

BIOLOGICAL SAFETY MANUAL

Including Institutional Exposure Control Plan



*Office of Prospective Health/Biological Safety
Biological Safety Committee
East Carolina University*

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Biological Safety Manual

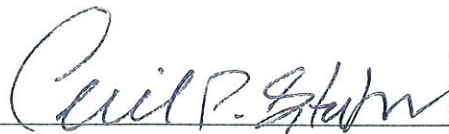
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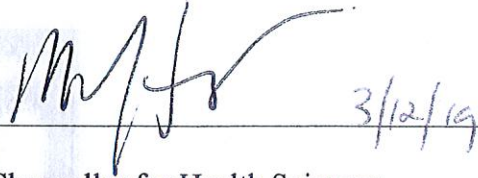
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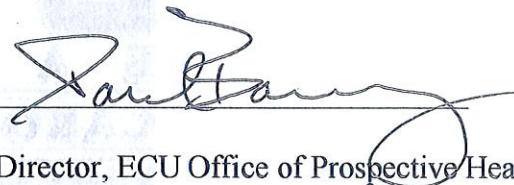
Chancellor
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Vice Chancellor for Health Sciences
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Chairman, ECU Institutional
Biological Safety Committee



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FOREWORD

The Institutional Biological Safety Committee develops Biosafety policy and procedures for East Carolina University research and teaching laboratories, reviews research proposals for use of infectious and biohazardous materials and recombinant DNA and oversees continued compliance by researchers.

East Carolina University is committed to maintaining a workplace that is free from recognized hazards and to complying with the Biological Safety guidelines from CDC and NIH, and other regulatory agencies.

All users of biological or biohazardous materials must become familiar with the requirements set forth in this manual, and conduct their operations in accordance with them.

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Chapter 1

ADMINISTRATION

Any work performed at East Carolina University which involve biohazardous biological agents will be conducted in a manner, which affords protection to workers, (ECU Faculty, staff, students, visitors, contract workers) and to the surrounding community and general environment. The Biological Safety Program is comprised of the Institutional Biological Safety Committee and the Biological Safety section of the Office of Prospective Health.

A. Definitions

1. *Biological Agent*: Any microorganism, including those which have been genetically modified, genetic elements, or materials containing or derived from microorganisms or biological materials (e.g. toxins, allergens, prions, cell cultures and parasites), which may cause an infection, create or provoke an immune reaction or exhibit toxicity in humans, animals, or plants or have an adverse effect on the environment. This includes any recombinant DNA molecule, DNA or RNA derived from recombinant DNA, or organisms or viruses containing recombinant DNA including plants and animals.
2. *Biohazard*: A Biological agent is considered to be biohazardous if exposure may be a hazard to laboratory workers, the community, livestock or the environment (WHO). Biohazardous agents include, but are not limited to conventional pathogens, non-exempt recombinant DNA molecules or recombinant organisms, human body fluids, tissues or cell cultures, or indigenous or experimental infections of laboratory animals or plants.
3. *Biosafety*: Laboratory Biosafety includes all containment principles, techniques and practices that are implemented to prevent the unintentional exposure to biological agents and toxins, or other accidental release (WHO/CDS/EPR, 2006).

B. Purpose

The Biological Safety program is established to ensure that adequate administrative, physical, and operational protective measures are in place in order to:

- Protect faculty, staff, students and visitors from biological agents that are stored or handled at ECU.
- Reduce the risk of unintentional release of biological agents and toxins, including the risk of infection or exposure.
- Comply with all regulatory and other legal requirements applicable to the biological agents and toxins that will be handled.

This manual:

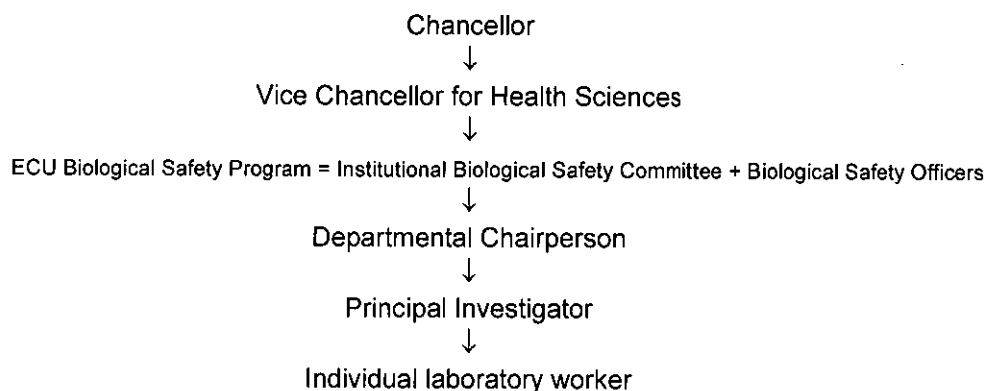
- Outlines the responsibilities of all parties involved in obtaining and using biohazardous agents.
- Specifies the training required by all individuals working with these agents
- Advises all individuals working with biohazardous agents of their rights and responsibilities under Federal and State regulations.
- Provides the Principle Investigator with reference materials or links to develop project-specific Biological Safety standard operating procedures.

- Serves as the Exposure Control Plan for research with human blood, serum, or tissue or other materials potentially infectious for Biosafety pathogen at the ECU.
- Outlines on the proper handling, treatment and disposal of biohazardous wastes.
- Describes the steps to be taken in the case of spills or other emergencies.
- Requires compliance with Federal Guidelines regarding Use of Recombinant DNA Molecules in research.
- Prescribes the use of medical preventive and surveillance procedures as applicable.
- Provides guidance for laboratories and individuals using Select Agents.

C. Organization/Authority

The Chancellor of East Carolina University has charged the Vice Chancellor for Health Sciences with the development of the Institutional Biological Safety program for the entire ECU campus. The Biological Safety Committee is authorized to review, and approve research projects and provide oversight of teaching or research laboratory activities which utilize biohazardous agents. Biological Safety administers the program on a day to day basis, and has the responsibility and operational authority to assist with the safe use of biological agents, ensure compliance, and interface with regulatory agencies.

Biological Safety Organization



The Biological Safety Program seeks to maximize protection for ECU personnel and the environment while minimizing interference with research activities. Where unsafe practices or conditions immediately dangerous to life or health or actions in violation of established guidelines or ECU policy are observed, the ECU Biological Safety Officer has the authority to suspend the work until a thorough review can be made by the Institutional Biological Safety Committee. If the Committee is not satisfied with the adequacy of the biological safety practices employed in a project, it may require all work involving the agent be deferred or suspended until satisfactory procedures are adopted.

D. Responsibilities

Office of Prospective Health/Biological Safety Office

The Biological Safety Officer(s) manages the Biological Safety Program for East Carolina University, under the administrative oversight of the Director of the Office of Prospective Health, and with the scientific oversight of the Chair of the Institutional Biological Safety Committee (IBC).

