3.1 Emergency Procedures

3.1.1. Biological Spills

Spill kit materials and written procedures shall be kept in each laboratory where work with microorganisms is conducted. Basic equipment includes concentrated disinfectant (such as chlorine bleach), absorbent material, latex or nitrile gloves, autoclave bags, sharps container, and forceps or other mechanical device to pick up broken glass. Do NOT handle broken glass with hands.

3.1.2. General Biological Spill Clean-Up Guidelines

- Wear gloves, protective eyewear and a lab coat.
- Use forceps or other mechanical means to pick up broken glass and discard into sharps container.
- Cover spilled material with paper towels.
- Add appropriate disinfectant in sufficient quantity to ensure effective microbial inactivation, let sit 15 minutes.
- Dispose of towels in waste container.
- Wipe spill area with diluted disinfectant. Discard of clean-up materials in waste container.
- Wash hands with soap and water when finished.
- Report all spills to IUEHS Biosafety for your respective campus.
- Post signage from Appendix D when the spill occurs outside the biosafety cabinet.

3.1.3. Specific Biological Spill Clean-Up Guidelines

3.1.3.1. Spill of BSL-1 material

- Wearing gloves and a lab coat, pick up broken glass with forceps and place in sharps container.
- Absorb the spill with paper towels or other absorbent material.
- Add appropriate disinfectant in sufficient quantity to ensure decontamination, let sit for 15 minutes.
- Discard these materials into waste container.
- Wipe the spill area with the appropriate dilution of a disinfectant effective against the organism. Discard of clean-up materials in waste container.
- Autoclave all gloves and other materials worn to clean up the spill.
- Wash hands with soap and water.
- Report all spills to IUEHS Biosafety for your respective campus.

3.1.3.2. Spill of Human Blood

- Wear gloves, face protection and lab coat to clean up spill.
- If broken glass is present, use forceps to pick up and place in sharps container.
- Absorb blood with paper towels and add appropriate disinfectant in sufficient quantity to ensure decontamination, let sit for 15 minutes.
- Clean the spill site of all visible blood.
- Discard all materials into trash container.
- Autoclave all gloves and other materials worn to clean up the spill.
- Wash hands with soap and water.
- Report all spills to IUEHS Biosafety for your respective campus.
- If an injury has occurred, complete an Occupational Injury/Illness Report and seek medical evaluation.

3.1.3.3. Spill of BSL-2 Material

- Keep other workers out of the area to prevent spreading of spill material.
- Post warning sign (Appendix D), if needed.
- Remove contaminated clothing and put in a biohazard bag for decontamination later.
- Wash hands and any exposed skin and inform the PI of the spill. Contact IUEHS Biosafety for your respective campus for assistance, if needed.
- Wear gloves, face protection and lab coat to clean up spill.
- If broken glass is present, use forceps to pick up and place in sharps container.
- Absorb the spill with paper towels and add appropriate disinfectant in sufficient quantity to ensure decontamination, let sit for 15 minutes.
- Discard all materials into waste container.
- Wipe the spill area with the appropriate dilution of a disinfectant effective against the organism. Discard of clean-up materials in waste container.
- Autoclave all gloves and other materials worn to clean up the spill.
- Wash hands with soap and water.
- Report all spills to IUEHS Biosafety for your respective campus.
- If an injury has occurred, complete an Occupational Injury/Illness Report and seek medical evaluation.

3.1.3.4. Spill of Recombinant or Synthetic DNA Material

- Keep other workers out of the area to prevent spreading of spill material.
- Post warning sign (Appendix D), if needed.
- Remove contaminated clothing and put in a biohazard bag for decontamination later.
- Wash hands and any exposed skin and inform the PI of the spill. Contact IUEHS Biosafety for your respective campus for assistance, if needed.
- Wear gloves, face protection and lab coat to clean up spill.
- If broken glass is present, use forceps to pick up and place in sharps container.

- Absorb the spill with paper towels and add diluted disinfectant in sufficient quantity to ensure decontamination, let sit for 15 minutes.
- Discard all materials into waste container.
- Wipe the spill area with the appropriate dilution of a disinfectant effective against the organism. Discard of clean-up materials in waste container.
- Autoclave all gloves and other materials worn to clean up the spill.
- Wash hands with soap and water.
- Report all recombinant or synthetic DNA spills to the IUEHS Biosafety for your respective campus immediately.
- If an injury has occurred, complete an Occupational Injury/Illness Report and seek medical evaluation.

3.1.3.5. Spill of BSL-3 Material

- Stop work immediately.
- Avoid inhaling airborne material while quickly leaving the room. Notify others to leave. Close door, and post with warning sign (Appendix D).
- Remove contaminated clothing, turn exposed area inward, and place in a biohazard bag. Wash hands with soap and water.
- Notify the PI and IUEHS Biosafety immediately. Do not reenter the lab until given permission from IUEHS Biosafety. After hours and weekends call 911.
- Following instruction from the Biological Safety Officer, allow 30 minutes for aerosols to disperse before re-entering the laboratory to begin clean-up.
- If given authority to clean the spill, put on personal protective equipment (HEPA filtered respirator, gown, gloves, and shoe covers) and assemble clean-up materials (disinfectant, autoclavable container or bag, forceps, sharps container, and paper towels).
- Contain the spill with absorbent paper towels or disposable pads. Carefully add appropriate disinfectant to the spill; avoid creating aerosols when pouring the disinfectant. Leave the room and allow 30 minutes for the disinfectant to inactivate the material.
- Pick up broken glass with forceps and discard in sharps container.
- Clean up liquid with paper towels and collect all contaminated materials into biohazard bag or container. Remove all spilled materials and decontaminate the area again with an appropriate disinfectant.
- Autoclave lab coat, gloves, and other protective equipment that was worn for clean-up.
- Wash hands thoroughly with soap and water.
- If a potential exposure has occurred, notify your immediate supervisor, complete an Occupational Injury/Illness Report and seek medical evaluation.

3.1.3.6. Spill in a Biological Safety Cabinet

- Leave the cabinet fan running.
- Wearing gloves and lab coat, spray or wipe cabinet walls, work surfaces, and equipment with disinfectant such as 70% ethanol. If necessary, flood work surface, as well as drain pans and catch basins below the work surface, with disinfectant. Allow at least 20 minutes contact time.
- Soak up the disinfectant and spill with paper towels, and drain catch basin into a container. Lift front exhaust grille and tray, and wipe all surfaces. Ensure that no paper towels or solid debris are blown into area below the grille.
- Surface disinfect all items that may have been spattered before removing them from the cabinet.
- Discard all clean-up materials into biohazard waste container. Wash hands and exposed skin areas with soap and water.
- IUEHS Biosafety for your respective campus should be notified if the spill overflows into the interior of the cabinet. It may be necessary to do a more extensive decontamination of the cabinet.

3.1.3.7. Spill of Radioactive Biological Material

A spill involving both radioactive and biological materials requires emergency procedures that are different from the procedures used for either material alone. As a general rule, disinfect the microorganism using a chemical disinfectant, then dispose of all clean-up materials in a separate bag/container labeled to indicate that the radioisotope is mixed with a chemically disinfected microorganism. **Do not use bleach solutions as a disinfectant on materials that contain iodinated compounds because radioactive iodine gas may be released.** Be sure to use procedures to protect yourself from the radionuclide while disinfecting the biological material. Before any clean-up, consider the type of radionuclide, the characteristics of the microorganism, and the volume of the spill. Contact your respective campus Radiation Safety Office for specific radioisotope clean-up procedures.

3.1.3.7.1. Preparation for Clean-up

- Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave.
- Close door and post with warning sign (Appendix D).
- Remove contaminated clothing, turn exposed area inward, and place in a biohazard bag.
- Wash all exposed skin with soap or hand washing antiseptic, followed by a three minute water rinse.
- Inform the PI, EHS Biosafety, and Radiation Safety for your respective campus of the spill and monitor all exposed personnel for radiation.
- Allow aerosols to disperse for at least 30 minutes before reentering the laboratory. Assemble clean-up materials (diluted disinfectant, autoclavable containers, forceps, paper towels, sharps container).

- Confirm with the Radiation Safety Officer that it is safe to enter the lab.

