acquired infection. A risk assessment should include potential laboratory routes of transmission and will determine the degree of correlation between an agent’s risk group classification and biosafety level.

The NIH and World Health Organization (WHO) have similar criteria for Risk Group classes. For the purposes of the IBC, Risk Groups are defined as the following:

**Risk Group 1** (RG1) agents are defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans or animals. Laboratory personnel may become infected through high doses or unusual routes of exposure that are not commonly encountered in a natural setting. Opportunistic RG1 pathogens may cause serious disease in elderly persons and infants, and persons with compromised immune systems. A risk assessment should be used for vaccine strains, as multiple passages in vivo do not ensure avirulence.

**Risk Group 2** (RG2) agents are associated with human or animal disease which is rarely serious and for which preventive or therapeutic interventions are often available. RG2 organisms have the capability to cause serious disease based on dose, route of exposure, and immune status. Laboratory exposures may cause serious infection, but the risk of spread of infection is limited. The risk assessment should give special attention to those RG2 organisms for which preventative or therapeutic interventions are not available.

**Risk Group 3** (RG3) agents are associated with serious or lethal human or animal disease and have the potential for respiratory transmission, and for which preventive or therapeutic interventions may be available. Preventative or therapeutic interventions may not be available, or are less available than for RG2 organisms.

**Risk Group 4** (RG4) agents are associated with serious or lethal human or animal disease. RG4 agents may be transmitted via the aerosol route, can be readily transmitted from one individual to another, directly or indirectly, and for which there is no available vaccine or therapy. Indiana University Bloomington is not authorized to possess RG4 agents.

**Section 6.3 Primary and Secondary Barriers**

Primary containment is provided by both good microbiological technique and the use of appropriate safety equipment. Examples of primary barriers include safety equipment such as biosafety cabinets, enclosed containers, and sealed centrifuge rotors or cups. Sealed centrifuge cups and rotors prevent aerosols from being released during centrifugation. Biosafety cabinets are described in more detail in Section 6.4.1 of this manual. Personal protective equipment also serves as a primary barrier, and may include gloves, laboratory coats or gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. When it is impractical to work with infectious agents or other research materials inside a
biosafety cabinet, a combination of additional personal protective equipment may serve as the primary barrier.

Secondary containment provides protection for the environment external to the laboratory from exposure to infectious materials. Examples of secondary barriers include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave), and hand washing facilities. When the risk of infection by exposure to an infectious aerosol is present, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features include specialized ventilation systems to ensure directional air flow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks at laboratory entrances, or separate buildings or modules to isolate the laboratory.

The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in Biosafety Level 1 and 2 facilities will be direct contact with the agents, or inadvertent contact exposures through contaminated work environments. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of secondary barriers.

Facilities should be commensurate with the laboratory's function and design, and the recommended biosafety level for the agents being manipulated. See Section 7 for details regarding facility requirements.

The use of vaccines may provide an increased level of personal protection, but are not to be relied upon in place of primary or secondary barriers.

6.3.1 Laboratory Practice and Technique

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or potentially infected materials must be aware of potential hazards, and must be trained and proficient in the practices and techniques required to handle such material safely. The director or person in charge of the laboratory is responsible for providing or arranging the appropriate training of personnel.

Each laboratory shall develop or adopt a laboratory specific biosafety manual that identifies the hazards that will or may be encountered, and the specific practices and procedures designed to minimize or eliminate exposures to these hazards. Personnel should be advised to read and follow the required practices and procedures. A scientist trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling infectious agents must be responsible for the conduct of work with any infectious agents or material. The PI should consult with the Biosafety Office.

When standard laboratory practices are not sufficient to control the hazards associated with a particular agent or laboratory procedure, additional measures may be needed. The PI is responsible for selecting additional safety practices, which must be in keeping with the hazards associated with the agent or procedure. Laboratory personnel, safety practices, and techniques must be
supplemented by appropriate facility design and engineering features, safety equipment, and management practices.

6.3.2 Laboratories that Manipulate or Analyze Clinical Specimens

Typically, clinical specimens are collected or received with little or no definitive information regarding the infectious nature of the clinical material. It is the responsibility of the Principal Investigator (PI) to establish standard procedures in the laboratory which realistically address the issue of the infective hazard of clinical specimens. Except in extraordinary circumstances (e.g. Sample is known to be contaminated with Risk Group 3 or 4 infectious agents) the initial processing of clinical specimens and serological identification of isolates can be done safely at Biosafety Level 2; the recommended level for work with bloodborne pathogens such as Hepatitis B virus and HIV. The containment elements described in Biosafety Level 2 are consistent with the OSHA standard, "Occupational Exposure to Bloodborne Pathogens."\textsuperscript{16,17,18} This requires the use of specific precautions with all clinical specimens of blood or other potentially infectious material (Universal Precautions).\textsuperscript{19}

Biosafety Level 2 recommendations and OSHA requirements focus on the prevention of percutaneous and mucous membrane exposures to clinical material. Primary barriers such as biological safety cabinets should be used when performing procedures that might cause splashing, spraying, or splattering of droplets. Biological safety cabinets also should be used for the initial processing of clinical specimens when the nature of the test requested or other information suggests the likely presence of an agent readily transmissible by infectious aerosols, or when the use of a biological safety cabinet is indicated to protect the integrity of the specimen.

The segregation of clinical laboratory functions and limited or restricted access to such areas is the responsibility of the PI. It is also the PI's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented.

6.4 Safety Equipment

6.4.1 Biological Safety Cabinets\textsuperscript{20,21,22}

Biological Safety Cabinets (BSCs) are among the most effective and the most commonly used primary containment devices in laboratories working with infectious agents.\textsuperscript{20} The BSC is designed to provide protection to the material in the biosafety cabinet, the user, and the environment when appropriate practices and procedures are followed and is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures.

Two types of biological safety cabinets, Class I and II, used in microbiological laboratories are described below. Contact the Biosafety Office for information or assistance with Class III biosafety cabinets. Open-fronted Class I and Class II biological safety cabinets are primary barriers which offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques. The Class II biological safety cabinet also
provides protection from external contamination of the materials (e.g., cell cultures, microbiological stocks) being manipulated inside the cabinet.

Both Class I and II BSCs have inward airflow velocities that provide containment to protect laboratory workers and the immediate environment from infectious aerosols generated within the cabinet. Class II BSCs also protect the research material itself through high-efficiency particulate air filtration (HEPA filtration) of the airflow down across the work surface.

The common element to all classes of biological safety cabinets is the high efficiency particulate air (HEPA) filter. This filter removes particulates of 0.3 microns with an efficiency of 99.97%, and removes particulates that are larger and smaller than 0.3 microns with even greater efficiency. HEPA filters do NOT remove volatile toxic chemicals from the exhaust air. Caution should be used for procedures that involve use of volatile chemicals. Only biosafety cabinets that are exhausted outside of the building and not into the laboratory should be used for volatile chemicals.

**Certification and Decontamination**
The biosafety cabinet requires regular maintenance and certification by a professional technician to assure that it protects the user, the research materials, and the environment.

- All biosafety cabinets should be certified when they are installed, and after being relocated.
- Biosafety cabinets used for manipulations in Biosafety Level 2 must be certified annually.
- Surface decontamination of all biosafety cabinets is required prior to certification, maintenance, repair, relocation, or decommissioning.
  - Spraying and wiping with 70% ethanol is recommended.
  - Wiping with 10% bleach followed by 70% ethanol may be appropriate based on the research organism. Bleach is corrosive and should not be sprayed within biosafety cabinets to prevent mist from circulating into, and corroding, inaccessible areas like plenums.
- Biosafety cabinets used for manipulations in Biosafety Level 2 may require gaseous decontamination when maintenance or repair requires access to plenums, HEPA filters, or other potentially contaminated areas in which surface decontamination is not feasible. The Biosafety Office should be contacted when maintenance or repairs include access to these areas. A determination will be made which considers the research organisms, and with input from the PI.
- Biosafety cabinets used for manipulations in Biosafety Level 2 may require gaseous decontamination prior to being relocated. The Biosafety Office should be contacted prior to moving biosafety cabinets out of BSL-2 lab space. A determination will be made which considers the research organisms, and with input from the PI.

**Working in a Biosafety Cabinet**
As with any other piece of laboratory equipment, personnel must be trained in the proper use of biological safety cabinets. Of particular note are activities that may disrupt the inward directional airflow. Repeated insertion and withdrawal of the workers' arms into and out of the work chamber, opening and closing doors to the laboratory or isolation cubicle, improper placement or operation of materials or equipment within the work chamber, or brisk walking past the BSC while it is in
use have been demonstrated to cause the escape of aerosolized particles from within the cabinet. Class I and II cabinets should be located away from traffic patterns and doors. Airflow from fans, room air supply louvers and other air moving devices can disrupt the airflow pattern at the face of the cabinet. Strict adherence to recommended practices for the use of BSCs and their proper placement in the laboratory are as important in attaining the maximum containment capability of the equipment as is the mechanical performance of the equipment itself.

Decommissioning
Decommissioning refers to biosafety cabinets which are being transported as salvage or waste from IU to the landfill or other receiving entity. All biosafety cabinets must be surface decontaminated prior to transport. Biosafety cabinets used in BSL-2 laboratories may be required to undergo gas decontamination. The Biosafety Office should be contacted prior to decommissioning biosafety cabinets. A determination will be made which considers the research organisms and with input from the PI.

6.4.2 Horizontal Laminar Flow Benches and Class I Biosafety Cabinets

Horizontal laminar flow "clean air benches" are not BSC’s. HEPA-filtered air is supplied to the work surface and blows out toward the user, providing protection for research materials only. They can be used for certain clean activities, such Polymerase Chain Reaction (PCR). They may never be used when handling cell culture materials or potentially infectious materials, or as a substitute for a biological safety cabinet in research laboratories.

Class I Biosafety Cabinets protect personnel and the environment, but provides no protection for research materials. Air is drawn into the biosafety cabinet through the sash and is supplied to the work surface is unfiltered, similar to a chemical fume hood, which protects the worker from the material in the cabinet. All of the air from the cabinet is exhausted through a HEPA filter either into the laboratory or to the outside, which protects the environment.

The use of Class I BSCs is not recommended. These cabinets are not appropriate for handling research materials that are vulnerable to airborne contamination, since the inward flow of air is unfiltered air and can carry microbial contaminants into the cabinet. Class I BSCs may be used to enclose aerosol generating equipment (e.g., centrifuges, harvesting equipment or small fermenters), or to perform procedures with potential to generate aerosols (e.g. cage dumping, culture aeration or tissue homogenation). Volatile chemicals may be handled in Class I biosafety cabinets if they exhaust only to the outside.

6.4.3 Class II Biosafety Cabinets

Class II biological safety cabinets provide protection to personnel, the environment, and the research materials. Air is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward flow of HEPA-filtered air within the cabinet provides protection to research materials by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air passes through the exhaust HEPA filter, it is contaminant-free, providing environmental protection, and may be recirculated back into the laboratory (Type A) or ducted out of the building (Type B).
Table 2 CLASS II BIOSAFETY CABINETS

<table>
<thead>
<tr>
<th>Type</th>
<th>Airflow Pattern</th>
<th>Nonvolatile Toxic Chemicals and Radionuclides</th>
<th>Volatile Toxic Chemicals and Radionuclides</th>
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<tr>
<td>Type A1 (Type A)</td>
<td>-70% of exhaust air is recirculated back into work area, 30% is exhausted to the room - Not connected to the building HVAC system, or thimble connected</td>
<td>Yes, minute amounts</td>
<td>No</td>
</tr>
<tr>
<td>Type A2</td>
<td>-70% of exhaust air is recirculated back into work area, 30% is exhausted to the room - Not connected to the building HVAC system</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Type A2 (Type B3)</td>
<td>-70% of exhaust air is recirculated back into work area, 30% is exhausted to the room - Thimble connected to building HVAC system</td>
<td>Yes</td>
<td>Yes&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Type B1</td>
<td>-70% of exhaust air is discharged to the building exhaust system, 30% is recirculated back to the work area - Hard Ducted to dedicated HVAC system</td>
<td>Yes</td>
<td>Yes&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Type B2</td>
<td>-100% of air is discharged - Hard Ducted to dedicated HVAC system</td>
<td>Yes</td>
<td>Yes&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Installation may require a special duct to the outside, an in-line charcoal filter, and a spark proof (explosion proof) motor and other electrical components in the cabinet. Discharge of a Class I or Class II, Type A2 cabinet into a room should not occur if volatile chemicals are used.