The responsibilities of the Biological Safety Officer(s) are to:

- Advise and assist potential user of designated biohazardous agents regarding biological safety procedures, supplies and equipment.
- Ensure that all regulations, standards and guidelines from the Centers for Disease Control (CDC), National Institutes of Health (NIH), Occupational Safety and Health Administration (OSHA), United States Department of Agriculture (USDA), Environmental Protection Agency (EPA), or the State of North Carolina are reasonably met.
- Review all applications to use biohazardous agents for completeness and clarify the content prior to presenting to the Institutional Biological Safety Committee.
- Review plans for all new construction or modifications to existing buildings where biohazardous agents may be used and recommend physical containment measures.
- Provide training and guidance to University employees regarding receipt or shipment of biohazardous agents. DOT/IATA training for shipping infectious material and diagnostic specimens and 2 year certification online.
- Manage biohazardous or biomedical waste collection and disposal program at ECU.
- Advise, supervise, or conduct decontamination activities in the event of accidental spill or release of biohazardous materials.
- Conduct periodic laboratory evaluations and inspections.
- Investigate and evaluate the circumstances surrounding biohazard exposure incidents; recommend preventative or remedial actions and ensure their implementation
- Ensure that research personnel receive needed training or occupational health services after Biological Safety registration review.
- Serve as member of the Institutional Biological Safety Committee.
- Suspend any operation causing an excessive and/or unnecessary biological hazard as rapidly and as safely as possible; conduct expeditious review of the situation with the Biological Safety Committee.
- Facilitate certification of Biological Safety Cabinets and coordinate and removal with Facilities Services.
- Act as central receiver for all Select Agent shipments, and ensure proper procedures and record keeping are followed.

Institutional Biological Safety Committee

The Biological Safety Committee is comprised of a minimum of five members selected based on education and work experience so that collectively the committee possesses knowledge of recombinant DNA technology, microbiologic procedures, biological safety, and physical containment techniques, institutional policies, applicable laws and standards, professional conduct and community standards. Background information and updated guidelines will be provided to the membership regularly. The committee meets on a bimonthly basis, during the academic year. Faculty and staff members represent various ECU Schools and Divisions, the community, and one member of the Department of Comparative Medicine veterinary staff.

Ad hoc members attend only when their special expertise is required, e.g., human gene transfer or plant-related projects.

The responsibilities of the Institutional Biological Safety Committee are to:

- Develop and update policies and guidelines for East Carolina University concerning the safe use of all potentially hazardous biologic agents.
- Review the Biological Safety Manual (Exposure Control Plan) **biannually**.
- Review and approve or modify all proposals for the use of biological agents. This includes Risk Group 2 or greater infectious agents, all recombinant DNA as specified by NIH guidelines, human blood or tissue cultures, or any project where the risk level is uncertain and must be determined.
- Review periodic reports from the Biological Safety Officer, such as the evaluation of any biohazard exposure incidents.
- Review any instances of alleged infractions of the ECU policy or procedures take necessary steps for correction.
- Ensure that all biological safety guidelines and regulations from the National Institutes of Health (NIH), Centers for Disease Control (CDC), Occupational Safety and Health Administration (OSHA), State of North Carolina Department of Environment and Natural Resources (DENR) and other applicable state and federal agencies are reasonably met.
- Suggest improvements to meet the goals of the committee.
- Provide the local review and oversight of research using recombinant DNA or organisms required by NIH based upon the category of work; approve any research project in which recombinant DNA material will be administered to human subjects after review by NIH and Office of Biotechnology Activities.
- Report significant problems, violations of NIH guidelines or research related accidents or illnesses to NIH/OBA within 30 days.
- The IBC will determine the Biosafety Level applicable to work performed at ECU.

The Chairman of the Biological Safety Committee will ensure:

- Appropriate membership/ representation on the committee, and that the membership is appropriately trained to perform the duties required.
- That any recombinant DNA research- related accidents/ illnesses are reported to NIH.
- That no research participant is enrolled in a human gene transfer experiment until the federal Recombinant DNA Advisory Committee (RAC) review process has been completed, and that the ECU Institutional Review Board approval and Biological Safety committee approval or other regulatory authorizations have been obtained.
- Maintain expertise in current research techniques in microbiology, use of vectors, and appropriate containment practices. Serve as content expert for the committee.
- Consult with NIH experts to determine appropriate containment practices if needed.
- Conduct meetings of the IBC

Department Chairperson

The Department Chairperson is responsible for the oversight of safe and appropriate use of biohazardous agents in the department. The Chairperson oversees the Principal Investigator obtaining and use of specified biohazardous agents at ECU. The chair is informed of major actions by the committee.

The responsibilities of the Department Chairperson are to:

- Ensure that laboratories are constructed, furnished, supplied and maintained to allow for safe operation and containment of the work planned.
- Ensure that faculty and staff members who desire to use biohazardous agents contact the Biological Safety Officer, obtain a copy of the Biological Safety Manual, and follow the required ECU registration and safety procedures.
- Ensure that plans for all new construction or modifications to existing structures where biohazardous agents are to be used are reviewed by Biological Safety prior to construction or modification.
- Request that areas where biohazardous agents were previously used are surveyed/inspected by Biological Safety before being renovated or adapted to non-laboratory use.
- Contact the Biological Safety Officer regarding the final disposition of any biohazardous agents in the possession of a departing staff person.
- Facilitate and ensure faculty and staff compliance with safety training, audits etc.
- Receive copies of communications about committee's actions and departmental researchers.

Principal Investigator

The Principal Investigator will complete and submit the Biological Safety registration before using biohazardous agents at ECU, and is **responsible** for adherence to all ECU policies, guidelines and regulations regarding use of biohazardous agents; The principle investigator is responsible for training and monitoring of students and staff in laboratory techniques, ensuring that safe work practices are followed and that protective devices and equipment are properly used and maintained.

Responsibilities of the Principal Investigator:

- Contact Biological Safety regarding any planned use of biohazardous agents. Submit registration form if work will present more than minimal risk (i.e. is BSL-2 or greater), containment level is uncertain, or uses recombinant DNA.
- Develop a written Laboratory Biosafety Plan, and specify the engineering and work practice controls to eliminate or minimize exposures. Develop agent specific Standard Operation Procedures (SOPs) for use of specific equipment or containment procedures for the process and agent used. Maintain an accessible copy of the lab Biosafety manual in the lab for ready reference.
- Arrange for the training of all employees ASAP (or within 10 days of employment in the use of human blood, body fluid, cells or tissue). Provide training on the agent-specific or laboratory-specific procedures, and the safety techniques and practices to be used in the laboratory. This is done initially, annually and as policies/procedures change. Ensure each person's proficiency in these tasks prior to allowing work with the agent; verify continued proficiency and performance of applicable biological safety practices by each individual.
- Limit personnel, student, or visitor exposure to biohazardous agents to the lowest practical level. Enforce the lab's Biosafety inventory and access policies.
- Consult with Biological Safety about special safety precautions, for minors, pregnant females or other vulnerable workers as needed. Verify that written parental consent has

been obtained for any individuals less than 18 years of age in the lab per the visitor policy of the school or college. (In general minors are limited to work at BSL-1 only).