3.1.3.7.2. *Clean-up of Radioactive Biological Spill*

- Put on protective clothing (lab coat, face protection, gloves, and shoe covers). Depending on the nature of the spill, it may be advisable to wear a HEPA filtered respirator instead of a surgical mask. In setting up your spill plan, contact IUEHS Biosafety for your respective campus for advice since the use of many types of respirators requires prior training, fit-testing, and medical approval.
- Pick up any sharp objects with forceps and put in sharps container labeled according to Radiation Safety guidelines.
- Cover the area with paper towels, and carefully pour appropriate disinfectant around and into the spill. Avoid enlarging the contaminated area. Use additional disinfectant as it becomes diluted by the spill. Allow at least 20 minutes contact time. Do not use bleach solutions on iodinated materials; radioactive iodine gas may be released. Instead, use an alternative disinfectant such as an iodophor.
- Wipe surrounding areas where the spill may have splashed with disinfectant.
- Absorb the disinfectant and spill materials with additional paper towels, and place into an approved radioactive waste container. Keep separate from other radioactive waste. Do not autoclave radioactive isotope-contaminated biological waste unless approved by the Radiation Safety Officer.
- Disinfect contaminated protective clothing prior to disposal as radioactive waste.
- Place contaminated item(s) on absorbent paper and scan for radioactivity. If none is detected, dispose of these items as biohazard waste.
- If radioactive, spray with disinfectant and allow a 20 minute contact time. Wrap the item(s) inside the absorbent paper and dispose of as radioactive waste.
- Wash hands and exposed skin areas with soap and water, and monitor personnel and spill area for residual radioactive contamination. If skin contamination is detected, repeat decontamination procedures under the direction of the Radiation Safety Officer. If spill area has residual activity, determine if it is fixed or removable and handle it accordingly.

3.2. Injury Involving Biological Materials

Any individual who receives an exposure or potential exposure will be offered a medical consultation and advised of available treatments by the Designated Medical Service Provider for your respective campus.

Exposure or potential exposure involving biological materials can occur from any of the following:

- Contact with non-intact skin such as cuts, rashes, or abrasions;
- Contact with mucosal membranes-eyes, nose, and mouth; and
- Sharps puncturing or cutting the skin, and
- Inhalation of biological aerosols.

Should an exposure occur:

- If immediate threat to life call 911; otherwise
- Wash the exposed area for 15 minutes;
- Report the incident to your work supervisor immediately;
- Notify IUEHS Biosafety for your respective campus of the exposure;
- Follow campus specific procedures to fill out an Occupational Injury/Illness Report to initiate medical consultation and treatment by the Designated Medical Service Provider for your respective campus.

Lab specific procedures may differ slightly and in such cases must be followed, while ensuring that the minimum above requirements are also met.

3.3. Introduction to Biohazardous Materials and Research

Laboratory research involving biological agents are subject to various federal and state regulations depending on the nature of the agents used and the experimental manipulations in which they will be employed. The following section of this Manual is intended to serve as a guide to the various federal and state agencies that govern biological research and their laws, regulations, and guidelines.

Principal Investigators are responsible for understanding the scope of their research program, identifying the regulations to which their work is subject, and complying with those regulations. IUEHS Biosafety for the respective campus is available to assist the Principal Investigator should guidance be needed in identifying and complying with those laws, regulations, and guidelines.

Principal Investigators should also note that many granting agencies require that grant recipients certify compliance with all relevant laws, regulations, and guidelines to which their research is subject. The scope of these regulations includes procedures and facilities involved in protecting laboratory workers, the public, and the environment from laboratory biological hazards.

3.3.1. Microorganisms

The National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) publish guidelines for work with infectious microorganisms. The publication, entitled Biosafety in Microbiological and Biomedical Laboratories (BMBL) recommends that work be done using one of four levels of containment: Biosafety Level 1 (BSL-1), BSL-2, BSL-3 and BSL-4 (see section 3.4). The *NIH Guidelines* (Appendix E1-3) classifies pathogenic agents into one of four risk groups according to specific criteria. It is required by Indiana University that all laboratories adhere to these NIH/CDC guidelines. Noteworthy, there are no BSL-4 laboratories on any of the IU campuses.

3.3.2. Microorganisms Capable of Causing Infection in Healthy Humans

Investigators must register any project involving a pathogenic agent with the IBC and receive its approval before work is begun. Following receipt of the completed IBC Protocol Submission Form, the laboratory will be inspected by IUEHS Biosafety to ensure that it meets the containment requirements listed in *BMBL* for the agent being studied. If the lab meets the requirements, the work will be reviewed and approved or disapproved by the IBC.

3.3.3. Genetically Engineered Organisms and/or Microorganisms

Work with all genetically engineered organisms must comply with the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*. These guidelines classify recombinant or synthetic nucleic acid molecules experiments into four levels of containment (BSL-1, BSL-2, BSL-3, and BSL-4) based on the hazard of the microorganism and the procedures and quantities being used. Additionally, the United States Department of Agriculture (USDA) requires permits for field testing of genetically engineered plants. It is required by Indiana University that all laboratories follow and ensure compliance with these guidelines.

3.3.4. Registration Document

Each PI is responsible for submitting protocols for all experiments involving biohazardous materials at BSL-2 or higher, biological toxins, and recombinant or synthetic nucleic acid molecules, including those exempt from *NIH Guidelines*. IUEHS Biosafety for your respective campus inspects all laboratories where BSL-2 or BSL-3 biocontainment is required, and all BSL-1 laboratories which are which require an IBC protocal prior to protocol approval.

3.3.5. Review and Approval of Experiments

The IBC, which oversees recombinant and synthetic nucleic acid molecule research at Indiana University, or the IUEHS Biosafety for your respective campus will review and approve the submitted protocol or amendment based on the submission status according to the NIH Guidelines, which are generally summarized below. More specific information about the categories and corresponding approval can be found with the Office of Research Compliance.

3.3.5.1. Experiments covered by the NIH Guidelines

Many experiments involving recombinant or synthetic nucleic acid molecules require registration and approval by the IBC before work may be initiated.

Experiments that require IBC approval before initiation include those that involve:

- Risk Group 2, 3, 4, or Restricted Agents as host-vector systems, cloning DNA from Risk Group 2, 3, 4, or Restricted Agents into nonpathogenic prokaryotic or lower eukaryotic host-vector systems, infectious virus, or defective virus in the presence of helper virus in tissue culture;
- Whole plants or animals; and
- More than 10 liters of culture.

Experiments that must be registered at the time of initiation include those that involve:

- The formation of recombinant or synthetic nucleic acid molecules containing no more than 2/3 of the genome of any eukaryotic virus propagated in tissue culture, recombinant or synthetic nucleic acid molecules-modified whole plants, and/or recombinant or synthetic nucleic acid molecules-modified organisms associated with whole plants, except those that fall under Section III-A, III-B, III-C, or III-D of the NIH Guidelines; and
- The generation of transgenic rodents that require BSL-1 containment.

3.3.5.2. Experiments exempt from the NIH Guidelines

Experiments exempt from the *NIH Guidelines*, although requiring registration with the IBC, may be initiated immediately. IUEHS Biosafety will review the registration and confirm that the work is classified correctly according to the *NIH Guidelines*. Exempt experiments are those that:

- Use synthetic nucleic acids that can neither replicate nor generate nucleic acids capable of replicating in any living cell; are not designed to integrate into DNA, and do not produce a toxin that is lethal for vertebrates at an LD50 of <100 ng/kg body weight;
- Use recombinant or synthetic DNA molecules that are not in organisms or viruses;
- Consist entirely of DNA segments from a single nonchromosomal or viral

DNA source, though one or more of the segments may be a synthetic equivalent;

- Consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well-established physiological means;
- Consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species);
- Consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent;
- Do not present a significant risk to health or the environment as determined by the NIH Director, with the advice of the Recombinant DNA Advisory Committee (RAC), and following appropriate notice and opportunity for public comment;
- Contain less than one-half of any eukaryotic viral genome propagated in cell culture;
- Use E. coli K12, Saccharomyces cerevisiae, or Bacillus subtilis host-vector systems, unless genes from Risk Group 3 or 4 pathogens are cloned into these hosts;
- Involve the purchase or transfer of transgenic rodents for experiments that require BSL-1 containment; and
- Work with biohazardous materials at BL2 that does not utilize recombinant or synthetic nucleic acid molecules.

3.3.6. Human Blood, Unfixed Tissue, and Cell Culture

Please refer to the Indiana University *Bloodborne Pathogens Exposure Control Plan* for detailed information on handling human material.

Work with human material is regulated by the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard, 29 CFR 910.1030. Human blood, unfixed tissue, cell culture, and certain other body fluids are considered potentially infectious for bloodborne pathogens such as epatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). All human clinical material shall be presumed infectious and handled using BSL-2 work practices. This concept is called Universal Precautions. Principal Investigators are responsible for registering their use of human materials so training and immunization can be provided as required by OSHA.