<sup>b</sup> In no instance should the chemical concentration approach the lower explosion limits of the compounds.

**Operation of Class II Biosafety Cabinets**

- Minimize traffic around the biosafety cabinet and avoid drafts from doors and air conditioning.
- Turn on cabinet fan 5 minutes before beginning work.
- Disinfect the cabinet work surface with 70% ethanol or other disinfectant.
- Place supplies in the cabinet. Place the container for disposal of pipettes inside the cabinet. (Movement of hands in and out of the cabinet to discard pipettes into a container located outside of the cabinet creates turbulence and disrupts the air barrier that maintains sterility inside the cabinet.)
- Work as far to the back (beyond the air split) of the BSC workspace as possible.
- **Avoid using open flames inside BSC’s. Flames disrupt the airflow and contribute to the heat load inside the BSC. Flames have burned holes through HEPA filters and have caused explosions in BSC’s.** If a flame is necessary, use a burner with a pilot light and place it to the rear of the workspace.
- Do not work in a BSC while a warning light or alarm is signaling.
• Locate liquid waste traps inside the cabinet and use a hydrophobic filter to protect the vacuum line. If traps must be located on the floor, place them in a secondary container to prevent spilling.

• Keep the work area of the BSC free of unnecessary equipment or supplies.

• Clutter inside the BSC may affect proper airflow and the level of protection provided. Also, keep the front and rear grilles clear. Adapt a “clean to dirty” pattern for BSC procedures (Figure 1).

• When work is completed, remove equipment and supplies from the cabinet. Wipe the work area with 70% ethanol and allow the cabinet to run for 5 minutes.

• Some BSC’s are equipped with ultraviolet (UV) lights. The UV light must be recertified annually, during the biosafety cabinet recertification.  
  o If a UV light is used, due to its limited penetrating ability, surfaces must be dust-free and the UV light tube should be wiped frequently with alcohol to remove dust.
  o Purple light does not signify the UV light is still working effectively to inactivate biological contaminants. UV radiation should not take the place of 70% ethanol for disinfection of the cabinet interior. If good procedures are followed, UV lights are not needed.
  o The UV lamp should never be on while an operator is working in the cabinet, or when the sash is not fully closed.

**Figure 1 A typical layout for working "clean to dirty" within a Class II BSC.**

Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded in the shallow pan and other contaminated materials can be placed in the biohazard bag (right). This arrangement is reversed for left-handed persons.

**6.4.4 Centrifuge Containment**

• Examine centrifuge tubes and bottles for cracks or stress marks before using them.

• Never overfill centrifuge tubes since leakage may occur when tubes are filled to capacity. Fill centrifuge tubes no more than 3/4 full.
• Centrifuge safety buckets and sealed rotors protect against release of aerosols. To further eliminate or reduce aerosols, sealed buckets and rotors (when possible) should be opened in a biosafety cabinet.

6.4.5 Protection of Vacuum Lines

All vacuum lines used to aspirate supernatants, tissue culture media, and other liquids that may contain microorganisms should be protected from contamination by the use of a collection flask and overflow flask (Figure 2). In addition, at BSL2 and above, a hydrophobic vacuum line filter must be used.

Collection and Overflow Flasks
Collection tubes should extend at least 2 inches below the sidearm of the flask. Locate the collection flask inside the biosafety cabinet instead of on the floor, so the liquid level can be seen easily and the flask emptied before it overflows. The second flask (overflow) may be located outside the cabinet.

If a glass flask is used at floor level, place it in a sturdy cardboard box or plastic container to prevent breakage by accidental kicking. In BSL2 or higher laboratories, the use of Nalgene flasks is recommended to reduce the risk of breakage.

Vacuum Line Filter
A hydrophobic filter will prevent fluid and aerosol contamination of central vacuum systems or vacuum pumps. The filter will also prevent microorganisms from being exhausted by a vacuum pump into the environment. Hydrophobic filters such as the Gelman Vacushield are available from several scientific supply companies such as Fisher Scientific, VWR, and Millipore.

Figure 2 Protection of Vacuum Lines from Contamination

One method to protect a house vacuum system during aspiration of infectious fluids is pictured. The left suction flask (A) is used to collect the contaminated fluids into a suitable decontamination solution; the
right flask serves as a fluid overflow collection vessel. A glass sparger in flask B minimizes splatter. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms.
SECTION 7 BIOLOGICAL CONTAINMENT LEVELS

Biological safety containment levels describe the combinations of laboratory practices, safety equipment including personal protective equipment (PPE), and laboratory facilities used in teaching and research activities to maximize the degree of protection provided to personnel, the environment, and the community. The purpose of containment is to reduce or eliminate exposure to recombinant and synthetic nucleic acid molecules and potentially hazardous agents.

The BMBL and NIH Guidelines provide guidance for work with infectious microorganisms. These publications recommend that work be done using one of four levels of containment: Biosafety Level 1 (BSL-1), BSL-2, BSL-3 and BSL-4. Containment levels correlate to Risk Groups, but the two are not equivalent. The risk assessment process should be used to determine if a procedure or experiment with a lower Risk Group organism should take place in a higher level of containment, or if experiments with a higher Risk Group organism may take place in a lower level of containment.

Indiana University does not have the facilities to support BSL-4, Animal BSL-4, Plant BSL-3, Plant BSL-4, or containment facilities for large or loose-housed animals. Facilities and practices associated with these containment levels are not described in this manual. Persons seeking to begin BSL-3 or ABSL-3 research should contact the Biosafety Office.

The following practices, safety equipment and facilities apply to all animal, plant and laboratory research:

Section 7.1 ALL Laboratories

Standard Practices

1. Access to the laboratory is limited or restricted at the discretion of the Principal Investigator (PI) when experiments are in progress. The PI must enforce the institutional policies that control access to the laboratory.

2. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work area.
   a. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
   b. The laboratory area includes all space adjacent to the laboratory work benches where there is not a physical door separating the areas.

3. Persons must wash their hands with soap and running water after they handle potentially hazardous materials or materials involving organisms containing recombinant or synthetic nucleic acid molecules and animals and before exiting the laboratory.

4. Mechanical pipetting devices must be used; mouth pipetting is prohibited.

5. All procedures are to be performed carefully to minimize the creation of aerosols or splatters of infectious waste.

6. Work surfaces and equipment shall be surface decontaminated with an appropriate disinfectant once a day and after any spill or splash of viable material.
7. Protective eyewear must be worn by all personnel, including persons wearing contact lenses, when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or be decontaminated before reuse.

8. When gloves are worn, the following rules apply:
   a. Glove selection should be based on an appropriate risk assessment. b. Alternatives to latex gloves (i.e., nitrile) should be available.
   b. Gloves must not be worn outside the laboratory, or the one-hand-glove rule must be followed.
   c. A glove is worn on the hand carrying research materials, while the hand used to open doors, etc., is bare.
   d. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.
   e. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste.
   f. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
   g. Hands must be washed with soap and running water before leaving the laboratory.


10. Persons handling blood, blood products, or other potentially infectious materials must participate in Bloodborne Pathogens Program training and laboratory inspections, administered by Environmental Health and Safety. See Section 7.2.2 Biosafety Level 2 for details.

11. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

12. Animals and plants not associated with the work being performed are not permitted
   a. in laboratory areas;
   b. in areas where animals are housed or manipulated;
   c. in areas where research plants are grown or manipulated.

13. The PI must ensure that laboratory personnel receive appropriate training regarding their duties.

14. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.

All Laboratory Facilities

1. Each laboratory must have a sink for hand washing.

2. The laboratory is designed so that it can be easily cleaned. Spaces between benches, cabinets, and equipment must be accessible for cleaning. Carpets and rugs are not permitted.

3. Laboratory furniture must be capable of supporting anticipated loads and uses.
   a. Bench tops are impervious to water and resistant to moderate heat, acids, alkalis, organic solvents, and other chemicals.
b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

4. Equipment containing sharp edges and corners should be avoided.

5. An effective insect and rodent control program must be in effect.

6. Refer to the IU Bloomington Laboratory Chemical Safety Plan for guidance on appropriate lab attire.

Use and Disposal of Sharps
Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Sharps such as needles, scalpels, pipettes, and broken glassware must be handled in accordance with IU Laboratory Chemical Safety Plan and the following standards:

- Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles.

- Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant or synthetic nucleic acid molecules.

- Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal.

- Needles must not be bent, sheared, recapped, replaced in the needle sheath or guard, or removed from disposable syringes before disposal.

- Used disposable needles and syringes must be carefully placed in conveniently located hard-walled containers used for sharps disposal and decontaminated (preferably autoclaved) before being discard.
  - Do not use thin cardboard or paper sleeves, soda bottles, or any object a sharp object will poke through under pressure. If cardboard boxes are used, tape the edges to prevent razor blades or sharps from sliding out.

- Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

- Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.

To prevent needle stick injuries:

- Avoid using needles whenever possible.
- Do not bend, break, or otherwise manipulate needles by hand.
- Do not recap needles by hand. Do not remove needles from syringes by hand.
- Discard needle and syringe as an intact unit immediately after use into puncture resistant sharps containers.
- Use care and caution when cleaning up after procedures that require the use of syringes and needles.
- Do not overfill sharps containers. Remove from service when 3/4 full, autoclave, and dispose of in accordance with University policy.
• Locate sharps containers in areas in which needles are commonly used. Make containers easily accessible.
• When performing animals experiments, load the syringe(s) prior to handling the animals.

In the event of a needle stick injury, notify the Principal Investigator and the Biosafety Office, and seek treatment, if needed.

Section 7.2 Laboratory Biological Safety

7.2.1 Biosafety Level 1 (BSL-1)

Biosafety Level 1 practices and facilities encompass all requirements described in Section 7.1 (ALL Laboratories).

BSL-1 practices, safety equipment, and facility design and construction include appropriate laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans, and are of minimal potential hazard to laboratory personnel and the environment. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is neither required nor generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

The following practices, safety equipment and facilities apply to laboratory BSL-1: Microbiological Practices (BSL-1)

1. All liquid or solid wastes that contain recombinant or synthetic nucleic acid molecules are decontaminated before disposal using an effective method.
2. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
3. Gloves are required to protect hands from exposure to material containing recombinant or synthetic nucleic acid molecules.

Laboratory Facilities (BSL-1)
1. Laboratories should have doors for access control.
2. If the laboratory has windows that open, they are fitted with fly screens.

7.2.2 Biosafety Level 2 (BSL-2)

Biosafety Level 2 practices and facilities encompass all requirements described in Section 7.1 (ALL Laboratories), and Section 7.2.1 (BSL-1). The most stringent practices must be followed wherever they are described (BSL-2).

BSL-2 practices, equipment, and facility design and construction are applicable to teaching and research laboratories in which work is done with the broad spectrum of indigenous moderate-risk
agents that are present in the community and associated with human disease of varying severity.
With good microbiological techniques, procedures with these agents that do not have a high potential for generating aerosols can be used safely in activities conducted on the open bench.

Biosafety Level 2 laboratories are defined as all areas where:
- agents infectious to humans or vertebrate animals are stored or manipulated;
- blood, blood products, or other potentially infectious materials, including commercial cell lines;
- animal tissues or fluids, especially those collected from nonhuman primates, that are contaminated with an agent infectious to humans or vertebrate animals, or materials that were collected from an animal known to be sick are manipulated, including commercial cell lines;
- certain types of research utilizing toxins or venom.

In BSL-1 laboratories containing a small BSL-2 room, (often housing a biosafety cabinet) in which the research meets the criteria above, but in which the small room does not meet inspection criteria as a BSL-2 laboratory, the outer room will be assessed as part of the BSL-2 laboratory. BSL-2 practices must be followed in all BSL-2 areas.

Biosafety Level 2 is also appropriate when work is done with any human or non-human primate blood, blood products, or other potentially infectious materials where the presence of an infectious agent may be unknown. (Laboratory personnel working with human-derived materials should refer to the OSHA Bloodborne Pathogen Standard for specific required precautions.)