- Implement the Biosafety level prescribed by the Institutional Biological Safety Committee.
- Ensure that any required medical surveillance or preventive health services, are obtained by all exposed laboratory personnel.
- Follow prescribed ECU procedures for procurement, storage, handling and packaging of biohazardous agents and waste.
- Maintain accurate records of the various agents, used in the laboratory.
- Provide current posting and labeling of laboratory entrance doors, stock materials, stored material or any equipment that may be contaminated.
- Adhere to good laboratory practices and follow any relevant ECU policy and procedures.
- Transport any infectious materials using appropriate containers, properly labeled and packaged to prevent or contain leakage. Ensure that personnel who ship infectious materials are trained and certified every two years in IATA packaging and shipping procedures.
- Provide personal protective equipment and instruction and monitoring of its proper use.
- Immediately report any hazardous spills, suspected exposures, or other incidents or adverse events to the Office of Prospective Health/Biological Safety.
- Ensure that the workplace is maintained in a clean and sanitary condition.
- Monitor that Biological Safety Cabinets are recertified annually or after being moved, and are maintained properly.
- Report any significant problems or any research related accident or illness with recombinant DNA organisms to the Biological Safety Officer(s) and NIH/OBA within 30 days.

Individual/Laboratory Worker

The individual worker must be familiar with good laboratory practice and laboratory specific procedures performed, the potential hazards associated with the agent, and the requisite safety procedures.

Responsibilities of the Individual:

- Limit exposure to the biohazardous agent to the greatest possible extent.
- Assist the Principal Investigator to maintain up to date postings and labeling of laboratory, materials, and equipment.
- Follow good laboratory practices at all times.
- Properly dispose of biohazardous wastes and maintain accurate disposal records.
- Report all hazardous spills, suspected exposures, and any other biohazard-related accidents to the Principle Investigator and/or Office of Prospective Health/Biological Safety immediately.
- Know the emergency and spill procedures and decontamination process for this lab and be prepared to implement.
- Comply with the laboratory and Biosafety policy, and procedures; perform assigned tasks safely; operate, maintain, and decontaminate equipment and use appropriate PPE following prescribed practices
- Self identify to Prospective Health if personal health conditions develop that may predispose to infection or complications due to occupational exposure (these include pregnancy, immune-suppression or cancer treatment). At Prospective Health risk

assessment will be performed by a health professional and possible additional or alternative protective measures recommended.

- Responsibility for managing biological risk is shared among the Principle investigator, Lab Manager, lab personnel and Department Chair.

Chapter 2

General Information: Hazard Levels & Procedures

Biological Risk Assessment

A risk assessment is performed by Biological Safety to determine the containment required for a laboratory research or teaching activity. The hazard characteristics of the procedures performed combined with the agent used determine the level of Biosafety containment required. Use of laboratory animals introduces additional opportunities for agent dissemination and containment considerations.

NOTE: *The Biosafety containment level will be, **at minimum**, the level of the agent itself. However, the nature of the procedures performed e.g. aerosol generation or other potential for dissemination into the environment may result in a higher Biosafety level, above that inherent to the organism agent alone.*

Risk assessment and risk management process:

1. Identify agent hazards:
 - Infectivity
 - Host susceptibility
 - Severity of resultant disease on individual or ecosystem
 - Availability of preventative treatment measures
 - Route of transmission
2. Identify laboratory hazards:
 - Agent concentration
 - Suspension volume
 - Equipment and procedures generating large droplets or small aerosol particles
 - Use of sharps
 - Use of animals
 - Complexity of procedures or manipulations, transport of biological agents within or between laboratories.
3. Determine Biosafety level and additional precautions indicated. When materials of unknown hazard characteristics are used, BL-2 practices are recommended.
4. Evaluate staff proficiency regarding safe practice and the integrity of safety equipment and containment facilities and the Principal Investigator's training processes.
5. Review the risk assessment with chair or members of the Institutional Biosafety Committee, or other subject matter experts if needed.
6. Conduct new risk assessment if:
 - New construction/modification to laboratory, physical plant, equipment or its operation.
 - Altered staffing arrangements, including contractors or visitors.
 - Change of work pattern, work flow or volume.
 - Significant alteration to SOP's or work practices (e.g. waste management, or PPE).
 - When actual or potential non-conformance is identified; e.g. new legislation or major accident or exposure.
 - New organism or agent is introduced

B. Biological Safety Levels (BMBL, 2007)

Four Biological safety risk levels for infectious microorganisms or biohazardous agents and laboratory animals have been established (See Tables 1&3). Good microbiological techniques and standard microbiological practices are common to all labs, beginning at Biosafety Level 1, upward.

Table 1- BMBL Risk Groups (2007)

**TABLE 1
CLASSIFICATION OF INFECTIOUS MICROORGANISMS BY RISK GROUP**

RISK GROUP CLASSIFICATION	NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES 2002²	WORLD HEALTH ORGANIZATION LABORATORY BIOSAFETY MANUAL 3RD EDITION 2004¹
Risk Group 1	Agents that are not associated with disease in healthy adult humans.	(No or low individual and community risk) A microorganism that is unlikely to cause human or animal disease.
Risk Group 2	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.	(Moderate individual risk; low community risk) A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.
Risk Group 3	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).	(High individual risk; low community risk) A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
Risk Group 4	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).	(High individual and community risk) A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available. ³

Good Microbiological Techniques include proper performance of:

- Culture, purification and storage techniques
- Use of biological safety cabinets
- Pipetting without generating aerosols
- Centrifugation avoiding spillage or aerosols
- Vacuum pump use and protecting vacuum lines.
- Sonication and other mechanical forms of cell/tissue disruption

- Use of disinfectant, spill control, routine decontamination, hand washing; emergency showering and clothing changes if needed
- Animal handling using safety devices for procedures

Standard Microbiologic Practices are used for all work with biological agents, including BSL-1:

- Wash hands after working with potentially hazardous materials and before leaving the laboratory. Personnel will wash their hands after all procedures involving animals or potentially contaminated materials and after glove removal.
- Gloves are worn to protect hands from hazardous materials. Gloves are removed and hands are washed when work is complete and before leaving the laboratory, or if glove integrity is compromised. **Do not wear gloves used for work in lab outside of the lab. Do not wear gloves outside the lab.** Materials to be transferred are placed in to a closed secondary container so that glove use outside the lab is not needed.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, or storing food for human consumption in the laboratory is prohibited.
- Mouth pipetting is prohibited: mechanical pipetting devices are used.
- All sharps such as needles, scalpels, pipettes and broken glass are handled carefully and disposed into puncture-resistant labeled containers. **Safety sharps are used routinely;** these include retractable needle/syringe systems or needle cover mechanism etc. Needles are not bent, sheared, broken recapped or removed from syringe or vacutainer holder before disposal; they are disposed of in puncture-resistant labeled containers. Used pipettes, pipette tips are carefully placed into puncture-resistant sharps containers for disposal. Glass is avoided and plastic materials are used when possible to avoid breakage.
- All procedures are performed to minimize creation of splashes or aerosols. All technical procedures will be performed in a manner that minimizes the creation of aerosols. All biological specimens should remain covered, capped, corked, or plugged at all times except at the time of collection, separation, pouring, or analysis. If there is potential for production of aerosols, the work must be conducted in a biological safety cabinet. Safety glasses, face shields, or other protective devices will be used to protect the eyes and face from splashes or impacting during mixing, vortexing, or decanting or other procedures which may create splashes.
- Hypodermic needles and syringes may be used for parenteral injection and aspiration of fluids from laboratory animals or bottles sealed with a diaphragm. Hypodermic needles and syringes will not be used as a substitute for automatic pipetting devices. **Safety needles/sharps must be used** unless incompatible with the planned procedures. If safety devices are not selected, the reason must be justified on the safety plan. (A current list of safety sharps is posted on the BSOM medical storeroom catalog site; Appendix I provides a partial list).
- Work surfaces are decontaminated **after completion of daily work** and after any spill or splash using appropriate disinfectant. Work surfaces which may have contacted potentially hazardous material should be decontaminated with an EPA approved disinfectant (See chart) at the beginning and end of the day and after any spill of potentially infectious material.
- Chairs are covered in a non-porous material to facilitate decontamination. Employees will not use work surfaces as seats.
- All cultures, stocks, and other potentially infectious materials are decontaminated before disposal using an effective method. Materials to be decontaminated outside of the

immediate laboratory are placed in a durable and leak-proof container and secured for transport.

- ***A sign incorporating the universal biohazard symbol is posted at the entrance to the laboratory when infectious agents are present.*** The sign includes the name of the agent, hazard level, and name and phone number(s) (day and evening) of the laboratory supervisor or other responsible personnel.
- Protective laboratory coats, gowns or uniforms or disposable gowns are worn by all laboratory employees/students working with potentially infectious or hazardous materials to prevent contamination of personal clothing. Laboratory clothing should not be worn in non-laboratory areas and should remain in the laboratory or change room at the end of the day. Laboratory clothing will be laundered by a contractor informed of potential contamination and experienced in safe handling of contaminated clothing.
- Employees will keep their hair at an appropriate length, covered, or tied in such a manner so that it does not become contaminated.
- Reusable instruments are washed and then autoclaved or disinfected
- All liquid or solid materials containing potentially infectious or hazardous material will be decontaminated before disposal (See Hazardous Waste Flow Chart and Autoclave Use Guidelines). Contaminated materials that are to be autoclaved will be placed in durable, leak proof containers with the outer container bearing the biohazard symbol. The container will be closed and loosely sealed before being removed from the laboratory for autoclave to prevent "popping". If the waste will not be autoclaved the bag can be securely closed.
- Spilled materials are wiped up to remove organic load before disinfection is performed.
- The laboratory supervisor must enforce institutional policy controlling access to the lab when agent is in use. Only persons who have been advised of potential hazards and who meet specific entry requirements (e.g., training, occupational medical clearance for animal use, immunization) will be allowed to enter the laboratory working area. Children are not permitted in laboratory work areas. Laboratory doors are kept closed when work is in progress. Access to animal facilities is restricted to authorized personnel.

Biosafety Level 2 (BSL-2) Practices

This includes all of the Standard Microbiological Practices (BL1) plus.

- Post the Biohazard symbol with Biosafety level at the laboratory entrance when infectious agents are present and in use.
- All persons entering the lab must be advised of the potential hazards and meet specific entry requirements.
- Post procedures for entering and exiting the lab at the entrance; for example, "Do not enter work in progress" or "Knock before entering". Laboratory supervisor will enforce the lab entry policy
- Prepare a laboratory specific Biosafety manual which is adopted as policy and maintained and accessible in the lab.
- Laboratory director ensures that the personnel "demonstrate proficiency" in safe work practices before working at this level, and evaluate or update annually.
- A spill control plan is available in the lab, including the basis for the disinfecting agent selected, and how it is to be used, and specify the duration of its contact time.
- Staff are trained and equipped to safely decontaminate spills of biohazard materials.
- Animals and plants are not permitted in the laboratory unless associated with the work being done.
- Laboratory doors should be self-closing and lockable doors are kept closed during active work. Access is restricted when work is being conducted. Laboratories should have a

handwashing sink near the exit door. Laboratories should be designed for easy cleaning and decontamination.

- Vacuum lines are protected with HEPA filters or equipment.
- Procedures creating infectious aerosols or splashes e.g. centrifugation, sonication are conducted in a Biosafety Cabinet or other physical containment. The user will verify inward airflow of the hood or biological safety cabinet before initiating work and work within the operationally effective zone of the hood or biological safety cabinet.
- Provisions to assure proper cabinet and air system performance must be verified.
- All cultures, stocks, and other potentially infectious/hazardous materials are decontaminated before disposal using an effective method. Materials to be decontaminated outside of the immediate laboratory are placed in a durable and leak-proof container and secured for transport.
- The exterior surface of the container will be wiped if contamination of the container is possible or the container will be placed in a secondary container or biohazard bag before leaving the lab.

Biosafety Level 3 (BSL-3) includes BSL-1 & 2 requirements plus:

- Laboratory is designed to contain airborne agents. Lab has anteroom at entry and is maintained under single-pass, direct exhaust ventilation under negative pressure vs. hallway. Penetrations are sealed to minimize air leakage and room functions to current standards, specifying-pressure differential, air-changes per hour, etc.
- Laboratories are designed so airflow will not be reversed under HVAC failure. A visual monitoring device confirming directional airflow is provided at laboratory entry.
- Decontamination of the laboratory is required after gross contamination of the space, significant change in laboratory use and before major renovations or maintenance shutdowns.
- If HEPA filtered exhaust is used, the HEPA filter housing will have gas-tight isolator dampers, decontamination parts and/or hazard/bag-out capabilities. The HEPA filter housing should allow for leak testing of each filter and assembly and both should be certified at least annually.

Table 2 Biosafety Containment Levels: Practices and Safety Equipment and Facilities

TABLE 2
SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FOR INFECTIOUS AGENTS

BSL	AGENTS	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to consistently cause diseases in healthy adults	Standard Microbiological Practices	None required	Open bench and sink required
2	<ul style="list-style-type: none"> Agents associated with human disease Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure 	BSL-1 practice plus: <ul style="list-style-type: none"> Limited access Biohazard warning signs "Sharps" precautions Biosafety manual defining any needed waste decontamination or medical surveillance policies 	Primary barriers: <ul style="list-style-type: none"> Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials PPEs: <ul style="list-style-type: none"> Laboratory coats; gloves; face protection as needed 	BSL-1 plus: <ul style="list-style-type: none"> Autoclave available
3	<ul style="list-style-type: none"> Indigenous or exotic agents with potential for aerosol transmission Disease may have serious or lethal consequences 	BSL-2 practice plus: <ul style="list-style-type: none"> Controlled access Decontamination of all waste Decontamination of laboratory clothing before laundering Baseline serum 	Primary barriers: <ul style="list-style-type: none"> Class I or II BSCs or other physical containment devices used for all open manipulation of agents PPEs: <ul style="list-style-type: none"> Protective laboratory clothing; gloves; respiratory protection as needed 	BSL-2 plus: <ul style="list-style-type: none"> Physical separation from access corridors Self-closing, double-door access Exhaust air not recirculated Negative airflow into laboratory
4	<ul style="list-style-type: none"> 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: <ul style="list-style-type: none"> Clothing change before entering Shower on exit All material decontaminated on exit from facility 	Primary barriers: <ul style="list-style-type: none"> 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: <ul style="list-style-type: none"> Separate building or isolated zone Dedicated supply and exhaust, vacuum, and decontamination systems Other requirements outlined in the text

* PPE – Personal Protective Equipment

As the agent biohazard risk level increases or if planned manipulations will generate aerosols or foster environmental spread, additional containment procedures are indicated (Table 2). Consult the full text of BMBL, 2007 for more information.