3.3.7. Select Agents

Select Agents are microorganisms and toxins that have potential for criminal misuse to cause harm. The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 restricts their possession and use, and requires the University to collect and maintain information on the location and use on campus of any select agents or toxins. Please contact IUEHS Biosafety for your respective campus immediately if you currently possess or plan to acquire any of the agents listed in Appendix A and have not yet reported that fact. Failure to provide notice may result in civil and criminal liability for individual researchers and/or the University. If you have questions, you may contact IUEHS Biosafety for your respective campus, or visit the federal Select Agent website www.selectagents.gov which provides links to select agent program information.

3.3.8. Non-Human Primate (NHP) Unfixed Tissue and Primary Cell Culture

Non-human primates and their tissues pose special zoonotic risks as many of their diseases are often transmissible to humans and can be a serious health hazard. Although there are a number of NHP viruses that can cause disease in humans, monkeys of the genus Macaca, or their unfixed tissues, can carry the virus Cercopithecine herpesvirus 1 (other terms used: Herpes B-virus, Herpesvirus simiae, or simply B-virus). B-virus is frequently carried by Rhesus and Cynomologus macaques, as well as other macaques. It can cause fatal encephalitis in humans.

Prior to working with any NHP primary cell cultures or unfixed tissues, PIs must register their work, and lab personnel must be trained in the safety procedures required for handling and post-exposure procedures. Sharps use with these materials is to be eliminated or restricted.

3.4. Biosafety Containment Levels

Four levels of biosafety are defined in the publication *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, published by the CDC and NIH. The levels, designated in ascending order by degree of protection provided to personnel, the environment, and the community, are combinations of laboratory practices, safety equipment, and laboratory facilities (see Appendices E1-3). Most microbiological work at Indiana University is conducted at BSL-1 or BSL-2 containment. The Indiana University Biosafety Manual supersedes the information in the BMBL Appendices E1-3 and must be followed should information differ.

Below is a summary of each biosafety level. Detailed criteria for each level are described in Appendix E1-3.

3.4.1. Biosafety Level 1

Suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science. Personal protective equipment shall be used as appropriate, including lab coats and gloves. Eye protection shall be used when splashing is likely.

Secondary barriers such as hand washing sinks and waste decontamination facilities must be available to reduce potential environmental contamination.

3.4.2. Biosafety Level 2

Practices, equipment, and facility design and construction are applicable to research, clinical, diagnostic, and teaching laboratories in which work is done with moderate- risk agents that are present in the community. Hepatitis B virus, HIV, salmonellae typhi, and *Toxoplasma gondii*. are representative of microorganisms assigned to this containment level. BSL-2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines (Laboratory personnel working with human-derived materials shall 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. Extreme caution shall be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment such as a biological safety cabinet (BSC) or safety centrifuge cups. Personal protective equipment shall be used as appropriate, including lab coats and gloves. Eye protection shall be used when splashing is likely.

Secondary barriers such as hand washing sinks and waste decontamination facilities must be available to reduce potential environmental contamination.

3.4.3. Biosafety Level 2+ BSL3 Practices

Used to describe biocontainment within a Biosafety Level 2 laboratory but using specific Biosafety Level 3 practices. This is not intended to be used as a substitute for Biosafety Level 3 with any Risk Group 3 biohazards. The final determination of this biocontainment is based on a risk assessment of the research planned. The risk assessment and review by the IBC may determine that safety practices above BSL-2 are required, but the research does not warrant the more complex BSL-3 laboratory suite.

No inclusive list of BSL-2+ viral vectors, microorganisms, biohazards, or experimental designs exists and decisions on IU research biocontainment is based on case by case risk assessment. The main focus of BSL-2 + BSL-3 Practices is a reduction in exposure to aerosols and/or particularly hazardous agents that do not quite meet the definition of Risk Group 3 biohazards. Some examples of experiments that may fall under the above definition would be:

- Viral vectors that have inserts of oncogenes or other gene products that may be toxic, particularly if injections are involved;
- Specific multi-drug resistant BSL-2 bacteria;
- High concentrations of Risk Group 2 viruses represented as inhalation hazards; and
- Large volumes of viral vectors, i.e., greater than 10 liters.

3.4.4. Biosafety Level 3

Practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii* are representative of the microorganisms assigned to this level. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols.

At BSL-3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all laboratory manipulations are performed in a BSC or other enclosed equipment, such as a gas-tight aerosol generation chamber. Secondary barriers for this level include controlled access to the laboratory and ventilation requirements that minimize the release of infectious aerosols from the laboratory.

3.4.5. Biosafety Level 4

Practices, safety equipment, and facility design and construction are applicable for work with dangerous and exotic agents that pose a high individual risk of life- threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. There are no BSL-4 level laboratories at Indiana University.

Summary of Recommended Biosafety Levels

BSL	AGENTS	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to consistently cause diseases in healthy adults	Standard Microbiological Practices	• PPE: Laboratory coats; latex or nitrile disposable gloves; eye/face protection as needed	Laboratory bench and sink required. Autoclave available
2	• Agents associated with human disease • Routes of transmission include percutaneous injury, ingestion, and mucous membrane exposure	BSL-1 practices plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety Manual defining any needed waste decontamination or medical evaluation program	BSL-1 Primary barriers plus: • Class I or II BSCs or other physical containment devices or appropriate PPE used for all manipulations of agents that cause splashes or aerosols of infectious materials	BSL-1 plus: • Recommended negative differential pressure • Readily available eyewash
3	• Indigenous or exotic agents with potential for aerosol transmission • Disease may have serious or lethal consequences	BSL-2 practices plus: • Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering • Baseline serum	Primary barriers: • Class I or II BSCs or other physical containment devices used for all open manipulation of agents • PPE: • Protective laboratory clothing; latex or nitrile disposable gloves; respiratory protection as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhaust air not recirculated • Negative airflow into laboratory
4	• Dangerous/exotic agents which pose high risk of life-threatening disease • Aerosol transmitted laboratory infections have occurred; or related agents with unknown risk of transmission	BSL-3 practices plus: • Clothing change before entering • Shower on exit • All material decontaminated on exit from facility	Primary barriers: • All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum • Decontamination systems • Other requirements outlined in the BMBL

3.5. Animal Facilities

Four standard biosafety levels are also described for activities involving infectious disease work with commonly used experimental animals. These four combinations of practices, safety equipment, and facilities are designated Animal Biosafety Levels 1, 2, 3, and 4, and provide increasing levels of protection to personnel and the environment.

One additional biosafety level, designated BSL-3-Agriculture (or BSL-3-Ag) addresses activities involving large or loose-housed animals and/or studies involving agents designated as High Consequence Pathogens by the USDA. BSL-3-Ag laboratories are designed so that the laboratory facility itself acts as a primary barrier to prevent release of infectious agents into the environment. More information on the design and operation of BSL-3-Ag facilities and USDA High Consequence Pathogens can be found in *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*.

A full description of requirements for animal facilities can be found in *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*

3.5.1. Animal Biosafety Level 1 (ABSL-1)

Assigned for animal work that does not involve biological agents or involves well-characterized agents that are not known to cause disease in immunocompetent humans, and that are of minimal potential hazard to laboratory personnel and the environment.

3.5.1.1. ABSL-1 Facility Requirements

In addition to the facility requirements listed for BSL-1 laboratories, ABSL-1 laboratories must meet the following requirements:

- Animal facilities must be separated from areas that are open to unrestricted personnel traffic.
- External facility doors must be self-closing and self-locking.
- Doors to animal rooms must open inward, be self-closing, and kept closed when experimental animals are present.
- The animal care facility must be designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) must be water-resistant.
- Windows are not recommended. Any windows must be resistant to breakage. Where possible, windows should be sealed.
- If floor drains are provided, the traps should always be filled with an appropriate disinfectant.
- Ventilation should be provided in accordance with the *Guide for Care and Use of Laboratory Animals, latest edition*. No recirculation of exhaust air may occur. It is recommended that animal rooms have inward directional airflow.
- The facility must have a hand washing sink.
- Cages are washed manually or in a cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F.
- Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
- Emergency eyewash and shower must be readily available.

3.5.2. Animal Biosafety Level 2 (ABSL-2)

Assigned for animal work with those agents associated with human disease that pose moderate hazards to personnel and the environment. ABSL-2 builds on the practices, procedures, containment equipment, and facility requirements of ABSL-1.