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Risk assessment should take into consideration for those Risk Group 2 organisms that have been shown to pose a risk for an inhalation exposure and illness through aerosolization. Extreme caution should be taken with contaminated needles or sharp instruments. Secondary barriers such as handwashing sinks and waste decontamination facilities must be available to reduce potential environmental contamination.

Though most organisms routinely manipulated at Biosafety Level 2 are not transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure through ingestion or direct contact of cuts or mucous membranes with infectious aerosols must be conducted in primary containment equipment, or in devices such as a BSC or safety centrifuge cups. Other primary barriers should be used as appropriate, such as splash shields, face protection, gowns, and gloves.

Laboratory personnel must have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures.

The following practices, safety equipment and facilities apply to laboratory BSL-2: Microbiological Practices (BSL-2)

1. The Principal Investigator limits access to the laboratory.
a. Access to the laboratory is limited or restricted when manipulations with biohazards or organisms containing recombinant or synthetic nucleic acid molecules are in progress.

b. The PI establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) may enter the laboratory or animal rooms.

c. The PI has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

2. All procedures are performed carefully to minimize the creation of aerosols. All procedures involving the manipulation of infectious materials that have the potential to generate aerosols must be conducted within a biosafety cabinet (BSC) or other physical containment device (See Containment Equipment (BSL-2) below for details).

3. Laboratory coats, gowns, smocks, or uniforms are worn while conducting research in the laboratory. Before exiting the laboratory for non-laboratory areas (e.g., cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory.

4. Gloves are required in BSL-2 to protect hands from exposure to hazardous materials, experimental animals, biohazards, and recombinant or synthetic nucleic acid molecules. Wear two pairs of gloves when appropriate.

5. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within the BSL-2 facility.

6. Laboratory equipment should be routinely surface decontaminated, as well as after spills, splashes, or other potential contamination.
   a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
   b. Equipment must be surface decontaminated before repair, maintenance, or removal from the laboratory.

7. All wastes from laboratories (and animal rooms) that have been in contact with biohazards or recombinant or synthetic nucleic acid molecules are decontaminated before disposal using an effective method.

8. A hazard warning sign is posted on the access door to the laboratory work area or animal laboratory work area. The hazard warning sign:
   a. lists the name and telephone number of the Principal Investigator or other responsible person(s)
   b. incorporates the universal biohazard symbol;
   c. includes the laboratory biosafety level;
   d. identifies the agent;
   e. indicates any special requirement(s) for entering the laboratory (immunizations, etc);
   f. security-sensitive information is posted in accordance with institutional policy.

9. If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring BSL-2 level physical containment, they shall be conducted in accordance with all BSL-2 level laboratory practices.
10. The PI must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with infectious agents.

11. A laboratory specific biosafety manual must be prepared and adopted as policy. Personnel are required to read and follow instructions on laboratory specific practices and procedures.

12. The PI must ensure that laboratory personnel receive training regarding the necessary precautions to prevent exposures, and training regarding exposure evaluation procedures.
   a. Personnel must receive annual updates or additional training when procedural or policy changes occur.
   b. Personal health status may impact an individual’s susceptibility to infection, ability to receive immunizations or prophylactic interventions.
      i. All laboratory personnel and particularly women of child-bearing age should be informed regarding immune competence and conditions that may predispose them to infection. The Biosafety Office may be contacted to assist PIs in meeting this requirement.
      ii. Individuals having any questions regarding immunity and proposed research are encouraged to self-identify to the institution’s healthcare provider for appropriate counseling and guidance.

13. Laboratory personnel and staff working in and around facilities where infectious agents are stored or handled must be provided medical surveillance, and be offered the appropriate immunizations, when immunizations are available.
   a. Medical evaluation, surveillance, and treatment are provided by the institutional healthcare provider as appropriate and written records are maintained by the healthcare provider. Baseline serum samples are collected when appropriate.
   b. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the PI and the Biosafety Office.

**Containment Equipment (BSL-2)**

1. Biological safety cabinets (Class I or II) or other appropriate personal protective or physical containment devices are used whenever:
   a. Procedures with a high potential for creating aerosols are conducted. These may include pipetting, centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs.
   b. High concentrations or large volumes of organisms containing recombinant or synthetic nucleic acid molecules are used. Such materials may be centrifuged in the open laboratory if sealed rotors or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

2. Eye and face protection (goggles, mask, face shield or other splatter guard) and/or a respirator is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device.
a. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.

Laboratory Facilities (BSL-2)
1. Laboratory doors should be self-closing and have locks.
2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.
3. An eyewash station must be readily available.
4. An autoclave for decontaminating laboratory wastes must be available.
   a. A method for decontaminating all laboratory wastes must be available in the facility (e.g., chemical disinfection or other validated decontamination method).
   b. An autoclave for decontaminating laboratory wastes is recommended within the BSL-2 facility.
5. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operation.
   a. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
   b. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer’s recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection.
   c. Provisions to assure proper safety cabinet performance and air system operation must be verified.
6. Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
7. There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
8. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.

Section 7.3 Plant Biological Safety (recombinant or synthetic nucleic acid molecule research only)

Containment described in this section applies ONLY to genetically modified plants, or genetically modified microorganisms or small animals, or genetically modified microorganisms associated with small animals used in plant research.

Laboratory containment practices are used in place of the greenhouse facility requirements described below for Plant BSL-1 and Plant BSL-2 when plant tissue culture rooms, growth chambers within laboratory facilities, or experiments are performed on open benches. Additional biological containment
practices should be added by the Greenhouse Director or Institutional Biosafety Committee as necessary if botanical reproductive structures are produced that have the potential of being released.

Definitions

- **Greenhouse**: a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.
- **Greenhouse facility**: the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area.
- **Exotic plant pathogens**: plant pathogens that are not known to occur within the U.S.
- **Noxious weeds**: plant species that have been designated by state or national agricultural authorities as a plant that is injurious to agricultural and/or horticultural crops and/or humans and livestock. They are often an invasive species, but can be a native species. Noxious weeds grow aggressively, multiply quickly, and adversely affect desirable plants or are somehow injurious to livestock or humans either by contact or when ingested.

Plant-associated microorganisms include viroids, virusoids, viruses, bacteria, fungi, protozoans, certain small algae, and microorganisms that have a benign or beneficial association with plants, such as certain *Rhizobium* species and microorganisms known to cause plant diseases.

Plant-associated small animals include those arthropods that:

1. are in obligate association with plants,
2. are plant pests,
3. are plant pollinators, or
4. transmit plant disease agents, as well as other small animals such as nematodes for which tests of biological properties necessitate the use of plants. Microorganisms associated with such small animals (e.g., pathogens or symbionts) are included.

The principal purpose of plant containment is to avoid the unintentional transmission of a recombinant DNA-containing plant genome, including nuclear or organelle hereditary material or release of recombinant or synthetic nucleic acid molecule -derived organisms associated with plants. The containment principles are based on the recognition that the organisms that are used pose no health threat to humans or higher animals (unless deliberately modified for that purpose), and that the containment conditions minimize the possibility of an unanticipated deleterious effect on organisms and ecosystems outside of the experimental facility, e.g., the inadvertent spread of a serious pathogen from a greenhouse to a local agricultural crop or the unintentional introduction and establishment of an organism in a new ecosystem.

Indiana University does not have the facilities to support research utilizing Plant BSL-3 or Plant BSL-4. Containment requirements for these facilities are not described here. These include experiments utilizing exotic agents infectious to plants, genetically modified organisms with recognized potential for serious detrimental impact on managed or natural ecosystems, and plants expressing cloned sequences of potent vertebrate toxins that are lethal to vertebrates.
7.3.1 Biosafety Level 1 - Plants (BSL1-P)

BSL1-P is appropriate for plant research in which the associated genetically modified non-exotic microorganism has no potential for rapid and widespread dissemination or serious detrimental impact on managed or natural ecosystems. Plant research utilizing *Rhizobium* spp. and *Agrobacterium* spp. meet these criteria. Also permissible are plant experiments with genetically modified arthropods or small animals in wild type plants, and experiments with wild type small animals in which the plant is genetically modified, that do not meet the criteria for BSL1-P Enhanced, BSL2-P, or BSL2-P Enhanced. BSL1-P may be used for research in which the genetically modified plant is not a noxious weed, or the genetically modified plant cannot interbreed with noxious weeds in geographic area.

1. Access to the greenhouse shall be limited or restricted, at the discretion of the PI and Greenhouse Director, when experiments are in progress.
2. A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens), by methods appropriate to the organisms and in accordance with applicable state and Federal laws.
3. Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.
4. Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.
5. Experiments involving other organisms that require a containment level lower than BL1-P may be conducted in the greenhouse concurrently with experiments that require BL1-P containment, provided that all work is conducted in accordance with BL1-P greenhouse practices.
6. Prior to entering the greenhouse, personnel shall be required to read and follow instructions on greenhouse practices and procedures. All procedures shall be performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism.
7. A record shall be kept of experiments currently in progress in the greenhouse facility.
8. The greenhouse floor may be composed of gravel or other porous material. At a minimum, impervious (e.g., concrete) walkways are recommended.
9. Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to contain or exclude pollen, microorganisms, or small flying animals (e.g., arthropods and birds); however, screens are recommended.

7.3.2 Biosafety Level 2 - Plants (BSL2-P)

Plant Biosafety Level 1 Enhanced and Plant Biosafety Level 2 are appropriate for recombinant or synthetic nucleic acid molecule experiments that utilize a genetically modified exotic microorganism, or that utilize a genetically modified microorganism associated with arthropods or small animals, where the genetically modified microorganism (in either case) has no recognized potential for serious detrimental impact on managed or natural ecosystems.
BSL1-P Enhanced or BSL2-P is also appropriate for plant research that utilizes a genetically modified non-exotic microorganism that has a recognized potential for serious detrimental impact on managed or natural ecosystems.

Research utilizing genetically modified plants in which introduced DNA represents the complete genome of a non-exotic plant pathogen, and experiments with genetically modified arthropods associated with plants that are vectors of human, animal or plant disease, and genetically modified plants that are noxious weeds, or that can interbreed with noxious weeds in the geographic area, are also conducted under BSL1-P Enhanced or BSL2-P containment.

**Plant BSL-2** incorporates all of the practices and facility requirements of BSL1-P. Where requirements described for BSL2-P are more stringent than BSL1-P, the more stringent requirement must be met.

1. Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, to individuals directly involved with the experiments when they are in progress.
2. Materials containing experimental microorganisms, which are brought into or removed from the greenhouse facility in a viable or intact state, shall be transferred in a closed non-breakable container.
3. Decontamination of run-off water is not necessarily required. If part of the greenhouse is composed of gravel or similar material, appropriate treatments should be made periodically to eliminate, or render inactive, any organisms potentially entrapped by the gravel.
4. A record shall be kept of
   a. experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.
   b. experiments currently in progress in the greenhouse facility.
5. A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following:
   a. the name of the responsible individual
   b. the plants in use
   c. any special requirements for using the area
   d. If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated on a sign posted on the greenhouse access doors.
   d. If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.
6. Experiments involving other organisms that require a containment level lower than BL2-P may be conducted in the greenhouse concurrently with experiments that require BL2-P containment provided that all work is conducted in accordance with BL2-P greenhouse practices.
7. A greenhouse practices manual shall be prepared or adopted. This manual shall:
   a. advise personnel of the potential consequences if such practices are not followed
   b. outline contingency plans to be implemented in the event of the unintentional release of organisms.
8. The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Greenhouse Director and Biosafety Office.
and other appropriate authorities immediately (if applicable). Documentation of any such accident shall be prepared and maintained.

BSL2-P Greenhouse Facilities
1. The greenhouse floor must be composed of an impervious material.
2. Concrete is recommended, but gravel or other porous material under benches is acceptable unless propagules containing experimental organisms are readily disseminated through soil.
3. Soil beds are acceptable unless propagules containing experimental organisms are readily disseminated through soil.
4. Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to exclude pollen or microorganisms. However, screens are required to exclude small flying animals (e.g., arthropods and birds).
5. An autoclave shall be available for the treatment of contaminated greenhouse materials.
6. If air intake fans are used, measures shall be taken to minimize the ingress of arthropods.
7. Louvers or fans that control intake air shall be constructed such that they can only be opened when the fan is in operation.