Biosafety Cabinets (preferably class II) must be used when:

- Procedures with a potential for creating infectious aerosols or splashes are conducted; e.g. pipetting centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissue from animal eggs.
- High concentration or large volumes of infectious agents are used.

Exception: Biohazardous material may be centrifuged in the open lab IF a centrifuge with sealed rotor heads and/or sealed centrifuge safety cups or other special engineering and design features to prevent microorganism dissemination (i.e. total containment) are used (See Centrifuge Use).

Laboratory Animal Containment Levels

Use of laboratory animals increases the possibility of agent dissemination. If animals are exposed, agent may be exhaled, excreted, settled on animal fur and skin or be transmitted to progeny. Handling animals for agent administration or bedding changes increases the possibility of environmental contamination. Special containment housing e.g. filter-top cages may be used to minimize the spread of biohazardous agents by exposed lab animals (See Table 3). Animal containment systems include use of microisolator cages, filter top cages equipped with HEPA filters, or dedicated cage rack ventilation systems. NOTE: Some commonly used cage top paper filters with 5 micron pores are not equivalent to HEPA filters (which have 95% filter efficiency for particles of 0.3 micron in diameter). Special facilities may be needed for research with livestock or arthropods. Consult BMBL 2007 for details.

Table 3

SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FOR ACTIVITIES IN WHICH EXPERIMENTALLY OR NATURALLY INFECTED VERTBRATE ANIMALS ARE USED.

ABSL	AGENTS	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to consistently cause diseases in healthy adults	Standard animal care and management practices, including appropriate medical surveillance programs	As required for normal care of each species	Standard animal facility: <ul style="list-style-type: none"> No recirculation of exhaust air Directional air flow recommended Hand washing sink is available
2	<ul style="list-style-type: none"> Associated with human disease Hazard: percutaneous exposure, ingestion, mucous membrane exposure 	ABSL-1 practice plus: <ul style="list-style-type: none"> Limited access Biohazard warning signs "Sharps" precautions Biosafety manual Decontamination of all infectious wastes and of animal cages prior to washing 	ABSL-1 equipment plus primary barriers: <ul style="list-style-type: none"> Containment equipment appropriate for animal species PPEs: <ul style="list-style-type: none"> Laboratory coats, gloves, face and respiratory protection as needed 	ABSL-1 plus: <ul style="list-style-type: none"> Autoclave available Hand washing sink available Mechanical cage washer recommended
3	<ul style="list-style-type: none"> Indigenous or exotic agents with potential for aerosol transmission Disease may have serious health effects 	ABSL-2 practice plus: <ul style="list-style-type: none"> Controlled access Decontamination of clothing before laundering Cages decontaminated before bedding removed Disinfectant foot bath as needed 	ABSL-2 equipment plus: <ul style="list-style-type: none"> Containment equipment for housing animals and cage dumping activities Class I, II or III BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols. PPE: <ul style="list-style-type: none"> Appropriate respiratory protection 	ABSL-2 facility plus: <ul style="list-style-type: none"> Physical separation from access corridors Self-closing, double-door access Sealed penetrations Sealed windows Autoclave available in facility
4	<ul style="list-style-type: none"> Dangerous/exotic agents that pose high risk of life threatening disease Aerosol transmission, or related agents with unknown risk of transmission 	ABSL-3 practice plus: <ul style="list-style-type: none"> Entrance through change room where personal clothing is removed and laboratory clothing is put on; shower on exiting All wastes are decontaminated before removal from the facility 	ABSL-3 equipment plus: <ul style="list-style-type: none"> Maximum containment equipment (i.e., Class III BSC or partial containment equipment in combination with full body, air-supplied positive-pressure personnel suit) used for all procedures and activities 	ABSL-3 facility plus: <ul style="list-style-type: none"> Separate building or isolated zone Dedicated supply and exhaust, vacuum and decontamination systems Other requirements outlined in the text

* PPE – Personal Protective Equipment

Eye, face and respiratory protection are to be used in rooms containing infected animals at ABSL-2 or higher. Eye and face protection must be disposed of with other contaminated waste or decontaminated before reuse. Respiratory protection may consist of surgical masks for many ABSL-2 agents. N-95 masks are used for agents causing infection or toxicity after aerosolization and inhalation. Specific masks used will be specified in the Biosafety Registration. Use of non-human primates or their tissues are handled at ABL-2 and BL-2 respectively.

C. Recombinant DNA Work Review and Requirements

Recombinant DNA work is defined as: (1) Molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell or the DNA or RNA molecules that result from the replication of these molecules or (2) organisms and viruses containing recombinant DNA.

The National Institute of Health (NIH) Guidelines must be followed at ECU in order for ECU to remain eligible for any NIH grant funding. All recombinant work must follow NIH rules whether it is funded by the NIH or not.

The ECU Biological Safety Committee functions as the Institutional **Biosafety Committee** as defined by the NIH Guidelines. The committee represents the University in ensuring compliance with relevant regulations and this policy. There are six NIH categories of experiments involving recombinant DNA. Three categories require review at the federal level, followed by Biological

Safety Committee review. These are: 1) Experiments that compromise the control of disease agents in medicine or agriculture through deliberate transfer of drug resistant trait into microorganisms not known to acquire the trait naturally (NIH category III-A-1-a), 2) cloning of DNA encoding molecules toxic to vertebrates that have an LD50 < 100 ng/kg body weight (NIH category III-B-1), 3) experiments involving the deliberate transfer of recombinant DNA into one or more human research participants (III-C-1). Two require only local committee review; the sixth is exempt but at ECU requires review of written material be reviewed by a Biological Safety representative to verify exemption. **No work with recombinant DNA at ECU will begin before Biological Safety has reviewed the registration or AUP, verified NIH category and assigned a containment level.**

The Biological Safety Committee will:

1. Review all recombinant DNA research for compliance with the NIH Guidelines as specified and approve projects found to be in conformity; independently assess containment levels facilities, procedures, practices, personnel training, and personnel expertise. Receive notice of NIH Section III-E work and signed and dated registration. Verify "exempt" status per the NIH guidelines when indicated or receive reports of "exempt" determination made by Biological Safety as a meeting agenda item.
2. Ensure that projects requiring federal review are forwarded to NIH/OBA or RAC as required per Section III, of the Guidelines.
3. Set containment levels for specified experiments including those involving whole animals or plants, or large scale work.
4. Review non-exempt recombinant DNA research being conducted to ensure fulfillment of the requirements of the Guidelines via annual inspection reports from Biological Safety Officer.
5. Adopt emergency plans covering accidental spills and personnel contamination; maintain copies of these plans for ready access in the event of an accident.
6. Receive reports of significant problems from the PI via the Biological Safety Officer immediately. Investigate all incidents, and jointly with the Principal Investigator, report appropriate information to the NIH Office of Recombinant DNA Activity within 30 days.
The Biological Safety Committee may not authorize initiation of recombinant DNA experiments not explicitly covered by the Guidelines unless authorized to do so by the NIH. The committee may require testing for replication competence if viral vectors are used in gene transfer to humans.