3.5.2.1. ABSL-2 Facility Requirements

In addition to the facility requirements listed for BSL-2 and ABSL-1 laboratories, ABSL-2 laboratories must meet the following requirements:

- Access to the facility is limited by secure locked doors.
- Ventilation should be provided in accordance with criteria from *Guide for Care and Use of Laboratory Animals, latest edition*. The direction of airflow in the animal care facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms.
- An autoclave should be available in the animal care facility to decontaminate infectious waste.
- A hand washing sink must be in the animal room where infected animals are housed or manipulated, as well as elsewhere in the facility.

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.

3.5.3. Animal Biosafety Level 3 (ABSL-3)

Assigned to animal work involving indigenous or exotic agents that present the potential of aerosol transmission and of causing serious or potentially lethal disease. ABSL-3 builds on the practices, procedures, containment equipment, and facility requirements of ABSL-2.

3.6. Clinical Laboratories

Clinical laboratories, especially those in health care facilities, receive clinical specimens with requests for a variety of diagnostic and clinical support services. Typically, the infectious nature of clinical material is unknown, and specimens are often submitted with a broad request for microbiological examination for multiple agents (e.g., sputa submitted for "routine," acid-fast, and fungal cultures). *It is the responsibility of the Laboratory Director to establish standard procedures in the laboratory that realistically address the issue of the infective hazard of clinical specimens*.

Except in extraordinary circumstances (e.g., suspected hemorrhagic fever), the initial processing of clinical specimens and serological identification of isolates can be done safely at BSL-2, the recommended level for work with bloodborne pathogens such as HBV and HIV. The containment elements described in BSL-2 are consistent with the OSHA standard*, "Occupational Exposure to Bloodborne Pathogens."* This requires the use of specific precautions with **all** clinical specimens of blood or other potentially infectious material (Universal or Standard Precautions). Additionally, other recommendations specific for clinical laboratories may be obtained from the Clinical Laboratory Standards Institute.

3.7. Laboratory Attire and Personal Protective Equipment

- Shoes must cover the entire foot. Open toed shoes and sandals are inappropriate footwear in laboratories. Fabric and athletic shoes offer little or no protection from biological spills. Leather shoes or equivalent (chemically resistant shoes) with slip resistant soles are required. Street clothing is to be chosen so as to minimize exposed skin below the neck. Long pants are required. Avoid rolled up sleeves. Shorts (including cargo shorts), capris, miniskirts, tank tops, sleeveless shirts and midriff-length shirts are inappropriate clothing in laboratories.
- Laboratory coats are designed to be fluid resistant and help protect the user from accidental splashes and spills of biological material. Laboratory coats are required whenever working with biological material.
- Gloves must be worn whenever handling or working with biological material. Latex and nitrile gloves are typically appropriate for work in biological laboratories. It is important to keep in mind what chemicals will be used alongside biologicals and chose gloves appropriate for the task that will be performed. Gloves must be chosen that are the appropriate size to help minimize the risk for incident.
- Safety glasses or goggles are required whenever there is a risk of splashing.
- Additional PPE, including a face shield, a face mask, or an N95 respirator may be required depending on the agent in use and the planned manipulations. These requirements will be outlined in any approved IBC protocols.

3.8. Use of Biohazard Labels

Biohazard labels are labels incorporating the universal biohazard symbol. They are stating that the item is either contaminated with biohazardous material or contains biohazardous material. Biohazard labels must be red or red-orange with biohazard symbol and the word "biohazard" in a contrasting color. Biohazard labels are required to be used in the following situations:

- On the outside door of where biological material is stored or manipulated;
- Bags/containers of biological waste;
- Bags/containers of contaminated laundry;
- Refrigerators and freezers used to store biological material;
    - Refrigerators and freezers in common storage rooms shall have individual emergency contact information on each.
- Bag/containers used to store, dispose of, transport, or ship biological material; and
- Contaminated equipment to be serviced or shipped.

3.9. Biological Safety in Teaching Laboratories

Teaching laboratories are frequently used for laboratory classes, demonstrations and lectures. Because hazards are present in these areas, the following rules and guidelines are provided to ensure that students and instructors are safe and compliant. These guidelines are intended to assist in outlining the requirements for use of personal protective equipment (PPE), the use of appropriate street clothing, and prohibition of food and drinks in laboratories. The classroom instructor is responsible for ensuring that participants adhere to these rules and guidelines.

3.9.1. Food and Drinks

- No food or drinks are permitted in teaching laboratories at any time.

3.9.2. Personal Protective Equipment (PPE)

3.9.2.1. When hazards are present or used in the laboratory:

- The instructor and students must utilize the appropriate PPE for the experiments. Manipulated or observed
- PPE is always used when biological materials are presented and nearby on bench tops.
- Safety eyewear (goggles, safety glasses) must be used to prevent injury or exposure of the eyes.
- Protective clothing (lab coats) must be worn to prevent contamination of the body and street clothes. Protective clothing must be left in the lab or locker at the end of the period and must be decontaminated prior to removal from the lab.
- Appropriate chemically resistant gloves must be used for handling chemicals to prevent contamination of the hands.

3.9.2.2. When biological demonstrations are being performed for observational purposes:

- The instructor and audience must be equipped with the appropriate PPE to protect them from the hazards associated with the demonstration.
- Instructors must not deviate from the established procedures or adjust quantities of materials during the demonstration without prior approval.

3.9.2.3. When hazards are not present in the laboratory (i.e. when all chemicals, biological, or radiological materials are secured in closed containers (chemical cabinets, biological freezers or incubators) and bench surfaces are clean and/or decontaminated:

- PPE is unnecessary and does not need to be utilized.

3.9.3. Street Clothes

Street clothing and footwear appropriate for laboratory work must be worn by the instructor and students for all activities (including lectures, lab sessions, and demonstrations) because some lectures are followed by lab sessions in the same course.

Street clothing should be chosen so as to minimize exposed skin below the neck. Long pants are required. Avoid rolled up sleeves. Shorts (including cargo shorts), capris, and miniskirts are inappropriate clothing in laboratories.

Shoes must cover the entire foot. Open-toed shoes and sandals are inappropriate footwear in laboratories. Fabric and athletic shoes offer little or no protection from biological spills. Leather shoes with slip-resistant soles are recommended.

When PPE is utilized for laboratory sessions and demonstrations (not lectures) long hair must be restrained and jewelry/watches removed.

3.10. Decontamination

Sterilization, disinfection, and antisepsis are all forms of decontamination. **Sterilization** implies the killing of all living organisms. **Disinfection** refers to the use of antimicrobial agents on inanimate objects; its purpose is to destroy all non-spore forming organisms. **Antisepsis** is the application of a liquid antimicrobial chemical to living tissue.

3.10.1. Chemical Disinfectants

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. Chemical disinfectants are registered by the EPA under the following categories:

- Sterilizer or Sterilant - will destroy all microorganisms including bacterial and fungal spores on inanimate surfaces.
- Disinfectant - will destroy or irreversibly inactivate specific viruses, bacteria, and pathogenic fungi, but not bacterial 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 - agent formulated to be used on skin or tissue - not a disinfectant.

3.10.2. Disinfectants Commonly Used in the Laboratory

3.10.2.1. Iodophors

- Recommended dilution is 75 ppm, or approximately 4.5 ml/liter water.
- Effective against vegetative bacteria, fungi, and viruses.
- Effectiveness reduced by organic matter (but not as much as with hypochlorites).
- 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.

3.10.2.2. Hypochlorites (bleach)

- Working dilution is 1:10 household bleach in water.
- Effective against vegetative bacteria, fungi, most viruses at 1:100 dilution.
- Effective against bacterial spores at 1:10 dilution.
- Very corrosive.
- Rapidly inactivated by organic matter.
- Solutions decompose rapidly; fresh solutions should be made daily.

3.10.2.3. Alcohols (ethanol, isopropanol)

- The effective dilution is 70-85%.
- Effective against a broad spectrum of bacteria and many viruses.
- Fast acting.
- Leaves no residue.
- Non-corrosive.
- Not effective against bacterial spores.