7.3.3 Plant Biosafety Level 2 Enhanced and Plant Biosafety Level 3

IU does not have the facilities to support BSL3-P research. In collaboration with the PI, the IBC must determine the appropriate containment for research that meets the criteria for BSL2-P Enhanced/BSL3-P. Experiments of this nature have potential for detrimental impact on managed or natural ecosystems, and include the following:

- Experiments involving an exotic plant pathogen.
- Plant experiments with genetically modified microorganisms or insects.
- Experiments involving plants containing cloned genomes of readily transmissible exotic plant pathogens in which there exists the possibility of reconstituting the complete and functional genome of the infectious agent by genomic complementation in planta.
- Plant experiments with microbial pathogens of insects or small animals.
SECTION 8 ANIMAL FACILITY BIOSAFETY MANUAL Section

8.1 Introduction

Laboratory animal facilities are a special type of laboratory. As a general principle, the biosafety level recommended for working with infectious agents in vivo and in vitro are comparable. The animal room can present unique problems. In the animal room, the activities of the animals themselves can present unique hazards not found in standard microbiological laboratories. Animals may generate aerosols, they may bite and scratch, and they may be infected with a zoonotic agent.

The recommendations detailed below describe combinations of practices, safety equipment, and facilities for experiments with animals involved in infectious disease research and other studies that may require containment. These three combinations describe the minimal standards for activities involving infected laboratory animals.

These recommendations presuppose that laboratory animal facilities, operational practices, and quality of animal care meet applicable standards and regulations (e.g., Guide for the Care and Use of Laboratory Animals and Laboratory Animal Welfare Regulations) and that appropriate species have been selected for animal experiments. Prior to beginning a study animal protocols must also be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee.

Facility standards and practices for invertebrate vectors of disease and hosts are not specifically addressed in this section. Refer to the Arthropod Containment Guidelines for containment requirements for experimentally infected arthropod vectors of disease.

Each animal biosafety containment level incorporates practices, personal protective equipment, and engineering controls equivalent to the corresponding laboratory biosafety containment level. As such, the policies and practices described in Section 7 must be followed in laboratories where animals are housed or manipulated. Additional requirements specific to animal containment biosafety are described below.

Animal Occupational Health

A medical surveillance program is in place for researchers who work with or around animals. All personnel working around research animals are required to enroll in the Animal Occupational Health Program administered by Environmental Health and Safety. PIs should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.

Personnel must have specific training in animal facility procedures and must be supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures.

Transgenic Animals and Experimentally Infected Animals

- Cages housing transgenic animals subject to the NIH Guidelines and animals experimentally infected with wild type or genetically modified microorganisms or a viral vector, should be
designated. Marking cage cards with biohazard stickers, designating the rack, or other IBC approved methods, meets with this requirement.

- Laboratory Animal Resource (LAR) staff handling of animals, cages, or bedding from cages housing animals infected with viral vectors is at the discretion of the Director of LAR.

- On the Bloomington campus, all experimental animals and tissues will be frozen after euthanization or death. Environmental Health and Safety must be contacted to collect frozen carcasses. All animal carcasses are subsequently removed from Indiana University and incinerated as medical waste.

Section 8.2 Animal Biosafety Level 1 (ABSL-1) and ALL Animal Laboratories

Animal Biosafety Level 1 practices and facilities encompass all requirements described in Section 7.1 ALL Laboratories, and Section 7.2.1 Biosafety Level 1.

ABSL-1 is suitable for work involving well-characterized agents that are not known to cause disease in immunocompetent adult humans, and present minimal potential hazard to personnel and the environment. Personnel must have specific training in animal facility procedures and must be supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures.

The following practices, safety equipment and facilities apply to all animal research:

Practices

1. An Animal Facility Safety Manual is prepared or adopted in consultation with the LAR Director and appropriate safety professionals.
   a. The safety manual must be available and accessible.
   b. Personnel are advised of potential hazards and are required to read and follow instructions on practices and procedures.
   c. Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.\textsuperscript{25,26,27}

2. The LAR Director establishes and enforces policies, procedures, and protocols for institutional policies and emergency situations.

3. LAR Supervisors and PIs must ensure that
   a. Staff and researchers under their direction such as animal care staff, laboratory personnel and support personnel, receive appropriate training regarding animal husbandry procedures, potential hazards, and manipulations of infectious agents;
   b. Records are maintained for all hazard evaluations, employee training sessions and staff attendance;
   c. The IU occupational healthcare provider is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.

4. All laboratory personnel, Laboratory Animal Resources (LAR) staff, IU staff and visitors must be advised of potential hazards and must be instructed on the appropriate safeguards.
a. The LAR Director is responsible for advising and instructing on hazards present in the facility to LAR staff.

b. The PI is responsible for advising and instructing on hazards to laboratory personnel.

5. A sign incorporating safety information must be posted at the entrance to the areas where infectious materials and/or animals are housed or are manipulated. The sign must include
   a. name of the PI (or other responsible personnel) and telephone number;
   b. name of the LAR Director and telephone number;
   c. the animal biosafety level;
   d. personal protective equipment requirements;
   e. required procedures for entering and exiting the animal areas;
   f. general occupational health requirements.

6. Access to the animal facility is restricted. Only those persons required for program or support purposes are authorized to enter the facility.

7. Laboratory coats, gowns, smocks, or uniforms may be required to prevent contamination of personal clothing. Protective outer clothing is not worn outside areas where infectious materials and/or animals are housed or manipulated. Before exiting the animal laboratory for non-laboratory areas (e.g., cafeteria, library, administrative offices), protective clothing is removed and left in the animal laboratory.

8. Impervious and/or protective gloves are required to protect hands from exposure to hazardous materials, experimental animals, biohazards, and recombinant or synthetic nucleic acid molecules.
   a. Wear two pairs of gloves when appropriate.
   b. Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.

9. Additional personal protective equipment, such as eye, face, respiratory protection, finger guards, etc can be used in rooms containing infected animals, as dictated by the risk assessment.

10. Needles and syringes or other sharp instruments are limited to use in the animal facility when there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.

11. Consideration should be given to the use of restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications, etc).

12. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in closed durable leak-proof container for appropriate disposal in compliance with applicable institutional, local and state requirements.

13. Persons must wash their hands after they handle animals.

Animal Facilities

1. The animal facility and individual animal containment areas must be in accordance with state and federal laws and animal care requirements.
2. Doors to areas where infectious materials and/or animals are housed open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open.

3. The animal facility must have a sink for hand washing. Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.

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

5. Emergency eyewash and shower are readily available; location is determined by risk assessment.

6. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping.
   a. The interior surfaces (walls, floors and ceilings) are water resistant.
   b. It is recommended that penetrations in floors, walls and ceiling surfaces are sealed, to include openings around ducts, doors and door frames, to facilitate pest control and proper cleaning.
   c. Floors must be slip resistant, impervious to liquids, and resistant to chemicals.

7. External windows are not recommended; if present windows must be resistant to breakage. Where possible, windows should be sealed. If the animal facility has windows that open, they are fitted with fly screens. The presence of windows may impact facility security and therefore should be assessed by security personnel.

8. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

9. Ventilation should be provided in accordance with the Guide for Care and Use of Laboratory Animals.  
   a. No recirculation of exhaust air should occur.
   b. It is recommended that animal rooms have inward directional airflow.
   c. Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

10. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.

11. Cage bedding from animals dosed with drugs or chemical toxins may contain un-metabolized residue or toxic metabolites. For occupational safety purposes this bedding must be emptied into bags at a ventilated dump station or within a fume hood. In addition to standard personal protective equipment (lab coat or gown, safety glasses and gloves), respiratory protection may be utilized if adequate ventilation is not available or fugitive dust is generated outside the containment.

12. Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F. The cage wash area should be designed to accommodate the use of high pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures, during the cage/equipment cleaning process.
8.3 Animal Biosafety Level 2 (ABSL-2)

Animal Biosafety Level 2 practices and facilities encompass all requirements described in
- Section 7.1 ALL Laboratories
- Section 7.2 Laboratory Biological Safety (BSL-1 and BSL-2)
- Section 8.2 ABSL-1 and ALL Animal Laboratories

When ABSL-2 specifies practices or facilities that are more stringent than BSL-2, ABSL-2 will supersede BSL-2.

ABSL-2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1. ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

ABSL-2 requires that personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of pathogenic agents. Personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures. Procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, should be conducted in BSCs or by use of other physical containment equipment.

The following practices, safety equipment and facilities apply to ABSL-2:

1. The PI establishes policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific facility entry requirements (e.g., vaccination) may enter the animal facility. The PI is responsible for establishing policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific facility entry requirements (e.g., vaccination) may enter the laboratory, enforces policies, procedures, and protocols for institutional policies and emergency situations.

2. A sign incorporating safety information must be posted at the entrance to the areas where infectious materials and/or animals are housed or are manipulated. The sign must include
   a. name of the PI (or other responsible personnel) and telephone number;
   b. name of the LAR Director and telephone number;
   c. the animal biosafety level;
   d. the universal biohazard symbol;
   e. infectious agents present in animal rooms or used in animal research;
   f. personal protective equipment requirements;
   g. required procedures for entering and exiting the animal areas;
   h. general occupational health requirements;
   i. security-sensitive information should be posted in accordance with institutional policy.

3. Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel and IU staff.

4. Gowns, uniforms, laboratory coats and personal protective equipment are worn while in the areas where infectious materials and/or animals are housed or manipulated and removed prior to exiting.
a. Scrub suits and uniforms are removed before leaving the animal facility. Laboratory and protective clothing should never be taken home.
b. Reusable clothing is appropriately contained and decontaminated before being laundered.
c. Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.

5. Eye and face protection (mask, goggles, face shield or other splatter guard) are used for anticipated splashes/ sprays from infectious or other hazardous materials and when the animal or microorganisms must be handled outside the BSC or containment device.

6. If the agent used as a vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste, etc.) should be prevented.

7. Properly maintained BSCs, personal protective equipment (e.g., gloves, lab coats, face shields, respirators, etc.) and/or other physical containment devices or equipment, are used whenever conducting procedures with a potential for creating aerosols or splashes. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals.

8. When indicated by risk assessment, animals are housed in primary biosafety containment equipment appropriate for the animal species, such as solid wall and bottom cages covered with filter bonnets for rodents, or larger cages placed in inward flow ventilated enclosures or other equivalent primary containment systems for larger animal cages.

9. Biological materials that are viable or in an intact state and that are being removed from the animal containment area for the purpose of further manipulation in a BSL-2 laboratory must be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container.
   a. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Providing a description of transfer procedures in an approved IBC protocol fulfills this requirement.
   b. All containers, primary and secondary, shall be disinfected before removal from the animal facility.
   c. Packages containing viable agents may only be opened in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction.

10. Equipment, cages, and racks should be handled in manner that minimizes contamination of other areas. Decontamination is recommended for all potentially infectious materials and animal waste before movement outside the areas where infectious materials and/or animals are housed or are manipulated by an appropriate method.

11. Cages and contents of cages must be autoclaved prior to washing. See Section 4.6, Standard Operating Procedure for Research Staff Autoclaving Dirty BSL-2 Animal Cages.

12. Materials to be decontaminated outside of the immediate areas where infectious materials and/or animals are housed or are manipulated, or at a site away from the laboratory, must be placed in a durable, leak proof, covered container and secured for transport.
a. The outer surface of the container is disinfected prior to moving materials.
   b. The transport container must contain a universal biohazard label.

13. Equipment must be surface decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated. Consideration should be given to means for decontaminating routine husbandry equipment, sensitive electronic and medical equipment.

14. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the Principal Investigator, LAR Director, and the Biosafety Office.

**ABSL-2 Facilities**

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

2. If vacuum service (i.e., central or local) is provided, each service connection should be fitted with liquid disinfectant traps and an in-line HEPA filter, placed as near as practicable to each use point or service connection. Filters are installed to permit in-place decontamination and replacement.