The **Biological Safety Officer(s)** shall be a member(s) of the Biological Safety Committee; duties in regard to work with recombinant DNA molecules will include:

1. Ensure that laboratory standards are rigorously followed by periodic inspection.
2. Report significant problems, violations of the guidelines, significant research related accidents and illnesses to the Chairperson of the Biological Safety Committee. The Committee Chair will investigate all incidents and violations with results reported to the Vice Chancellor for Health Sciences; if appropriate, the details will be reported to the NIH Office of Recombinant DNA Activity.
3. Develop emergency plans for dealing with accidental spills, personnel contamination and investigate laboratory accidents with assistance and consultation of members of the Biological Safety Committee.
4. Provide advice on laboratory security and research safety.
5. Schedule and organize the meetings of the Biological Safety Committee

On behalf of the institution, **the Principal Investigator** is responsible for complying fully with the Guidelines in conducting recombinant DNA research. The Principal Investigator will be knowledgeable of the current NIH Guidelines and regarding containment and safety, and will:

1. Not initiate or modify recombinant DNA research requiring Institutional Biological Safety Committee approval, until that research or proposed modification has been approved by the Committee.
2. Make the initial determination of the required levels of containment in accordance with the Guidelines. Select microbiological practices and laboratory techniques to be used. Request consultation from Biological Safety, if needed to assist with project safety and occupational health plans.
3. Submit the initial research proposal registration to the Institutional Biological Safety Committee for review and approval/disapproval. The Committee will verify whether the project is covered under NIH Section IIIA, IIIB, IIIC, or not.
4. Remain in communication with the Biological Safety Committee throughout the conduct of the project. Report significant protocol changes or adverse events to Biological Safety.
5. Before project initiation, provide the laboratory staff copies of the safety protocols that describe potential hazards and routine precautions to be taken and in the event of an accident. Ensure that a copy of the safety protocol is maintained in the laboratory.
6. Instruct and train the staff in practices and techniques to ensure safety and deal with accidents; document the training by maintaining a record of date, time, attendees, and discussion content.
7. Inform the staff of any precautionary medical practices (e.g. vaccinations, serum collection) advised or requested:
 - a. Employees and students will be informed of any symptoms of illness caused by the agent(s) under investigation by the PI or lab supervisor. A laboratory worker who develops illness that could be of laboratory origin will inform his/her supervisor and report to Prospective Health.
 - b. A periodic health history will be reviewed for each employee exposed to biohazard agents at risk level 3 or higher, or using laboratory animals. A baseline serum sample may be collected and frozen if indicated by the Biosafety level/protocol.
 - c. All employees who will work with or are exposed to infectious microorganisms will be immunized against those agents if a vaccine is available.
8. Report significant problems or violations of the Guidelines, or any research-related accidents or illnesses to the chairman of Biological Safety Committee or Biological Safety Officer immediately. The Chairman or Biological Safety Officer will investigate the problem, and with the Principal Investigator, will report appropriate details to the NIH Office of Recombinant DNA Activity within 30 days.
9. Report new information bearing on laboratory compliance with the Guidelines to the Institutional Biological Safety Committee and NIH.
10. Be adequately trained in proper microbiological techniques. (If inexperienced in safe methods of conducting recombinant DNA research, the IBC may advise or require individual or group training by the department chair or other mechanism.)
11. Comply with shipping requirements for recombinant DNA molecules.
12. Adhere to Institutional Biological Safety Committee-approved emergency plans in the event of accidental spills and/or personal contamination.
13. Submit information to NIH if required; obtain Institutional Biological Safety Committee concurrence if direct communication to NIH is required.

See Appendix E for more on the NIH Guidelines and Appendix F for more information on human gene transfer.

D. Transgenic Organisms: Animals and Plants

All research at ECU involving transgenic organisms must be reviewed by Biological Safety prior to the start of any work. Although some projects will qualify as exempt under the NIH Guidelines, all projects that involve transgenic organisms must be reviewed. Frequently Biological Safety review of the Animal Use Protocol submitted to IACUC will allow a determination of NIH "Exempt". If the AUP information is not sufficient, completion of a Biosafety registration for IBC review will be requested.

The creation of transgenic rodents falls under one of two sections of the NIH Guidelines depending on the containment level required to house the rodents:

The creation of transgenic rodents that can be housed under Biosafety Level 1 conditions are covered under ***NIH Section III-E-3***.

The generation of transgenic rodents requiring BL2, BL3 and BL4 containment are covered under ***NIH Section III-D-4***.

The breeding of a transgenic animal with a non transgenic strain potentially results in a new transgenic animal being created and thus the activity is subject to the same requirements as described above.

E. Bio aerosols: Creation and Control

Bioaerosols consist of airborne particles generated from microorganisms (viruses, bacteria, molds, protozoa), originating from living organisms (biotoxins) or their fragments, e.g. toxins, dead microorganisms, spores, allergens (ACGIH, 1999). Particles of concern typically are 5µm or less in diameter. Due to their small size, aerosols do not settle and can remain airborne for long periods of time. If inhaled, bioaerosols can be carried to the alveoli and present a significant risk for laboratory-acquired infection or systemic reaction.