3.10.3. Important Characteristics of Disinfectants

	Hypochorites "Bleach"	Iodoform "Wescodyne"	Ethyl Alcohol
Shelf-life > 1 week		X	X
Corrosive	X	X	
Residue	X	X	
Inactivation by Organic Matter	X	X	
Skin Irritant	X	X	
Respiratory Irritant	X		
Eye Irritant	X	X	X
Toxic	X	X	X

3.10.4. Dilution of Disinfectants

3.10.4.1. Chlorine compounds (Household Bleach)

Dilution in Water	% Available Chlorine	Available Chlorine (mg/l or ppm)
Not diluted	5.25	50,000
1/10 (10% v/v)	0.5	5,000
1/100	0.05	500

Bleach solutions decompose at room temperature and need to be made fresh daily. However, if stored away from direct sunlight in tightly closed brown bottles, bleach solutions retain activity for 30 days. The use concentration is dependent on the organic load of the material to be decontaminated. Use a 10% solution to disinfect clean surfaces and surfaces mildly contaminated with organic material. A higher bleach concentration may be needed to disinfect surfaces contaminated with a heavy organic load. To disinfect liquid biological waste before disposal, add concentrated bleach to a final concentration of 10%.

3.10.4.2. Iodophor

Manufacturer's recommended dilution is 3 ounces (90 ml) into 5 gallons water, or approximately 4.5 ml/liter. For porous surfaces, use 6 ounces into 5 gallons water, or approximately 9 ml/liter.

3.10.4.3. Alcohols

Ethyl alcohol and isopropyl alcohol diluted to 70 - 85% in water are useful for surface disinfection of materials that may be corroded by a halogen or other chemical disinfectant.

3.11. Autoclaving Procedures for Biological Waste

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 if blocked. If the sieve is blocked with debris, a layer of air may form at the bottom of the autoclave, preventing efficient operation.

3.11.1. Container Selection

3.11.1.1. Polypropylene bags

Commonly called biohazard or autoclave bags, these bags are able to withstand autoclaving and are tear resistant, but can be punctured or burst during autoclaving. Therefore, place bags in a rigid container such as a polypropylene or stainless steel pan during autoclaving. Bags are available in a variety of sizes, and some are printed with an indicator that changes color when processed. The biohazard bags should be clear or translucent. Red and orange autoclave bags are no longer permitted on Indiana University campuses.

Polypropylene bags are impermeable to steam, and for this reason mustnot 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.

3.11.1.2. 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 do the following:

- Remove the lid (if applicable).
- Turn the container on its side when possible.
- Select a container with the lowest sides and widest diameter possible for the autoclave.

3.11.1.3. Stainless Steel Containers and Pans

Stainless steel is an efficient conductor of heat and is less likely to increase sterilizing time, though is more expensive than polypropylene.

3.11.2. Preparation and Loading of Materials

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

- Add water to loads containing dry or absorbent material to facilitate proper steam generation and sterilization.

3.11.3. Cycle Selection

- Use liquid cycle when autoclaving liquids, to prevent contents from boiling over.
- Select fast exhaust cycle for glassware.
- Use fast exhaust and dry cycle for wrapped items.

3.11.4. Time Selection

- Bags of biological waste mustbe autoclaved in cycles that allow for a minimum of 20 minutes at 121°C and 15 psi to assure decontamination.
- 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.
- Non-select agent biological toxins may require extended autoclave times for inactivation. See **Appendix F** for additional instruction on biological toxin inactivation

3.11.5. Removing the Load Safely

CAUTION - AUTOCLAVES MAY CAUSE SERIOUS BURNS. TO PREVENT INJURY:

- Check that chamber pressure has returned to zero before opening door.
- Wear eye and face protection. Wear thermal protective gloves to handle materials.
- Stand behind door when opening it.
- Slowly open door only a crack. Beware rush of steam as a burn hazard is present.
- Keep face away from door as it opens. Escaping steam may burn face.
- Wait 5 minutes after opening door before removing liquids.
- Liquids removed too soon may boil up and out of container, burning operator.

It is the responsibility of the autoclave user to transport autoclaved waste to the regular trash or dumpster. Follow the departmental specific procedures for your respective campus.

3.12. Autoclave Monitoring and Validation

Autoclaves used to decontaminate laboratory waste should be tested periodically to assure effectiveness. As an institutional practice, IUEHS Biosafety advises semi-annually or quarterly QA testing of autoclaves. Two types of tests are used: 1) a chemical indicator that fuses when the temperature reaches 121°C, and 2) heat-resistant spores (*Bacillus stearothermophilis*) that are killed by exposure to 121°C for approximately 15 minutes. 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.

The chemical test should be used first to determine that the temperature in the center of the container reaches 121°C.

Ampules of heat-resistant spores should be used in subsequent test runs to determine the length of time necessary to achieve sterilization.

If you need assistance, contact the IUEHS Biosafety for your respective campus.

3.13. Use and Disposal of Sharps

3.13.1. To prevent needlestick injuries:

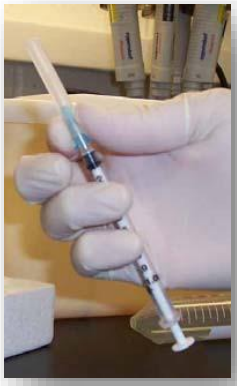
- Avoid using needles whenever possible.
- Do not bend, break, or otherwise manipulate needles by hand.
- Do not recap needles by hand. Do not remove needles from syringes by hand.
- Immediately after use, discard needle and syringe (whether contaminated or not) into puncture resistant sharps containers.
- Never discard sharps into regular trash.
- Never discard sharps into bags of biological waste.
- Use care and caution when cleaning up after procedures that require the use of syringes and needles.
- Use extra care when two persons are working together. Locate sharps container between the workers when possible. Do not overfill sharps containers.
- Locate sharps containers in areas in which needles are commonly used.
- Make containers easily accessible.
- Occasionally needles must be filled, recapped, and set aside for use later. In these cases, recapping may be performed by the one-handed scoop technique, or by placing the needle in a sterile conical tube.

3.13.2. In the event of a needlestick injury

- Follow procedures identified in section 3.2 of this document. Notify supervisor and go immediately to your Designated Medical Service Provider for your respective campus.

3.13.3. To dispose of sharps other than needles

- Do not handle broken glassware directly. Instead, remove it to a sharps container or other puncture-resistant container using a brush and dustpan, tongs or forceps.
- Discard razor blades and scalpel blades into sharps containers.



3.14. Biological Waste Disposal Procedures

3.14.1. Biological Waste

All biological waste from BSL-1, BSL-2, and BSL-3 laboratories must be decontaminated prior to disposal.

If you do not have access to an autoclave or the autoclave is not functioning, contact IUEHS Biosafety for your respective campus for assistance arranging pick-up.

If the waste will be picked up by a contracted vendor, packaging requirements for that vendor must be followed which may differ from the packaging and labeling requirements outlined by IUEHS.

Decontamination and disposal are the responsibility of the person/laboratory generating the waste.

Collect disposable, solid materials contaminated by an infectious agent, **excluding sharps, or broken or unbroken glass,** into autoclave-proof bags (bag must have biohazard symbol and be clear or translucent and not red) within a sturdy container with biohazard symbol. When full, these bags are autoclaved, cooled, biohazard symbol defaced, put into plain opaque household trash bags, and then placed in the building's dumpster. Please refer to Appendix B for specific Indiana University waste guidelines.

Decontaminate liquids containing a biological agent by the addition of a chemical disinfectant such as sodium hypochlorite (household bleach) or an iodophor, **or** by autoclaving, then dispose of by pouring down the sink. It is not necessary (or advisable) to autoclave liquids that have been chemically disinfected.

Non-select agent biological toxins may be chemically treated or may require extended autoclave times for inactivation. See Appendix F for additional instruction on biological toxin inactivation.

3.14.2. Reusable Labware

Items such as culture flasks and centrifuge bottles are decontaminated by lab personnel before washing by one of two methods.

- Autoclave items that have been collected in an autoclavable container.
- Chemically disinfect items by soaking in diluted disinfectant for one hour before washing.

3.14.3. Disposal of Blood Products and Body Fluids

All human blood and other potentially infectious materials (OPIM) must be handled using Universal Precautions under BSL-2 biocontainment. Refer to Appendix B for Indiana University waste guidelines.

Discard disposable items contaminated with human blood or body fluids (**excluding sharps and glassware)** into autoclavable biohazard containers or bags. Material must be packaged and decontaminated as BL2 biohazardous waste, refer to 3.14.1 or disposal procedures.

3.14.4. Disposal of Sharps and Disposable Glassware

Discard all needles, needle and syringe units, scalpels, and razor blades, **whether contaminated or not,** directly into rigid, labeled sharps containers (clear or translucent and not red). Do not recap, bend, remove or clip needles. Sharps containers must not be overfilled. Biohazardous sharps containers must be autoclaved as above.