3. An autoclave should be present in the animal facility to facilitate decontamination of infectious materials and waste.
SECTION 9 SHIPMENTS OF BIOLOGICAL MATERIALS

9.1 Introduction

The Biosafety Office MUST be contacted prior to packaging and transporting any biological material classified as a Dangerous Good.

Shipment of infectious agents, biological products, and clinical specimens is regulated by many agencies. Multiple permits may be needed from multiple agencies, and requirements are not always uniform. In addition, regulations are continually modified and new ones are added. A summary of current requirements is presented in Section 9.2, but it is recommended that the investigator check with the various agencies before shipping any material that may be regulated.

In general, first determine whether the material you wish to ship requires a federal permit, and begin the application process, if required. Depending on the type of permit applied for, permits may take as few as 10 days, or as many as 120-150 days. Second, contact the Biosafety Office for assistance with U.S. Department of Transportation (DOT) and International Air Transport Association (IATA) packaging, labeling, and documentation requirements. Third, decide on a carrier, and learn the packaging and labeling requirements of that carrier.

- DOT defines *Hazardous Material* as “a substance or material that the Secretary of Transportation has determined is capable of posing an unreasonable risk to health, safety and property when transported in commerce.”
- IATA defines *Dangerous Goods* as “articles or substances which are capable of posing a risk to health, safety, property, or the environment.”

For transport purposes, infectious substances are those that are known or reasonably expected to contain pathogens. *Pathogens* are defined as microorganisms and other agents which can cause disease in humans or animals. The DOT definition of an infectious substance may be more stringent than that of the Occupational Safety and Health Administration (OSHA) or the Center for Disease Control and Prevention (CDC). The DOT definition must be used when determining if a substance intended for transport is regulated.

Plant pathogens are not considered “infectious” for the purposes of transport. Consult the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) for regulations pertaining to transport of plant material.

9.2 Permits

Permits and licenses, if applicable, must be in place prior to initiating shipment or receipt of Dangerous Goods. The Biosafety Office may be contacted to provide assistance with import permits. See Section 9.2.2 and 9.3 for information regarding Export licenses.

Transport of Select Agent Toxins: In addition to import permits or export licenses, domestic transport of select agent toxins may require a USDA/APHIS/Veterinary Services (VS) permit.
9.2.1 Public Health Service
A U.S. Public Health Service (PHS) permit is required to import material containing etiologic (infectious) agents. Etiologic agents, vectors, and materials containing etiologic agents are recognized as hazardous materials. Etiologic agents are those microorganisms and microbial toxins that cause disease in humans and include bacteria, bacterial toxins, viruses, fungi, rickettsiae, protozoans, and parasites. Arthropods and other organisms that transmit pathogens to animals (including humans) are called vectors. The importer is legally responsible for assuring that the foreign personnel package, label, and ship the infectious materials according to Federal and international regulations.

Permits are required for
1) any microorganism that causes disease in humans;
2) biological materials, such as blood and tissues, when known or reasonably suspected to contain an infectious agent;
3) live insects or vectors of disease, such as mosquitoes, known or reasonably suspected of being infected with any disease transmissible to humans; and
4) any animal known or reasonably suspected of being infected with any disease transmissible to humans.

Detailed information about PHS import permits may be accessed at [www.cdc.gov/od/eaipp/](http://www.cdc.gov/od/eaipp/).

9.2.2 Animal and Plant Health Inspection Service

APHIS serves to facilitate
- Safe international trade by monitoring the health of animals presented at the border and regulating the import and export of animals, animal products, and biologics.
- Monitoring the movement of hazardous material, protecting against the introduction of pests, regulating the import and export of plants, and helping exporters meet the entry requirements of other countries.

Animals

Plants
9.2.3 Fish and Wildlife Service
U.S. Fish and Wildlife Service permits are required for certain live animals, including bats. Consult [www.fws.gov](http://www.fws.gov/) for details.

9.2.4 Import of Select Agents
Individuals wishing to import select agents and toxins must be registered with CDC’s Select Agent Program in accordance with 42 CFR Part 73 (Possession, Use, and Transfer of Select Agents and Toxins; Interim Final Rule) for the select agent(s) and toxin(s) listed on the import permit application. Contact the Biosafety Office prior to applying for any permit for materials known or suspected to contain viable or non-viable select agents.

9.3 Export of Infectious Materials
Contact the Office of Research Administration Compliance Services for assistance with export control questions and assistance with evaluating requirements or applying for licenses.

The export of a wide variety of etiologic agents and toxins of human, plant, and animal diseases may require a license from the U.S. Department of Commerce (DOC). **These include all microorganisms and toxins designated as Select Agents, including attenuated strains.** However, the list of organisms and toxins regulated for the purpose of export also includes several microorganisms that may be used for research conducted in Biosafety Level 2 laboratories. The following are just a few examples of nonselect agent microorganisms and toxins, or genetic elements thereof, that require an export license prior to shipment outside the United States:

- *Escherichia coli* O157:H7
- *Salmonella typhi*
- *Shigella dysenteriae*
- *Vibrio cholera*
- *Clostridium perfringens*
- Dengue Fever virus
- Yellow fever virus
- St. Louis encephalitis virus
- Western equine encephalitis virus
- Vesicular stomatitis virus (all strains)
- Cholera toxin, Aflatoxin

**Genetically modified organisms or genetic elements**

- that contain nucleic acid sequences associated with pathogenicity of regulated organisms
- that contain nucleic acid sequences associated with pathogenicity and are derived from regulated plant pathogens
- Microorganisms genetically modified to produce a regulated toxin

Genetic elements include chromosomes, genomes, plasmids, transposons, and vectors, whether genetically modified or unmodified. Nucleic acid sequences associated with the pathogenicity of any of the *microorganisms means any sequence specific to the relevant controlled microorganism that:

- In itself or through its transcribed or translated products represents a significant hazard to human, animal or plant health; or
- Is known to enhance the ability of a “microorganism or any other organism into which it may be inserted or otherwise integrated, to cause serious harm to human, animal or plant health
9.4 Shipping Biological Materials

9.4.1 Responsibilities, Classification and Identification

Title 49 of the Code of Federal Regulations contain the Hazardous Materials Regulations (HMR). These regulations pre-empt all local, state and federal regulations for the transport of hazardous materials. Violations may result in civil penalties up to $50,000 for each violation, and possible criminal penalties of up to $500,000 or up to 5 years imprisonment.

The HMR are applicable to anyone who handles or transports hazardous materials, offers hazardous materials for transport, or causes hazardous materials to be transported, or performs any function related to the transport of hazardous materials in commerce. Biological Dangerous Goods are included in the HMR. Anyone who performs the functions listed above should contact the Biosafety Office for assistance and training.

Individual states/countries or carrier’s regulations may vary from the IATA Dangerous Goods Regulation (DGR). These variations are generally more restrictive than IATA DGR. The shipper must take into account these more restrictive regulations, as well as the regulations for states/countries that shipment will fly over, and the destination state/country.

Infectious agents, blood, body fluids, tissues, cultures, and other potentially biohazardous materials are not permitted in carry-on bags or in checked luggage on aircraft. Unlike the exceptions for other small amounts of dangerous goods, there are no “small quantity” or “limited quantity” exceptions for infectious substances or dry ice.

The Shipper (Consignor): the person or people who prepare the material for shipment and offer the material to the Carrier.

The Carrier (Operator): the party that moves the material from point A to point B.

The Receiver (Consignee): receives the material.

Transportation refers to the packaging and shipping of these materials by air, land, or sea, generally by a commercial conveyance.

Transfer refers to the process of exchanging these materials between facilities.

Shipper Responsibilities

Classification: The Shipper establishes whether or not a material is a Dangerous Good.

Identification: The Shipper selects the Proper Shipping Name (PSN). All Dangerous Goods must be assigned a PSN. PSNs are standardized throughout the world.

Packing Instruction: The Shipper is responsible for following the proper instructions for selection of the appropriate packaging material. Each PSN has a designated Packing Instruction associated with it. The corresponding Packing Instruction must be used to package the material, if packaging
does not meet these standards, in the eyes of the regulatory agencies the fault lies entirely with the shipper.

**Marking and Labeling:** The Shipper communicates information about the hazards present in the package to the carrier, consignee, and general public. The shipper must ensure that marks and labels meet regulatory requirements and are correct and legible.

**Documentation:** All shipments of Dangerous Goods require documentation. The shipper must ensure that documentation is correct and legible, and meets regulatory requirements.

**Carrier Responsibilities**
The operator/carrier is responsible to ensure all Dangerous Goods have been packaged, marked, labeled and documented appropriately. Operators who accept improperly packaged Dangerous Goods are liable for those packages, thus they will reject or refuse packages that do not meet all regulatory standards. Operators have the authority to open packages suspected to contain hidden or undeclared Dangerous Goods or improperly packaged material.

**Receiver Responsibilities**
The consignee is responsible to obtain necessary import permits when the shipment is arriving internationally. DGR do not apply to consignees. However, consignees should inspect packages containing infectious substances prior to opening. If a package of infectious substances is damaged or leaks during shipment, or if damage or leaking is observed on receipt of the package, the CDC must be notified by the Biosafety Office.

Packages containing infectious substances should arrive with an itemized list of contents. Consignees should compare the list with the contents and report any discrepancies to shipper.

**9.4.2 Packaging, Labeling and Documentation**

All biological materials must be packaged so that they do not leak to ensure the shipper, carrier, receiver and general public are protected from possible exposure. Triple Packaging is required for all **Category A, Category B, Exempt Human Specimens, Exempt Animal Specimens**, and **genetically modified organisms** (GMOs). Triple Packaging is recommended for unregulated biological material.

Basic requirements for Triple Packaging are described here; however these instructions are intended for unregulated and exempt material. The specific requirements for Triple Packing for Category A and B Dangerous Goods vary, and increase in stringency as the hazard associated with the material increases. Note that when genetically modified organisms are shipped internationally, they are Class 9 Miscellaneous Dangerous Goods, and the corresponding Dangerous Goods requirements apply. **Persons who have not been trained and certified to package and ship Category A and B Dangerous Goods are not authorized to do so. The Biosafety Office must be contacted for assistance.**

“Certified” packaging is required to ship category A Infectious Substances. It is not required to ship Category B Infectious Substances, exempt and non-regulated materials. Packaging systems previously used to ship Category A and or Category B Infectious Substances may be re-used to
ship Category B Infectious Substances, exempt and nonregulated materials, provided all hazard marks and labels have been completely removed or defaced and the packing has been disinfected.

Packaging systems and labels for exempt and non-regulated items are available through online vendors, or from the Biosafety Office. Some shipping supplies are provided by the Biosafety Office at no cost, packaging systems are provided at the same rate as the manufacturer cost. An account number must be provided to the Biosafety Office prior to receiving shipping packaging systems.

9.5 Dangerous Goods

All Infectious Substances are classified as Biological Dangerous Goods. They are divided into category A and Category B Infectious Substances. All Infectious Substances must be packaged so that they do not leak, and to ensure the shipper, carrier, receiver and general public are protected from possible exposure. Triple Packaging is required for all Category A and Category B Infectious Substances.

Non-pathogenic genetically modified organisms and microorganisms (GMOs) are unregulated when shipped within the United States. When shipped internationally, GMOs are classified as Miscellaneous Dangerous Goods. Consult Appendix F and contact the Biosafety Office for international shipment of nonpathogenic GMOs.

Consult Appendix F, Program for Shipping Biological Dangerous Goods and contact the Biosafety Office for training and assistance packaging, marking, labeling, and shipping Biological Dangerous Goods (Category A and or Category B Infectious Substances).

Refrigerants
- **Wet ice** should not be used. If wet ice is used, it must be placed outside the secondary container, in a leakproof container, and outer packaging must be leakproof.
- **Gel paks** are not regulated, there are no requirements for marking or labeling
- **Dry ice (a Class 9 Dangerous Good):**
  - An insulated package must be used.
  - Dry ice may not be placed inside a sealed primary or secondary container because of the risk of explosion. The outer package must be secured such that venting may occur.
  - The secondary container must be secured such that the original orientation is maintained after refrigerant has melted or dissipated.
  - A UN1845 Dry Ice handling label and a Class 9 Miscellaneous Goods hazard label are required on the outside of the package, along with the weight of dry ice within the package.
- **Liquid nitrogen (a Class 2 Dangerous Good):**
  - An insulated package must be used.
  - Liquid nitrogen may not be placed inside a sealed primary or secondary container because of the risk of explosion. The outer package must be secured such that venting may occur.
  - The secondary container must be secured such that the original orientation is maintained after refrigerant has melted or dissipated.
  - **Additional requirements for liquid nitrogen**
Dry Shipper: Liquid nitrogen is fully adsorbed in a porous material where the design and insulated packaging would not allow the build-up of pressure in the container and would not permit the release of any liquid nitrogen regardless of package orientation.