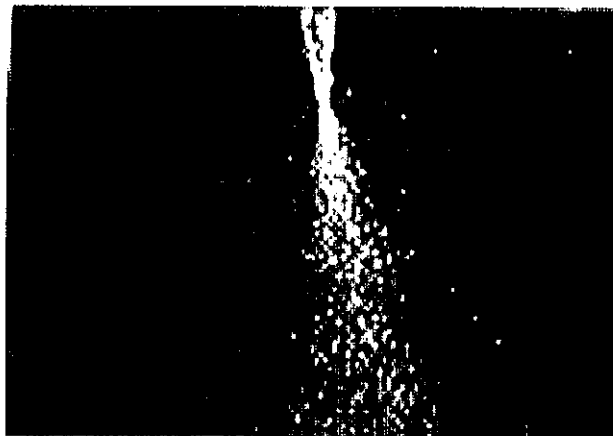
Aerosol from syringe removed from viral diaphragm



Aerosols can be generated by many common laboratory techniques. Almost any handling of liquids or of dry powders may generate aerosols or larger droplets. Examples of procedures generating aerosols include:

- High speed blending
- Mixing
- Agitation
- Pipetting
- Shaking
- Grinding
- Filtering
- Sonicating
- Flaming
- Opening vials of lyophilized cultures
- Centrifuging
- Opening containers of infectious materials or Petri dishes
- Inoculating animals intranasally
- Harvesting infected tissues from animals or eggs

To minimize aerosol production, **pipettes** should be drained gently with the tip against the inner wall of the receiving tube or vessel. Infectious material is not expelled forcibly from pipettes. Air is never bubbled through a suspension of infectious agents in an open container. (See below pipette aerosol)



Improper technique in flaming **inoculating loops** can result in spatter and release of droplets or aerosols. This can be prevented by heating the shaft until the sample has been heat-dried before “flaming” the loop itself using a die-arm burner or electric microincinerator. The process of flaming can be avoided by using sterile, disposable loops. **Bunsen burners are not used in Biological Safety Cabinets.**

A properly maintained biological safety cabinet (BSC) (preferably class II) and other appropriate personal protective equipment or other physical containment devices **must** be used for procedures that can create aerosols or when concentrated or high volumes of the agent are used.

Biohazardous material may be centrifuged in the open laboratory if sealed rotorheads or capped trunnion cups are used and the rotor or capped cups are opened under the BSC. Centrifuges and safety trunnion cups should be visually inspected on a regular basis to ensure that leakage does not occur during operation. When an ultracentrifuge is used, HEPA filters are installed between the chamber and the vacuum pump of the ultracentrifuge.

Some centrifuges and other laboratory instruments are specifically designed for use in a biological safety cabinet. Only a biological safety cabinet intended for this purpose should be used, as the air curtain in the Biosafety Cabinet may be compromised by use of the centrifuge, and the expected containment may not be achieved.

See Appendix K for more information on aerosol generation by opening culture plate, tubes, ampoules, Appendix L about blenders and mixers, and Appendix J about pipetting.

F. Toxins

All toxins are presumed to pose a hazard in an aerosol form, although most toxins exert their effects only after parenteral exposure or ingestion. A few toxins present a dermal hazard. In general, toxins of biological origin are not intrinsically volatile. The laboratory safety precautions appropriate for handling these materials closely parallel those for handling infectious organisms. Lyophilized materials are especially susceptible to aerosolization. Whether hazardous chemicals or toxins are handled as biological or chemical agents can be an arbitrary distinction; agents which have the characteristics of particles are best handled as a biological hazard. Agents used in solution or vapor form may be more suited to handling as chemical agents.

Venomous or poisonous animals are found in all animal classes with the exception of birds. Venomous animals produce a poison in a highly developed secretory gland or group of cells and deliver the toxin by biting or stinging. Poisonous animals or plants have toxic tissues and poisoning results from ingestion, a hazard that should be minimized in the laboratory setting or by skin contact.

Some sources of animal toxins:

Table 4

Reptiles	Cnidaria (Coelenterates)
Snakes	Echinodermata
Lizards	Mollusca
Amphibians	Fishes
Frogs	Arthropods
Toads	Spiders
Marine Animals	Scorpions
Marine toxins	Centipedes and millipedes
Protista	Hymenoptera (ants, bees, wasps, hornets)
Porifera (Sponges)	Tick and mite

Phytotoxins are common and formed the basis of early pharmacology. Toxins may be alkaloids, glycosides, phenols, resins oxalates, or peptides. Fungi are a source of microbial biotoxins known as mycotoxins. Algal toxins are produced by a variety of microorganisms including true algae, blue-green algae, and dinoflagellates. Mold particles may induce allergic and inflammatory reactions.

Several biotoxins of both plant and animal origin are regulated as Select Agents if the investigator possesses more than a specified quantity. (See Select Agents, Appendix A)

Standard Practices for work with toxins

Standard laboratory practices listed under on pages 13-14 should be incorporated into all protocols for work with toxins. Special practices for BSL 2 and BSL 3 should be reviewed and incorporated as appropriate into protocols for work with toxins when aerosolization is possible. Each laboratory will develop a biological safety plan specific to the toxin(s) used. The plan will A) identify the hazards that will be encountered in normal use of the toxin, or could be encountered in case of a spill or other accident, and B) specify the policies and practices to be used to minimize risks (e.g., containment and personal protective equipment, management of spills, management of accidental exposures, medical surveillance). A Chemical Hazard Plan is required in addition to a Biosafety plan. Training specific to the toxin(s) is required and documented for all laboratory personnel; training is provided before starting work with the toxin and at yearly intervals thereafter.

Training

This includes the theory and practice re handling the toxin and practical aspects such as liquid and solid transfer, waste disposal, decontamination, and spill management. Workers must be proficient with required manipulation before being provided with the toxin. When biotoxins are in use, the room is posted with a sign with the Biohazard Symbol indicating "Toxins in Use Authorized Personnel Only." Contact information, and any special entry requirements (PPE used) are posted on the entrance(s) to the room. Only personnel whose presence is required should be permitted in the room while toxins are in use.

Storage

Toxins should be stored in locked storage rooms, cabinets, or freezers when not in use. Access to areas containing toxins is restricted to those whose work assignments require access. An inventory control system should be in place. Preparation of primary containers of toxin stock solutions and manipulations of primary containers of dry forms of toxins will be conducted in a chemical fume hood, a glove box, or a biological safety cabinet or equivalent containment system. HEPA and/or charcoal filtration of the exhaust air may be required, depending on the toxin characteristics and particle generation. The user will verify inward airflow of the hood or biological safety cabinet before initiating work and work within the operationally effective zone of the hood or biological safety cabinet. Before containers are removed from the hood, cabinet, or glove box, the exterior of the closed primary container will be decontaminated and placed in a clean secondary container. Toxins will be transported only in leak/spill-proof secondary containers. The interior of the hood, glove box, or cabinet will be decontaminated periodically, for example, at the end of a series of related experiments or daily. Any "high hazard" operation will 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. Intentional generation of aerosols is extremely hazardous, and should only be conducted after validation of equipment and personnel using non-toxic simulation. (These will be specified in the Biosafety Registration)

Contaminated and potentially contaminated protective clothing and equipment will be decontaminated using methods known to be effective against the toxin or by autoclaving before removal from the laboratory for disposal, cleaning or repair (Table 5A and 5B). If decontamination is not possible/practical, contaminated materials (e.g., used gloves) should be contained and disposed of as contaminated/toxic waste. Materials contaminated with infectious agents as well as toxins should also be autoclaved, decontaminated or otherwise rendered non-hazardous before leaving the laboratory.