Uncontaminated (no biological materials have been used) Pasteur pipettes and broken or unbroken glassware are discarded into containers specifically designed for broken glass disposal, or into heavy-duty cardboard boxes that are closeable. When boxes are full, tape closed and place in the building's dumpster.

Biologically Contaminated Pasteur pipettes and broken or unbroken glassware may be treated in one of two ways:

- Discarded into approved biological sharps containers, or
- Decontaminated by autoclaving or chemical disinfection, then discarded into glass disposal boxes or bins.

Biologically contaminated plastic sharps (including serological pipettes) may be packaged and treated in the following ways:

- An approved biological sharps container
  - To be autoclaved and disposed of as referenced above for glassware and other sharps waste.
- Decontaminated by chemical disinfection; either in a vertical or horizontal tray fully submerged in disinfectant.
  - Pipettes must be fully submerged in disinfectant for a minimum of 30 minutes.
  - Once fully decontaminated, the pipettes can be packaged as non-hazardous sharps waste and disposed of in the building dumpster.
- Rigid plastic tub with a lid that can withstand autoclaving
  - Tub must be large enough to fully enclose the pipettes and be lined with a biohazard bag to facilitate transfer of decontaminated pipettes to a box for non-hazardous sharps waste disposal.
  - Autoclave the tub with lid as described in section 3.14.1.
  - Once autoclaved, remove the bag and place into a cardboard box for non-hazardous sharps disposal.
- Placed in a lined cardboard box that can be fully closed
  - The box must be lined with a biohazard bag to ensure that any liquid biological material cannot leak onto the ground during collection.
  - Box must be closed, with bag open, before transport to the autoclave.
  - Box must be placed in an autoclaveable tray (plastic or metal) before autoclaving.
  - Autoclave as described in section 3.14.1.

- Sharps that are contaminated with radioactive materials or hazardous chemicals must be discarded into separate sharps containers labeled with the name of the isotope or chemical. Contact IUEHS Biosafety or Radiation Safety for your respective campus for disposal information.

3.14.5. Multi-hazard or Mixed Waste

Avoid generating mixed waste if possible. Keep volume to minimum.

Do not autoclave mixed waste, i.e., chemical waste combined with biological waste.

When discarding waste containing an infectious agent and radioactive material, inactivate the infectious agent first, then dispose as radioactive waste. Seek advice from the Radiation Safety Officer (RSO) for your respective campus before beginning inactivation procedures.

When discarding waste containing an infectious agent and a hazardous chemical, inactivate the infectious agent first, then dispose as chemical waste. Seek advice before beginning inactivation procedures. Contact IUEHS Biosafety for your respective campus for assistance.

3.14.6. Disposal of Animal Tissues and Carcasses

Disposal of animal carcasses/tissues is coordinated through the Animal Care Facility for your respective campus.

- Place animal carcasses/tissues into non-transparent bag. Double-bag when carcass contains zoonotic agent (transmissible from animals to humans).
- Place bag in freezer at Animal Care Facility or other designated location for your respective campus.
- Contact IUEHS for your campus with any questions.

Disposal of animal carcasses/tissues that are contaminated with radioactive materials requires special handling. Disposal instructions are available by contacting Radiation Safety for your respective campus.

3.14.7. Disposal Containers

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

3.14.7.1. Biological Sharps Containers

Sharps containers may be purchased from laboratory product distributors. They are available in various sizes, and must be puncture resistant, not red in color, labeled as "sharps," have a visible biohazard symbol, and have a tightly closing lid. Do not purchase “needle-cutter” devices, which may produce aerosols when used.

3.14.7.2. Hard plastic autoclave tub

Hard plastic autoclave tubs may be purchased from laboratory product distributors. They are available in various sizes. They must have a lid.

Example of an acceptable autoclave tub:

3.14.7.3. Pipette washers

Pipette washers can be used to chemically decontaminate serological pipettes. They can be purchased from laboratory product distributors and are available in various sizes.

Example of an acceptable pipette washer and basket

3.14.7.4. Cardboard box

Cardboard box must be closable and must be placed into an autoclave tub large enough to fit the entire box.

3.14.7.5. Biohazard Autoclave Bags

May be purchased from various laboratory product distributors, such as Fisher Scientific, VWR, and Baxter. Be sure to select polypropylene bags that are able to withstand autoclaving. Red biohazard autoclave bags are no longer permitted for laboratory use. They should be placed inside a rigid container with lid while waste is being collected. The rigid container must have the biohazard symbol.

3.14.7.6. Glass Disposal Boxes

May be purchased from various laboratory product distributors. Alternatively, heavy-duty, closeable cardboard boxes may be used for disposal of broken glass. They should be lined with a clear plastic bag and the bottoms reinforced with tape. Glass disposal boxes are only to be used for the disposal of non-biohazard glass.

3.15. Laboratory Inspections and Corrective Action Procedures

Laboratory inspections are conducted to ensure that laboratories utilizing biological materials meet specific requirements and follow certain safety guidelines. Inspections are intended to promote a safe laboratory working environment and to ensure compliance with the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, the Biosafety in Microbiological and Biomedical Laboratories, 5th ed., the IU Biosafety Manual, and the OSHA Bloodborne Pathogens Standard. Interactive inspections are conducted where the inspector makes observations as well as speaks with a laboratory designee to answer and discuss specific laboratory procedures and safety practices.

3.15.1. Annual Inspections

Annual Biological Safety Inspections are conducted for all laboratories utilizing biological materials for teaching or research purposes at BL1 or higher. Annual inspections are PI specific, thus more than one inspection may be conducted per room for shared spaces. Upon inspection of the laboratory, deficiencies will be documented and an inspection report sent to the PI. The inspection report will contain a description of the individual deficiencies as well as recommended or required corrective actions.

It is expected that all deficiencies be addressed and corrected as soon as possible. PIs will be given 2-3 weeks from the receipt of their inspection report to begin corrective action. A written verification of complete or partial correction is required. Corrective action can be emailed to the general IUEHS Biosafety email address for your respective campus or a hard copy can be sent via campus mail to IUEHS Biosafety or your respective campus. IUEHS Biosafety for your respective campus is available to provide advice on how to address the deficiencies.

If sufficient progress has not been documented, a follow up inspection can be conducted 2-3 weeks after the inspection report is sent to verify progress. Follow-up inspections at the regional campuses are the responsibility of the IUEHS representatives for the respective campus.

Imminent danger or egregious violations are cause to terminate laboratory operations immediately.

3.15.2. Corrective Action Procedures

3.15.2.1. Level 1

Failure to take sufficient corrective action by the end of the initial 2-3 week corrective action period or the severity of remaining violations will determine if the process proceeds to Level 1. If very little or no progress has been made a Level 1 response will be necessary and a re-inspection of the laboratory will be conducted if necessary. IUEHS Biosafety will send copies of the Level 1 re-inspection report to the PI.

IUEHS Biosafety will discuss the Level 1 re-inspection with the PI to agree upon corrective actions. The PI will be given an additional ten (10) business days to correct all violations. Written verification of corrected deficiencies must be submitted to IUEHS Biosafety

within that time period. A follow-up inspection will be conducted to verify that all corrections have been made unless written verification is deemed sufficient.

3.15.2.2. Level 2

If written verification has not been submitted within the additional ten (10) day time period, a re-inspection and follow up inspection will be conducted by IUEHS Biosafety or other personnel if necessary. The IUEHS Biological Safety Manager for the respective campus will send a letter and copies of inspections and any PI, lab manager, or lab supervisor responses to the PI, the IBC, and the Department Chair or Director. The letter will give the PI an additional five (5) business days to correct remaining violations and submit written verification.

Mandatory retraining of laboratory personnel will be considered if the violations reveal a lack of understanding or deliberate avoidance of biological safety guidelines.

3.15.2.3. Level 3

If written verification of completed corrective actions has not been submitted to IUEHS Biosafety by the end of the process through Level 2 (a total of 25-30 business days), IUEHS Biosafety will send a letter of non-compliance to the PI, the IBC, the Department Chair or Director, and the administrative head of the college, school, or unit. A re-inspection and follow-up inspection will be conducted as necessary.

Failure of the PI to submit verification of corrections will impact their ability to obtain approvals for permits and grant certifications requiring validation of compliance with applicable state and federal regulations. If the laboratory involves work with non-exempt recombinant or synthetic nucleic acid molecules an incident report of non-compliance will be sent to the NIH.

Extensions to provide corrective action may be requested in writing at any stage of this process from IUEHS Biosafety staff.