Dry Shippers and excepted from all packaging, marking, labeling and documentations requirements for liquid nitrogen.

The Biosafety Office MUST be contacted for assistance with packages containing Dry Shippers in order to complete additional required paperwork.

- **Free standing liquid nitrogen**
  - A Class 2 Non-flammable Non-toxic Compressed Gas hazard label and “Contains Cryogenic Liquid” handling label are required on the outside of the package.
  - Contact the Biosafety Office for additional requirements for receptacles containing liquid nitrogen.

### 9.6 Non-Dangerous Goods:

**Exempt and Unregulated Specimens**

**Exempt Specimens** are specimens with low probability that pathogens are present. Exempt specimens are not regulated as Dangerous Goods. However, federal packaging, marking and labeling requirements do apply. There are two types of Exempt Patient Specimens, Exempt Human Specimens and Exempt Animal Specimens. The sample must be collected directly from a human or animal to meet criteria as an exempt patient specimen.

Judgment is based on known medical history, symptoms, individual circumstances of the source, and endemic local conditions. These types of samples are collected and transported for the purpose of testing for cholesterol, glucose, or hormone levels, prostate specific antigens, organ function of humans or animals with noninfectious disease, drug or alcohol monitoring, pregnancy tests, biopsies, and antibody detection in humans or animals in the absence of concern for infection. Exempt specimens may be transported for the purpose of screening for infectious agents, provided the source is not reasonably expected to be infected, or sources experiencing illness are not being targeted.

**Unregulated materials** are those that do not contain infectious substances or substances that are likely to cause disease in humans or animals. Some examples are:

- Non-pathogenic wild type organisms or microorganisms.
- Non-pathogenic genetically modified organisms and microorganisms (GMOs) are unregulated when shipped within the United States.
- Materials that have been inactivated or neutralized such that they no longer pose a health risk.
- Environmental samples which are not considered to pose a significant risk of infection. This includes decontaminated medical or clinical waste.
- Dried blood spots, fecal occult blood screening tests, blood or blood components, tissues and organs collected for the purpose of transfusion or transplantation.

### Packaging and Triple Packaging

All biological materials must be packaged so that they do not leak, and to ensure the shipper, carrier, receiver and general public are protected from possible exposure.
Triple Packaging is required for all Exempt Human or Animal Specimens, and genetically modified organisms (GMOs). Triple Packaging is recommended for unregulated biological material.

An Overpak is a large outer package (box or crate) or packing (wrapped in shrink wrap) containing multiple individual packages. Each individual package within the overpak must be fully compliant for packing, labels and marks, and documentation.

**Markings and Labeling**
- The complete name and address of the shipper and consignee must be listed on the outside of the package.
- “Package Orientation” labels and “Cargo Aircraft Only” labels are included as appropriate.
- Biohazard symbols and infectious substances markings or labels present on any primary, secondary or outer packages must be defaced prior to transporting unregulated and Exempt Patient Specimens in them.
- Exempt Patient Specimens must bear an “Exempt Human Specimen” or “Exempt Animal Specimen” label on the outside of the box.
- There are no marking or labeling requirements for unregulated biological materials

**Transportation; Unregulated and Exempt Patient Specimens**
Exempt specimens and non-regulated specimens may be transported in a private motor vehicle provided these three conditions are met:
- Material is a direct human or animal specimen, human or animal material. Cultures are not permitted in personal vehicles. University owned vehicles may be used.
- The vehicle cannot be used for any other purpose during transport including transport of passengers or food.
- The material must not meet the definition for a Category A infectious substance.

**Documentation**
- An itemized list of contents is not required for unregulated biological materials or Exempt Patient Specimens.
- If dry ice or liquid nitrogen are included, they must be listed as Dangerous Goods on the air waybill.

**9.7 Additional resources for shipping biological materials**

- **Dangerous Goods Regulations (DGR).** International Air Transport Association (IATA).
  - These regulations provide packaging and labeling requirements for infectious substances and materials, as well as clinical specimens that have a low probability of containing an infectious substance. These are the regulations followed by the airlines.
  - These regulations are derived from the Committee of Experts on the Transport of Dangerous Goods, United Nations Secretariat, and the Technical Instructions for the Transport of Dangerous Goods by air which is provided by the International Civil Aviation Organization (ICAO). A copy of the DGR may be obtained by calling 1-800-716-6326 or through the Internet at: [http://www.iata.org/](http://www.iata.org/), or [http://www.who.org](http://www.who.org)

- Centers for Disease Control and Prevention
Importation of Plant Pests. 7 CFR Part 330. Federal Plant Pest Regulations; General; Plant Pests; Soil; Stone and Quarry Products; Garbage.
- This regulation requires a permit to import or domestically transfer a plant pest, plant biological agent, or any material that might contain them.
- Information can be obtained by calling (301) 734-3277 or through the Internet at: http://www.aphis.usda.gov/ppq/permits/

Importation of Etiologic Agents of Livestock, Poultry and Other Animal Diseases. 9 CFR Parts 92, 94, 95, 96, 122 and 130.
- These regulations require an import permit from the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services to import or domestically transfer etiologic agents of livestock, poultry, other animals, and any materials that might contain these etiologic agents.
- Information may be obtained at (301) 734-3277, or from the Internet at: http://www.aphis.usda.gov/vs/ncie

- Provides minimal packaging and labeling requirements for transport of blood and body fluids within the laboratory and outside of it. Information may be obtained from your local OSHA office or from the Internet: http://osha.gov


Public Health Service 42 CFR Part 72.

Importation of Etiologic Agents of Human Disease. 42 CFR Part 71 Foreign Quarantine. Part 71.54 Etiologic Agents, Hosts and Vectors.
- This regulation requires an import permit from the Centers for Disease Control and Prevention for importing etiologic agents of human disease and any materials, including live animals or insects, that may contain them. An application and information on importation permits may be obtained by calling 1-888- CDC-FAXX and enter document number 101000 or on the Internet at: http://www.cdc.gov/od/ohs/biosfty/imprtper.htm

- Applies to the shipment of both biological agents and clinical specimens. Information may be obtained from the Internet at: http://www.text-trieve.com/dotrspa/
APPENDIX A References

3. [http://www.autoclavesporetesting.com/chemical_indicators.htm](http://www.autoclavesporetesting.com/chemical_indicators.htm)
6. Chatigny MA, Barkley WE, Vogl WF. Aerosol biohazard in microbiological laboratories and how it is affected by air conditioning systems. ASHRAE Transactions. 1974;80 (Pt 1):463-469.
10. Sewell, David L. Laboratory Associated Infections and Biosafety. Clinical Microbiology Reviews, 8:389-405, 1995
17. U.S. Department of Labor, Occupational Safety and Health Administration. 1991. (2)


25. National Institutes of Health, Office of Laboratory Animal Welfare. Public Health Service policy on humane care and use of laboratory animals, Bethesda (MD); The National Institutes of Health (US); 2000.


APPENDIX B  Federally Regulated Microorganisms and Toxins

The following information is current as of April 9, 2013.  Lists and information are updated periodically; see the National Select Agent Registry at http://www.selectagents.gov/ for updated information.

Infectious biological agents and toxins that have the potential to pose a severe threat to public, animal or plant health, or to animal or plant products have been classified by the U.S. Department of Health and Human Services (HHS) or U.S. Department of Agriculture (USDA) as biological Select Agents and Toxins. The National Select Agents Registry Program (NSAR) oversees activities utilizing select agents including possession, use and transfer. The HHS select agent program (42 CFR 73) is administered by the Center for Disease Control (CDC) Division of Select Agents and Toxins. The USDA select agent program (7 CFR 331, and 9 CFR 121) is administered by the Animal and Plant Health Inspection Service (APHIS) Veterinary Services and Plant Protection and Quarantine Programs.

Possession, use and transfer of select agents and toxins is not permitted without an approved registration. An approved registration provides information about the entity, facility, research, and personnel, and includes detailed plans for safety, security, and incident response.

Excluded Strains of Select Agents
A small number of attenuated strains of select agent organisms have been excluded from the HHS and USDA lists of select biological agents and toxins and are not subject to the requirements described in 42 CFR 73. An individual or entity that possesses, uses, or transfers an excluded attenuated strain will be subject to the regulations if there is any reintroduction of factor(s) associated with virulence or other manipulations that modify the attenuation such that virulence is restored or enhanced.

Identification of Select Agents
The identification of a select agent or toxin in a clinical, diagnostic, or environmental sample must be described on an approved Institutional Biosafety Committee (IBC) protocol prior to initiation of the research. Positive identification must be reported to the CDC or APHIS within 7 calendar days. The reporting requirement includes samples that have been rendered nonviable or nonfunctional prior to identification. Certain select agents must be reported immediately (within 24 hours) upon identification in a clinical, diagnostic, or environmental sample.

Additional requirements must be met, and protocols have been developed to assist PI’s. Contact the Biosafety Office for additional information or assistance.

Also regulated under 42 CFR 73
• Select agents and toxins that have been genetically modified.
• Genetic elements, recombinant or synthetic nucleic acid molecules, and organisms carrying them:
  o Nucleic acids that can produce infectious forms of any of the select agent viruses listed above.
  o Recombinant or synthetic nucleic acids that encode for the functional form(s) of any of the toxins listed above, if the nucleic acids can be expressed in vivo or in vitro or are in a vector or recombinant host genome and can be expressed in vivo or in vitro.
Exemptions to 42 CFR 73

- Non-viable select agents or nonfunctional toxins.
  - Fixed tissues are exempt, provided the agent(s) that may be present have been rendered non-viable
  - Subunits of toxins that do not cause harm or illness are exempt from the select agent regulations

  *Note: “Nonviable” or “nonfunctional”, regardless of the environment in which an organism can or cannot survive.*

- A select agent or toxin that is in its naturally occurring environment.
  - Examples of a natural environment:
    - Animals naturally infected with a select agent or toxin, e.g., rodents naturally infected with *Y. pestis.*
    - Milk samples that contain *Coxiella burnetti.*

  *Note: A select agent or toxin that has been intentionally introduced, cultivated, collected, or otherwise extracted from its natural source, is regulated under the select agent regulations. As such the entity is required to report the select agent or toxin upon identification.*
APPENDIX C Guidelines for Work with Toxins of Biological Origin

Biological toxins comprise a broad range of poisons, predominantly of natural origin but increasingly accessible by modern synthetic methods, which may cause death or severe incapacitation at relatively low exposure levels. The main laboratory risks are accidental exposure by direct contamination of mouth, eyes or other mucous membranes; by inadvertent aerosol generation; and by needle-sticks or other accidents that may compromise the normal barrier of the skin. An approved IBC protocol must be on file prior to any research that includes the use of natural, synthetic, or cloned biological toxins.

Routine operations with dilute toxin solutions are conducted under Biosafety Level 2 (BSL-2) policies, practices and containment with the aid of personal protective equipment and a well-maintained biosafety cabinet (BSC), chemical fume hood, or comparable engineering controls. If toxins and infectious agents are used together, then both must be considered when containment equipment is selected and safety procedures are developed. Animal safety practices must be considered for toxin work involving animals. Selected operations with toxins may require modified BSL-3 practices and procedures. This determination is made by the IBC in consultation with the PI and appropriate safety officials.

Training and Laboratory Planning
Each laboratory handling biological toxins must participate in the Indiana University Laboratory Safety and Chemical Safety Programs administered by Environmental Health and Safety, and comply with all applicable Occupational Safety and Health Association (OSHA) rules.

1. Training specific to the toxin(s) used should be required and documented for all laboratory personnel working with toxins, before starting work with the toxin and at intervals thereafter. Training should include
   a. how to handle transfers of liquids containing toxin;
   b. where to place waste solutions and contaminated materials or equipment;
   c. how to decontaminate work areas after routine operations and accidental spills.
2. Toxins should be stored in locked storage rooms, cabinets, or freezers when not in use to prevent theft or loss. An inventory control system should be in place to account for toxin use and disposition.
3. Access to areas containing toxins should be restricted to those whose work assignments require access.
   a. When toxins are in use, the room should be posted to indicate “Toxins in Use—Authorized Personnel Only.”
   b. Any special entry requirements should be posted on the entrance(s) to the room.
   c. Only personnel whose presence is required should be permitted in the room while toxins are in use.
4. Laboratory personnel must wear suitable laboratory personal protective equipment (PPE) to protect the hands and arms, such as laboratory coats, smocks, or coveralls and disposable gloves.
   a. When handling toxins that are percutaneous hazards (irritants, necrotic to tissue, or extremely toxic from dermal exposure), select gloves that are known to be impervious to the toxin.
   b. Consider both toxin and diluent when selecting gloves and other protective clothing.
c. When conducting liquid transfers and other operations that pose a potential splash or droplet hazard in an open-fronted hood or BSC, safety glasses and disposable facemask, or a face shield, should be worn.

5. Other protective equipment may be required, depending on the characteristics of the toxin and the containment system. Additional respiratory protection may be required if aerosols may be generated and it is not possible to use containment equipment or other engineering controls.

6. When handling dry forms of toxins that are electrostatic:
   a. Do not wear gloves (such as latex) that help to generate static electricity
   b. Use glove bag within a hood or biological safety cabinet, a glove box, or a class III biological safety cabinet.

7. Toxins must be centrifuged using the same practices and containment as infectious agents.
   a. Safety centrifuge cups or sealed rotors;
   b. Cleaned before each use to prevent contamination that may create an aerosol;
   c. Cups or rotor opened in a biosafety cabinet or chemical fume hood.

8. Pressurized tubes or other containers holding toxins, operations that expose toxin solutions to vacuum or pressure (e.g., sterilization of toxin solutions by membrane filtration), should be opened in a BSC, chemical fume hood, or other ventilated enclosure.

9. Preparation of primary containers of toxin stock solutions and manipulations of primary containers of dry forms of toxins should be conducted in a chemical fume hood, a glove box, or a biological safety cabinet or equivalent containment system approved by the IBC and Chemical Hygiene Officer. HEPA and/or charcoal filtration of the exhaust air may be required, depending on the toxin.

10. When vacuum lines are used with systems containing toxins should be protected with a HEPA filter to prevent entry of toxins into the lines. Sink drains should be similarly protected when water aspirators are used.

11. All high-risk operations should be conducted with two knowledgeable individuals present. Each must be familiar with the applicable procedures, maintain visual contact with the other, and be ready to assist in the event of an accident.

12. Before containers are removed from the hood, cabinet, or glove box, the exterior of the closed primary container should be decontaminated using a method known to be effective against the toxin, and placed in a clean secondary container. Toxins must be transported only in leakproof secondary containers.

13. Contaminated and potentially contaminated protective clothing and equipment should be decontaminated using methods known to be effective against the toxin before removal from the laboratory for disposal, cleaning or repair.
   a. If decontamination is not possible/practical, materials (e.g., used gloves) should be disposed of as toxic waste.
   b. Materials contaminated with infectious agents as well as toxins should also be autoclaved or otherwise rendered non-infectious before leaving the laboratory.

14. The interior of the hood, glove box, or cabinet should be decontaminated at the end of a series of related experiments. Until decontaminated, a sign must be posted on the hood, box, or cabinet to indicate that toxins are in use, and access to the equipment and apparatus restricted to necessary, authorized personnel.
Toxin Inactivation and Disposal
Toxin stability varies considerably outside of physiological conditions depending upon the temperature, pH, ionic strength, and availability of co-factors and other characteristics of the surrounding matrix. Literature values for dry heat inactivation of toxins can be misleading due to variations in experimental conditions, matrix composition, and experimental criteria for assessing toxin activity. Moreover, inactivation is not always a linear function of heating time, and some protein toxins possess a capacity to re-fold, and partially reverse inactivation caused by heating. In addition, the conditions for denaturizing toxins in aqueous solutions are not necessarily applicable for inactivating dry, powdered toxin preparations.

While the Biosafety Office can offer guidance, the Chemical Hygiene Officer should be contacted with all questions and for detailed instructions on spill cleanup, inactivation and disposal of biological toxins.

References for Working with Biological Toxins
APPENDIX D Working with Human and Nonhuman Tissues, Fluids and Cell Lines

The Centers for Disease Control and Prevention and the National Institutes of Health wish to express their appreciation to Frank P. Simione, M.S., and Jane Caputo, B.A., of the American Type Culture Collection (ATCC), a global bioscience organization dedicated to biological standards and biodiversity, for their contributions to the preparation of this Appendix.

At least 24 documented cases of infection of laboratory workers handling primary cell cultures (e.g., primary rhesus monkey kidney cells) have occurred in the past 30 years. While a limited number of laboratory-associated infections have been reported as resulting from the handling of human and other primate cells, there is a more significant risk to acquiring infection with HBV or HIV from exposure to human blood and other body fluids, and OSHA has developed a bloodborne pathogens standard. Procedures have been published to reduce contamination of cell cultures with microorganisms or other cells.

Potential Laboratory Hazards: The potential laboratory hazards associated with human cells and tissues include the bloodborne pathogens HBV and HIV, as well as agents such as Mycobacterium tuberculosis that may be present in human lung tissues. Other primate cells and tissues also present risks to laboratory workers. Potential hazards to laboratory workers are presented by cells transformed with viral agents, such as SV-40, EBV, or HBV, as well as cells carrying viral genomic material. Tumorigenic human cells also are potential hazards as a result of self-inoculation.

Recommended Practices: Human and other primate cells should be handled using Biosafety Level 2 practices and containment. All work should be performed in a biosafety cabinet, and all material should be decontaminated by autoclaving or disinfection before discarding. All employees working with human cells and tissues should be enrolled in the institutional Bloodborne Pathogens Program, and should practice Universal Precautions and work under the policies and guidelines established by the institutional Exposure Control Plan.

Employees should be offered hepatitis B immunization, and be evaluated by a health care professional following an exposure incident.

References for Working with Human and Nonhuman Tissues, Fluids and Cell Lines


**APPENDIX E Integrated Pest Management**

Pest management is an important part of managing a research facility. Many pests, such as flies and cockroaches, can mechanically vector disease pathogens and compromise the research environment. Even the presence of innocuous insects can contribute to the perception of unsanitary conditions.

The most common approach to pest control has been the application of pesticides, either as a preventive or remedial measure. Pesticidal treatments can be effective and may be necessary as a corrective measure, but they have limited long-term effect when used alone. Pesticidal applications also present the potential to contaminate the research environment through pesticide drift and volatilization.

To control pests and minimize the use of pesticides, it is necessary to employ a comprehensive program approach to pest management that integrates housekeeping, maintenance, and pest control services. This method of pest control is often referred to as Integrated Pest Management (IPM). The primary goal of an IPM program is to prevent pest problems by managing the facility environment in such a way as to make it less conducive to pest infestation. Along with limited applications of pesticides to control pests, pest control is achieved through proactive operational and administrative intervention strategies to correct conditions that foster pest problems.

IPM is a strategy-based service. The decision to implement an IPM program should be based not only on the cost of the services, but also on the effectiveness of the program's components. IPM is site-specific, and each program should be tailored to the environment where it is applied. IPM services in a laboratory will be different from those in an office building or an animal care facility.

Integrated pest management programs can be delineated into various interrelated components which contribute to the "environmental management" approach to controlling pests. These are:

- **Facility Design**: The inclusion of pest management issues and requirements in a facility's planning, design, and construction provides an opportunity to incorporate features that help to exclude pests, minimize pest habitat, and promote proper sanitation. This can help to reduce the need for future corrective pest management services that can be disruptive to research operations.
- **Monitoring**: Traps, visual inspections, and staff interviews are used to identify areas and conditions that may foster pest activity. Monitoring is the central activity of an IPM program and is used in place of preventive pesticidal treatments.
- **Sanitation and Facility Maintenance**: Many pest problems can be prevented or corrected by using proper sanitation, reducing clutter and pest habitat, and by performing repairs that exclude pests and reduce pest habitat. Maintaining records of structural deficiencies and housekeeping conditions can help to track problems and determine if corrective actions are completed in a timely manner.
- **Communication**: A staff member can be designated to meet with pest management personnel to assist in resolving facility issues that impact on pest management. Information on pest activity, and recommendations on personnel practices and facility conditions that impact pest management, can be relayed verbally and in writing to that person. Training on subjects such as
pest identification, biology, and sanitation can also promote understanding and cooperation with the goals of the IPM program.

- **Record Keeping**: A logbook can be used to record pest activity and conditions pertinent to the IPM program. It may contain protocols and procedures for IPM services in that facility; Material Safety Data Sheets on pesticides; pesticide labels; treatment records; floor plans; survey reports; etc.

- **Nonpesticidal Pest Control**: Pest control methods such as trapping, exclusion, caulking, washing, and freezing can be applied safely and effectively when used in conjunction with proper sanitation and structural repair.

- **Pest Control With Pesticides**: Preventive applications of pesticides should be discouraged, and treatments should be restricted to areas of known pest activity. When pesticides are applied, the least toxic product(s) available should be used and applied in the most effective and safe manner.

- **Program Evaluation and Quality Assurance**: Quality assurance and program review should be performed to provide an objective, ongoing evaluation of IPM activities and effectiveness. This is to ensure that the program is controlling pests and meeting the specific needs of the facility program(s) and its occupants. Based upon this review, current pest management protocols can be modified and new procedures implemented.

- **Technical Expertise**: A qualified entomologist can provide helpful technical guidance in developing and implementing an IPM program. Pest management personnel should be licensed and certified through examination by the appropriate regulatory agency.

- **Safety**: By limiting the scope of pesticidal treatments and using nonpesticidal control practices, IPM can minimize the potential of pesticide exposure to the research environment and the staff.

Prior to initiating any type of pest management program, development of an operational framework for IPM services can help to promote collaboration between pest management specialists and facility personnel. This framework can also be used to incorporate facility restrictions and operational and procedural issues into the IPM program. An effective pest management program is an integral part of the facility's management. Including an IPM policy statement in the facility's standard operating procedures can increase awareness of the program.

Training on the principles and practices of structural (indoor) integrated pest management and information on IPM programs is available from many sources. Some of these are university entomology departments, county extension offices, the Entomological Society of America, state departments of agriculture, state pest control associations, the National Pest Control Association, suppliers of pest control equipment, and pest management consultants or pest management firms. There are also correspondence courses available from several universities as well as short courses and training conferences on structural pest management.

**Additional Information**

- National Pest Control Association: [http://www.pestworld.org](http://www.pestworld.org)
- Biocontrol Network: [http://www.bioconet.com](http://www.bioconet.com)
- United States Environmental Protection Agency (EPA): [http://www.epa.gov/pesticides/](http://www.epa.gov/pesticides/)
- Indiana State Chemist and Seed Commissioner (OISC): [http://www.isco.purdue.edu](http://www.isco.purdue.edu)
APPENDIX F Program for Shipping Biological Dangerous Goods

Personnel planning to ship Biological Dangerous Goods should read and be familiar with Section 9 and this Appendix. Training MUST be obtained from the Biosafety Office prior to shipping biological Dangerous Goods.

Dangerous Goods Classification

There are 9 DOT/IATA Dangerous Goods classifications. The Classifications appropriate for biological materials and refrigerants are listed here, along with a brief description of the materials that belong under that classification.