3.15.2.4. Level 4

If the steps taken in the previous action levels have not resulted in the submission of a written verification of completed corrective actions to IUEHS Biosafety within the established timeline then the laboratory will be deemed noncompliant. The chief academic officer of the campus where the laboratory is located and the University Director of Environmental Health and Safety will be notified of the noncompliant laboratory and punitive action will be requested which may include prohibiting employee access to the laboratory until corrective action has been taken. The IBC may terminate approved protocols and place a hold on funding until appropriate action is taken. If the action taken by the chief academic officer does not result in compliance by the noncompliant laboratory then the Executive Vice President for University

Academic Affairs will be requested to take punitive action to ensure compliance.

3.15.3. IBC Approval Inspections

Protocol specific laboratory inspections will be conducted prior to protocol approval for protocols utilizing material that fall under the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, all protocols and research that have been otherwise required to be reviewed and approved by the IBC. Upon inspection of the laboratory any deficiencies must be corrected or addressed before approval of the proposed IBC protocol. Deficiencies will be documented and an inspection report sent to the PI. IUEHS Biosafety for your respective campus would be available to provide advice to the PI to address and correct any deficiencies in a timely manner for approval. An annual inspection may be accepted in place of a separate IBC inspection as deemed appropriate by IUEHS Biosafety for your respective campus.

3.16. Research Related Vaccinations

In certain cases, while working with biological agents that can cause disease in humans, it is advisable to offer vaccination for those agents. The need for research related vaccination will be dependent on the research description given in the Institutional Biosafety Committee (IBC) protocol. The IBC along with the IUEHS Biosafety for your respective campus will conduct a risk assessment to evaluate if the research warrants recommendation or requirement of a specific vaccine. In some cases vaccination would be a requirement of conducting proposed research. The identification of Indiana University employees who may become exposed to infectious biological agents and need vaccination is based on the IBC protocol and the Center for Disease Control and Prevention list of vaccine preventable diseases. The vaccine preventable diseases list is available for your review at http://www.cdc.gov/vaccines/vpd-vac/vpd-list.htm

Medical evaluation and vaccination at Indiana University will be performed by the Designated Medical Service Provider for the respective campus. Evaluation and vaccination is considered confidential. Some physical conditions may affect the ability of an individual to be vaccinated. The Designated Medical Service Provider for the campus may ask for a medical history to assist in determination of these conditions. If an employee elects to decline the vaccine, but continues to work under the protocol, they must sign the declination section of the Vaccination Acceptance/Declination form (Appendix C1-2). A copy of the form must be kept with the Designated Medical Service Provider. Medical records for Regional Campus employees must also be included in Indiana University Human Resources.

Obtaining vaccines which have been recommended or required by IUEHS Biosafety and the IBC will be at no cost to employees of Indiana University. The IBC and IUEHS Biosafety may recommend or require vaccination of non-Indiana University employees who have been listed on IBC protocols. IUEHS Biosafety for the respective campus will cover the expense of vaccinations for Indiana University employees. The cost of vaccination for non-Indiana University employees is the responsibility of the Principal Investigator.

Contact the IUEHS Biosafety for your respective campus to request consideration of a research related vaccination and to obtain necessary forms. Vaccination Acceptance/Declination forms are required to be filled out and signed by IUEHS Biosafety before a vaccine is obtained. For billing purposes, IUEHS Biosafety for the respective campus will be notified that personnel have completed recommended vaccination(s). Evaluation and vaccination is considered confidential and no personal medical information will be shared with staff or faculty at Indiana University.

3.17. Biosafety Equipment

3.17.1. Biosafety Cabinets (BSCs)

The BSC is designed to provide protection to the product, the user, and the environment when appropriate practices and procedures are followed. Three types of BSCs (Class I, II, III) and the horizontal laminar flow cabinet are described below.

The common element to all classes of BSCs is the high efficiency particulate air (HEPA) filter. This filter removes particles of 0.3 microns with an efficiency of 99.97%. However, it does not remove vapors or gases.

The BSC requires regular maintenance and certification and should be completed by an NSF 49 accredited vendor to assure that it protects you, your experiments, and the environment. Each cabinet must be certified when it is installed, each time it is moved or repaired, and at least annually. Annual certification is a requirement under the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (Appendix G-II-C-4-j of the Guidelines). Individual departments or PIs are responsible for costs of certification and repairs or replacement of HEPA filters. Annual certification is verified by IUEHS Biosafety during annual inspections and before IBC protocol approvals.

3.17.1.1. Types of Biosafety Cabinets

- **Class I BSCs** protect personnel and the environment, but not research materials. They provide an inward flow of unfiltered air, similar to a chemical fume hood, which protects the worker from the material in the cabinet. The environment is protected by HEPA filtration of the exhaust air before it is discharged into the laboratory or ducted outside via the building exhaust.

- **Class II BSCs** (Types A1, A2, B1, B2) provide personnel, environment, and product protection. Air is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward laminar flow of HEPA-filtered air within the cabinet provides product protection 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 (environmental protection), and may be recirculated back into the laboratory (Type A) or ducted out of the building (Type B).

- **Class III BSCs** (sometimes called Class III glove boxes) were designed for work with infectious agents that require BSL-4 containment, and provide maximum protection to the environment and the worker. The cabinet is gas-tight with a non-opening view window, and has rubber gloves attached to ports in the cabinet that allow for manipulation of materials in the cabinet. Air is filtered through one HEPA filter as it enters the cabinet, and through 2 HEPA filters before it is

exhausted to the outdoors. This type of cabinet provides the highest level of product, environmental, and personnel protection.

3.17.1.2. Installing or Relocating Biosafety Cabinets

After installing or relocating a BSC, work may not begin until the BSC has been certified and tested by an outside vendor to ensure proper functionality. Care should be taken when deciding on the initial placement or relocation of a BSC. BSCs should be placed away from doorways and high traffic areas. They should also be placed away from heating and cooling vents to help maintain proper airflow within the cabinet.

Please contact IUEHS Biosafety for consultation on proper placement of a biological safety cabinet.

3.17.1.3. Disposal of Biosafety Cabinets

BSC must be space decontaminated before disposal. Contact IUEHS Biosafety prior to the decontamination and disposal of the cabinet.

3.17.1.4. Repairs of Biosafety Cabinets

Repairs may only be conducted by NSF-accredited technicians. If your BSC is in need of a repair, contact IUEHS Biosafety for your respective campus for assistance locating a repair technician.

3.17.1.5. Operation of Class II Biological Safety Cabinets

- Turn on cabinet fan 15 minutes before beginning work.
- Disinfect the cabinet work surface with 70% ethanol or other disinfectant.
- Place supplies in the cabinet. Locate container inside the cabinet for disposal of pipettes (Movement of hands in and out of the cabinet to discard pipettes into an outside container disrupts the air barrier that maintains sterility inside the cabinet.).
- Work as far to the back (beyond the air split) of the BSC work space as possible. Always use mechanical pipetting aids.
- Do not work in a BSC while a warning light or alarm is signaling.
- Locate liquid waste traps inside 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 (such as a cardboard box) to prevent spilling. It is recommended that a secondary flask be utilized. See 3.17.6
- Wear gloves and laboratory coat when working within the biosafety cabinet
- Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC may affect proper air flow and the level of protection provided. Also, keep the front and rear grilles clear.
- When work is completed, remove equipment and supplies from

the cabinet. Wipe the work area with 70% ethanol and allow cabinet to run for 15 minutes.

- Some BSCs are equipped with ultraviolet (UV) lights. However, if good procedures are followed, UV lights are not needed. If one is used, due to the limited penetrating ability of UV light the tube should be wiped with alcohol every two weeks, while turned off, to remove dust. UV radiation must not take the place of 70% ethanol for disinfection of the cabinet interior.
- The UV lamp must never be on while an operator is working in the cabinet.
- Minimize traffic around the BSC and avoid drafts from doors and air conditioning.
- Do not put your head inside the BSC. This compromises the sterility of the environment and, more importantly, could expose you to infectious pathogens.
- Do not tamper with the BSC or interfere with its designed function. It was engineered to operate optimally with no obstructions around the sash or grilles.
- Open flames are not required in the near microbe-free environment of a biological safety cabinet. On an open bench, flaming the neck of a culture vessel will create an upward air current which prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates turbulence which disrupts the pattern of HEPA-filtered air supplied to the work surface. Therefore, the **use of open flames is strongly discouraged and gas burners are prohibited in in biosafety cabinets.** When deemed absolutely necessary, touch-plate micro-burners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized. The burner must be turned off when work is completed. Small electric "furnaces" are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable sterile loops can also be used.

3.17.2. Clean Air Benches

Horizontal laminar low benches are not BSCs. They discharge HEPA-filtered air across the work surface and toward the user, providing only product protection. They can be used for certain clean activities, such as dust-free assembly of sterile equipment or electronic devices. However, clean air benches are not a substitute for a BSC in research laboratories and use when handling BSL-2 cell culture materials or potentially infectious materials is inappropriate.

3.17.3. 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.

3.17.4. Protection of Vacuum Lines

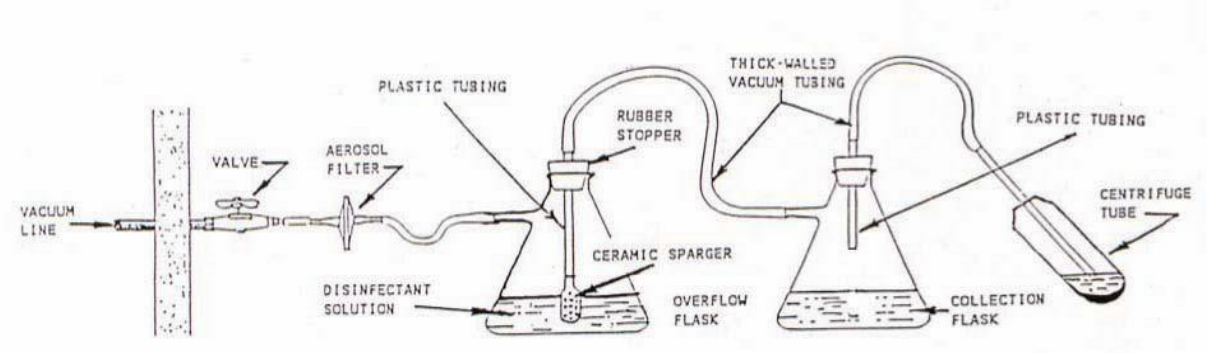
All central vacuum lines used to aspirate supernatants, tissue culture media, and other liquids that may contain microorganisms must be protected from contamination by the use of a collection flask and overflow flask. In addition a hydrophobic vacuum line filter must be used.

3.17.5. 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 BSL-2 and BSL-3 laboratories, the use of Nalgene flasks is recommended to reduce the risk of breakage.

3.17.6. 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 Whatman HEPA-Vent Filter are available from several scientific supply companies (Fisher Scientific, catalog #09-744-79).



An alternative to this setup is a medical grade suction canister, which is an increasingly popular option.

It can be found at:
http://extww02a.cardinal.com/us/en/distributedproducts/ASP/65651-212.asp?cat=surgerycenter

3.18. Shipment of Biological Materials

3.18.1. General Information

Anyone who prepares or ships packages containing biological or infectious substances (human or animal pathogens) or biological substances containing recombinant or synthetic DNA, must attend a training class before providing a package for transport by commercial carrier. The U.S. Department of Transportation (DOT) and the International Air Transport Association (IATA) regulate shipment of human and animal pathogens. The regulations are complex and exacting. They require that researchers who prepare infectious materials for shipment receive periodic training (every 2 years). In addition, packages must be marked and labeled exactly as the regulations specify, and packaging materials must have been tested and certified to withstand certain durability and pressure tests. Cardboard boxes in which supplies have been received cannot be used to ship infectious materials. Recent events have led to greater scrutiny for compliance with these regulations. Training is also required when receiving and signing for packages containing infectious substances. Please contact IUEHS Biosafety for your respective campus for assistance with packaging and shipping biohazardous material and for information regarding required training.

3.18.2. Permits

Permits are required from the Centers for Disease Control and Prevention (CDC) to import or transport 1) any microorganism that causes disease in humans; 2) biological materials, such as blood and tissues, when known or suspected to contain an infectious agent; 3) live insects, such as mosquitoes, known or suspected of being infected with any disease transmissible to humans; and 4) any animal known or suspected of being infected with any disease transmissible to humans. Importation permits are issued only to the importer, who must be located in the U.S. The importation permit, with the proper packaging and labeling, will expedite clearance of the package of infectious materials through the U.S. Public Health Service Division of Quarantine and release by U.S. Customs. Transfers of previously imported material within the U.S. also require a permit. IUEHS Biosafety for the respective campus must be notified prior to submission of application for permit and are available to assist through the permitting process.

Application for the permit should be made at least 10 working days in advance of the anticipated shipment date. Further information and application forms may be obtained by calling the CDC at (404) 639-3235, or through the CDC website at http://www.cdc.gov/od/eaipp/

Permits are required from the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) for importation or domestic transport of agents infectious to livestock; and of biological reagents containing animal, particularly livestock, material (this includes tissue culture media containing growth stimulants of bovine origin such as calf serum). Further information and application forms may be obtained by calling the USDA/APHIS at (301) 734-4401, or http://www.aphis.usda.gov/animal_health/permits/.

Permits are also required from the USDA/APHIS for **interstate movement, importation, or release into the environment (i.e., field tests)** of genetically engineered organisms that are **plant pests**, or that contain portions (plasmids, DNA fragments, etc.) of **plant pests.** Application should be made at least 120 days in advance of the anticipated release or shipment date. IUEHS Biosafety for the respective campus must be notified prior to the submission of application for permit and are available to assist through the permitting process.

Information and application forms may be obtained by calling the USDA/APHIS at (301) 734-4401, or through the APHIS web site at http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/biotechnology.

Facility registration and completion of the CDC/USDA Form 2 are required by the CDC prior to transfer of **select agents and toxins** (42 CFR Part 73). If proposed research involves the use of any agents listed in Appendix A the PI must contact IUEHS Biosafety for your respective campus immediately to initiate the registration process. It is strictly forbidden to use, transport, or possess any of the agents listed without an active registration. Your IUEHS Biosafety will assist you with obtaining approval for research involving Select Agents and should be your first contact if your research designs include Select Agents.

A validated license is required by the Department of Commerce for **export** of certain microorganisms and toxins (listed in 15 CFR Part 774) to all destinations. Investigators wishing to ship these items must contact the IU Export Control Office for assistance in meeting these requirements (export@iu.edu). .

3.18.3. Packaging

Various carriers (FedEx, UPS, Postal Service or others) have different requirements for packaging and labeling infectious substances. In addition, various agencies such as the International Air Transport Association (IATA), and the Department of Transportation (DOT) have developed guidelines and procedures to facilitate the safe shipment of infectious substances. Therefore, it is important to check with the carrier you have chosen to determine their specific requirements for shipping infectious agents. In addition to the materials listed above that require permits, the following materials are likely to require special packaging and/or labeling:

- Infectious Substance: a viable microorganism, or its toxin, which causes or may cause disease in humans. DOT requires shippers of infectious substances to attend training every 2 years.
- Diagnostic Specimen: any human or animal material including blood, tissue, and tissue fluids, shipped for the purpose of diagnosis.
- Biological Product: a product for human or veterinary use, such as vaccines and investigational new drugs.

The basic component of all shipping requirements, with various minor modifications, is triple packaging, as follows:

- A primary container that contains the specimen;

- A secondary container that contains the primary container and packaging capable of absorbing the specimen; and
- An outer rigid shipping container that contains the secondary container and other material.

3.18.4. Genetically Modified Microorganisms (GMOs)

The International Air Transport Association's Dangerous Goods Regulations (50th ed.) states that:

- GMOs of Category A agents must be shipped as Category A.
- GMOs of Category B agents must be shipped as Category B.
- If a GMO is not classified as Category A or B it would be classified as UN 3245 Category 9

3.18.5. Human Clinical Materials

The OSHA Bloodborne Pathogens Standard requires that all packages containing human blood and other potentially infectious materials be labeled with the universal biohazard symbol or color-coded. Various carriers may have additional requirements. For more information regarding OSHA Bloodborne Pathogens Standard and the handling of blood and OPIM refer to the Indiana University Bloodborne Pathogens Exposure Control Plan.

3.18.6. On-Campus Transport Between Laboratories or Buildings

When moving infectious substances between labs or buildings on campus, the following minimum procedures must be followed:

- Sample must be in sealed primary container. Utilize plastic containers whenever possible.
- Place primary container in sealed secondary container, with absorbent (paper towels) between primary and secondary container suitable for the volume transported.
- If dry ice is needed, the secondary container should be placed in an outer container, with the dry ice placed between the secondary and tertiary container (never place dry ice in a sealed container).
- Place biohazard label on outer container.