Class 6, Division 6.2 Infectious Substances

- **Category A** “An infectious substance which is transported in a form that, when exposure occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals.” Typically Risk Group 3 and 4 microorganisms, and includes toxins that meet this criteria. However, Category A Infectious Substances may include new or emerging pathogens and some Risk Group 2 microorganisms, when cultured for transport. If any of the materials below meet the criteria for a Category A Infectious substance, the package must ship as Category A.
  - Cultures
  - Infectious GMOs
  - Patient specimens
  - Medical or Clinical waste, biohazardous waste

The following non-select agent pathogenic microorganisms are indicative of the Infectious Materials that must be shipped as Category A when shipped as cultures. These Infectious Materials may ship as Category B when shipped in a patient specimen. The list is not meant to be comprehensive:

<table>
<thead>
<tr>
<th>Risk Group 2</th>
<th>Risk Group 3</th>
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<tbody>
<tr>
<td>Dengue virus</td>
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<tr>
<td>Poliovirus</td>
<td>Hantavirus</td>
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<tr>
<td>Yellow Fever virus</td>
<td>Human immunodeficiency virus</td>
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<td>Hepatitis B virus</td>
<td><em>Mycobacterium tuberculosis</em></td>
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<td>West Nile virus</td>
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<td>Chlamydia psittaci</td>
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<tr>
<td><em>Escherichia coli</em>, verotoxigenic</td>
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<tr>
<td><em>Shigella dysenteriae</em> type 1</td>
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<tr>
<td>Rabies virus</td>
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- **Category B** “An infectious substance which does not meet the criteria for inclusion into Category A.” If any of the materials below meet the criteria for a Category B Infectious substance, the package must ship as Category B. Includes toxins.
  - Cultures
  - Infectious GMOs
  - Patient specimens
  - Medical or Clinical waste, biohazardous waste

Human or animal specimens known to contain infectious agents, or collected from sources reasonably expected to be infected must ship as category A or B (example, blood samples collected from patients...
participating in an anti-HIV therapy trial) depending on the agent that is known or suspected to be present. These samples may ship as Exempt Human Specimens or Exempt Animal Specimens ONLY if they have been killed or inactivated prior to shipment. Specimens in which the presence of an infectious agent is not known, and an infectious agent is not reasonably expected to be present, may ship as exempt specimens.

Specimens known to contain select agents must be shipped as Category A. Under Title 42 Code of Federal Regulations Part 73 it is illegal to receive samples known to contain viable select agents without an approved CDC Registration (CDC Form 1) that lists the select agent being transported. In addition, transport of select agents requires prior approval from the CDC and notification to the CDC on receipt of the package (CDC Form 2).

**Dangerous Goods Identification**

Each Dangerous Good Infectious Material has a Proper Shipping Name (PSN). Each PSN has a corresponding UN identification number. For example, the Proper Shipping Name for a Category B Infectious Material is Biological Substance, Category B, the UN identification number is UN3373. This information must be included on the outside of Dangerous Goods packages. The UN identification number and PSN for Category A, Category B, medical waste and genetically modified organisms are listed below:

- UN 2814 Infectious Substance, affecting humans. (This is a Category A Infectious Substance.)
- UN 2900 Infectious Substance, affecting animals. (This is a Category A Infectious Substance.)
- UN3373 Biological Substance, Category B
- UN3291: Identification used for medical or clinical waste containing a Category B infectious substance, and medical or clinical waste with a low probability of containing an infectious substance. The PSNs are:
  - UN3291 Clinical waste, unspecified, n.o.s
  - UN3291 (Bio) Medical waste, n.o.s
  - UN3291 Regulated medical waste, n.o.s
- UN3245 Genetically Modified Organism, UN3245 Genetically Modified Microorganism
- UN1845 Dry Ice, UN1845 Carbon Dioxide, solid
- UN1997 Nitrogen, refrigerated liquid.

**Packaging**

When packaging Dangerous Goods for transport, quantity limits apply. The quantity limits differ for passenger and cargo aircraft.

All biological materials must be packaged so that they do not leak, and to ensure the shipper, carrier, receiver and general public are protected from possible exposure. Triple Packaging is required for all shipments of Category A and Category B Infectious Materials.

Basic requirements for Triple Packaging are described in Section 9; however these instructions are intended for unregulated and exempt material. The specific requirements for Triple Packing for Category A and B vary, and increase in stringency as the hazard associated with the material increases. Persons
who have not been trained and certified to package and ship Dangerous Goods are not authorized to do so. The Biosafety Office must be contacted for training or assistance.

Category A Infectious Materials MUST be shipped in certified shipping systems. “Certified” packaging meets the requirements set out by the DOT and IATA, have been certified by the governing national authority, and bear the UN Specification Mark. Packaging that does not meet these requirements is not permitted to be used to ship Category A Infectious Materials. Substitutions of packing materials are not allowed, and invalidate the UN Specification Mark and the certification. For example, the secondary pressure vessel from one manufacturer may not be used in the outer package from another manufacturer.

The following packing systems are not considered certified by state, federal and international regulatory agencies, and are not permitted for shipping Category A Infectious Substances at Indiana University:
- Uncertified packing systems;
- Certified packaging that is not used according to the instructions provided by the vendor;
- Packaging systems that utilize certified packing materials from different vendors;
- Packaging that displays the UN number and Proper Shipping Name is not necessarily certified. Verify certification with the vendor.

Certified shipping containers are not required to ship Category B Infectious Materials. Category B packages must be tested as a system. Substitutions in packing materials are allowed. For example, a secondary pressure vessel from one manufacturer can be used in another manufacturer’s outer package. However, when substituting packing materials the shipper assumes the responsibility of providing proof that the system was tested according to regulations.

Certified packaging containers for Category A and containers used to ship Category B and supplies are available through online vendors, or from the Biosafety Office. Individual labels are provided by the Biosafety Office at no cost, however packaging systems are provided at the same rate as the manufacturer cost. An account number must be provided to the Biosafety Office prior to receiving shipping packaging systems.

An Overpak is a large outer package (box or crate) or packing (wrapped in shrink wrap) containing multiple individual packages. Each individual package within the overpak must be fully compliant for packing, labels and marks, and documentation.

Division 6.2 Category B materials may be transported in a private motor vehicle provided these three conditions are met:
- Material is a direct human or animal specimen, human or animal material. Cultures are not permitted in personal vehicles. University owned vehicles may be used.
- The vehicle cannot be used for any other purpose during transport including transport of passengers or food.
- The material must not meet the definition for a Category A infectious substance.
Transportation
Category A Dangerous Goods may not be transported in personal vehicles. Category B Dangerous Goods may be transported in private or University owned vehicles provided the following three conditions are met:

- Material is a direct human or animal specimen, human or animal material. Cultures are not permitted in personal vehicles. University owned vehicles may be used.
- The vehicle cannot be used for any other purpose during transport including transport of passengers or food.
- The material must not meet the definition for a Category A infectious substance.

Note: The transport of infectious substances on private property is not regulated.

Biological products that meet the definition for Category A or B must be classified, packaged and transported according to the appropriate regulations for that classification. Persons who have not been trained and certified to package and ship Dangerous Goods are not authorized to do so. Contact the Biosafety Office for training and assistance packaging, marking, labeling, and shipping Dangerous Goods.
## APPENDIX G Acronyms and Abbreviations

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<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>ABSL-1</td>
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<td>Animal and Plant Health Inspection Service</td>
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<td>ATCC</td>
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<td>Bloodborne Pathogens</td>
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<td>BI</td>
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<td>BIACUC</td>
<td>Bloomington Institutional Animal Care and Use Committee</td>
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<tr>
<td>BMBL</td>
<td>Biosafety in Microbiological and Biomedical Laboratories</td>
</tr>
<tr>
<td>BRS</td>
<td>Biotechnology Regulatory Services</td>
</tr>
<tr>
<td>BSC</td>
<td>Biological Safety Cabinet/Biosafety cabinet</td>
</tr>
<tr>
<td>BSL-1</td>
<td>Biosafety Level 1</td>
</tr>
<tr>
<td>BSL-2</td>
<td>BIOSAFETY Level 2</td>
</tr>
<tr>
<td>BSL-3</td>
<td>Biosafety Level 3</td>
</tr>
<tr>
<td>BSL-4</td>
<td>Biosafety Level 4</td>
</tr>
<tr>
<td>BSL1-P</td>
<td>Plant Biosafety Level 1</td>
</tr>
<tr>
<td>BSL2-P</td>
<td>Plant Biosafety Level 2</td>
</tr>
<tr>
<td>BSL3-P</td>
<td>Plant Biosafety Level 3</td>
</tr>
<tr>
<td>BSL4-P</td>
<td>Plant Biosafety Level 4</td>
</tr>
<tr>
<td>BSO</td>
<td>Biosafety Office/Biosafety Officer</td>
</tr>
<tr>
<td>CDC</td>
<td>Center for Disease Control and Prevention</td>
</tr>
<tr>
<td>CFR</td>
<td>Code of Federal Regulations</td>
</tr>
<tr>
<td>DOC</td>
<td>Department of Commerce</td>
</tr>
<tr>
<td>DOT</td>
<td>Department of Transportation</td>
</tr>
<tr>
<td>ECP</td>
<td>Exposure Control Plan</td>
</tr>
<tr>
<td>EH&amp;S</td>
<td>Environmental Health and Safety</td>
</tr>
<tr>
<td>EO</td>
<td>Ethylene Oxide</td>
</tr>
<tr>
<td>DGR</td>
<td>Dangerous Goods Regulations</td>
</tr>
<tr>
<td>GMO</td>
<td>Genetically Modified Organism, Genetically Modified Microorganism</td>
</tr>
<tr>
<td>HEPA</td>
<td>High-Efficiency Particulate Air Filtration</td>
</tr>
<tr>
<td>HMR</td>
<td>Hazardous Materials Regulations</td>
</tr>
<tr>
<td>HHS</td>
<td>Health and Human Services</td>
</tr>
<tr>
<td>IACUC</td>
<td>Institutional Animal Care and Use Committee</td>
</tr>
<tr>
<td>IATA</td>
<td>International Air Transport Association</td>
</tr>
<tr>
<td>IBC</td>
<td>Institutional Biosafety Committee</td>
</tr>
<tr>
<td>IBM</td>
<td>Institutional Biosafety Manual</td>
</tr>
<tr>
<td>ID$_{50}$</td>
<td>Infectious Dose</td>
</tr>
<tr>
<td>IPM</td>
<td>Integrated Pest Management</td>
</tr>
<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
</tr>
<tr>
<td>IUB</td>
<td>Indiana University Bloomington</td>
</tr>
<tr>
<td>IUPD</td>
<td>Indiana University Police Department</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
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<tr>
<td>IUPUI</td>
<td>Indiana University Purdue University Indianapolis</td>
</tr>
<tr>
<td>LAI</td>
<td>Lab Acquired Infection</td>
</tr>
<tr>
<td>LAR</td>
<td>Lab Animal Resources</td>
</tr>
<tr>
<td>LD50</td>
<td>Lethal Dose</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
</tr>
<tr>
<td>n.o.s</td>
<td>Not Otherwise Specified</td>
</tr>
<tr>
<td>NSAR</td>
<td>National Select Agent Registry</td>
</tr>
<tr>
<td>OBA</td>
<td>Office of Biotechnology Activities</td>
</tr>
<tr>
<td>OSHA</td>
<td>Occupational Health and Safety Administration</td>
</tr>
<tr>
<td>PHS</td>
<td>Public Health Service</td>
</tr>
<tr>
<td>PI</td>
<td>Principal Investigator</td>
</tr>
<tr>
<td>PPE</td>
<td>Personal Protective Equipment</td>
</tr>
<tr>
<td>PPQ</td>
<td>Plant Protection Quarantine</td>
</tr>
<tr>
<td>PSDS</td>
<td>Pathogen Safety Date Sheet</td>
</tr>
<tr>
<td>PSI</td>
<td>Pounds per square inch</td>
</tr>
<tr>
<td>PSN</td>
<td>Proper Shipping Name</td>
</tr>
<tr>
<td>QAC</td>
<td>Quaternary Ammonium Compound</td>
</tr>
<tr>
<td>RG1</td>
<td>Risk Group 1</td>
</tr>
<tr>
<td>RG2</td>
<td>Risk Group 2</td>
</tr>
<tr>
<td>RG3</td>
<td>Risk Group 3</td>
</tr>
<tr>
<td>RG4</td>
<td>Risk Group 4</td>
</tr>
<tr>
<td>SCBA</td>
<td>Self-Contained Breathing Apparatus</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
<tr>
<td>USDA</td>
<td>United States Department of Agriculture</td>
</tr>
<tr>
<td>VS</td>
<td>Veterinary Services</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</table>