Table 5A

Complete Inactivation of Different Toxins With A 30 Minute Exposure Time to Varying Concentrations of Sodium Hypochlorite (NaOCl) + Sodium Hydroxide (NaOH)				
Toxin	2.5% NaOCl + 0.25 N NaOH	2.5% NaOCl	1.0% NaOCl	0.1% NaOCl
T-2 Mycotoxin	Yes	No	No	No
Brevetoxin	Yes	Yes	No	No
Microcystin	Yes	Yes	Yes	No
Tetrodotoxin	Yes	Yes	Yes	No
Saxitoxin	Yes	Yes	Yes	Yes
Palytoxin	Yes	Yes	Yes	Yes
Ricin	Yes	Yes	Yes	Yes
Botulinum Toxin	Yes	Yes	Yes	Yes
Staphylococcal Enterotoxin B	Yes (?)	Yes (?)	Yes (?)	Yes (?)
Wannemacher, R.W. 1989. Procedures for Inactivation and Safety Containment of Toxins. In: Proceedings of the Symposium on Agents of Biological Origin, U.S. Army Research, Development and Engineering Center, Aberdeen Proving Ground, MD, pp. 115-122.				

Table 5B

Complete Inactivation of Different Toxins By Autoclaving or 10 Minute Exposure to Varying Temperatures of Dry Heat					
Toxin	Autoclaving	Dry Heat (°F)			
		200	500	1000	1500
T-2 Mycotoxin	No	No	No	No	Yes
Brevetoxin	No	No	No	No	Yes
Microcystin	No	No	Yes	Yes	Yes
Tetrodotoxin	No	No	Yes	Yes	Yes
Saxitoxin	No	No	Yes	Yes	Yes
Palytoxin	No	No	Yes	Yes	Yes
Ricin	Yes	No	Yes	Yes	Yes
Botulinum Toxin	Yes	Yes	Yes	Yes	Yes
Staphylococcal Enterotoxin B	Yes (?)	Yes (?)	Yes (?)	Yes (?)	Yes (?)
Wannemacher, R.W. 1989. Procedures for Inactivation and Safety Containment of Toxins. In: Proceedings of the Symposium on Agents of Biological Origin, U.S. Army Research, Development and Engineering Center, Aberdeen Proving Ground, MD, pp. 115-122.					

Safety Equipment

The safety equipment guidelines for BSL 2 or BSL 3 should be incorporated as appropriate into protocols for work with toxins.

When using an open-fronted fume hood or biological safety cabinet, protective clothing, including gloves and a disposable long-sleeved body covering (gown, laboratory coat, smock, coverall, or similar garment) will be worn so that hands and arms are completely covered. Eye protection will be worn if an open-fronted containment system is used.

Other protective equipment may be required, depending on the characteristics of the toxin and the containment system. Use additional respiratory protection if aerosols may be generated and it is not possible to use containment equipment or other engineering controls. When handling toxins, select gloves that are known to be impervious to the toxin, and resistant to its diluents. Nitrile gloves generally provide better solvent/chemical resistance than latex. When handling dry forms of toxins that are electrostatic, do not wear gloves (such as latex) that help to generate static electricity. If infectious agents and toxins are used together in an experimental system, consider both hazards and select protective clothing and equipment that protects for both.

Laboratory Facilities

Laboratory facility recommendations for BSL 2 and BSL 3 are incorporated as appropriate into protocols for work with toxins.

Vacuum lines: When vacuum lines are used with systems containing toxins, they 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.

Occupational Health: Prospective Health will provide preventive measures as available. Prospective Health will be notified if exposure to or injury with contaminated materials occurs. Post-exposure prophylaxis will be provided if available or referral made to the Emergency Department for treatment.

LD50's or references must be provided to Biosafety or included in the IBC registration for all toxins and toxin alternatives. If an inactive form such as a toxoid, or fraction that is thought to be non-toxic is used (based on provided LD50's or references); then normal biosafety precautions can be followed in accordance with the lab's biosafety level.

Guidelines for Toxin Use in Animals

- 1.) Lab specific SOPs, including SDS sheets, should be sent to Biosafety or attached to IBC registration and AUP. Deconning, concentrations stocked or amount on hand, and concentrations used during experiments.
- 2.) Toxin is kept locked up.
- 3.) PPE required to work with toxin: lab coat or disposable gown, nitrile gloves, and eye protection.
- 4.) Toxin is resuspended in a chemical fume hood or biosafety cabinet (BSC).
- 5.) Toxin is transported in secondary containment between locations. This can be something as simple as a 50ml screw-cap conical.
- 6.) A buddy system should be in place, so nobody works with toxin alone.
- 7.) Work will take place in a designated room in a designated BSC.
- 8.) During experiments that use toxin a "DO NOT ENTER" sign should be hung on the door to the designated room.
- 9.) Before injecting animals with toxin, they should be restrained properly or anesthetized.
- 10.) Wipe the injection site clean after toxin injections.

- 11.) Date and toxin concentration should be written on procedure cards for 7 days post-injection.
- 12.) Appendix 1 of AUP should be filled out and posted for animal care staff.
- 13.) Work space and BSC should be wiped down with appropriate deconning agent after work with toxin is completed.
- 14.) Contact DCM staff regarding cage changing within the first 7 days post-infection.
- 15.) All waste potentially contaminated with toxin, including animal waste, should be autoclaved if within 7 days post-injection. After 7 days waste can be disposed of following normal protocols.

G. Human Cell and Tissue Culture Systems

Most animal cell cultures harbor viruses, either adventitiously or deliberately. Primary and permanent cell lines from mice, hamsters, and rats should be handled as if they carry low risk infections. Primate cells and tissues may contain hazards such as Herpes B virus latent in neural tissue, simian retrovirus, or if transformed with viral agents, such as SV-40, EBV, or HBV, or carry viral genomic material.

All human cell lines and primate cell lines are potential sources of infection with Biosafety pathogens or other viral agents. Even commercial cell lines are not certified to be pathogen-free of all potential Biosafety pathogens.

Recommended Practices. All Human and other primate cells are handled using a minimum 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 disposal. All personnel working with human cells and tissues will be enrolled in the ECU Bloodborne Pathogens Program.

When occupational exposure to human blood, body fluid or unfixed tissues or tissue culture human blood, body fluid, infected tissue or tissue culture is anticipated.

- a. Employees will be offered the Hepatitis B vaccination series free of charge. The vaccination will be made available after the employee has received the required ECU BBP training and within 10 working days of initial assignment. Exceptions are if the employee has previously received the complete hepatitis B vaccination series, antibody testing reveals that the employee is immune, or the vaccine is contraindicated for medical reasons. Employees who decline to accept the Hepatitis B vaccination must sign a mandatory waiver form.
- b. Training on Biosafety pathogens for employees is provided by the Office of Prospective Health. Initial training is provided twice a month at BSOM New Employee Orientation. Records of Bloodborne Pathogens and Tuberculosis training will be maintained by Office of Prospective Health for individuals listed on a Biosafety Registration using human cell culture, blood or tissue.
- c. Students who will have curricular exposure to human blood, body fluids or unfixed tissue will follow the Hepatitis B immunization policy of their department. Training on blood borne pathogens will be provided by the department, supplemented by education on the project-specific exposures, PPE, and work practices by the

laboratory director or designee (Self-help student workers or paid graduate workers are considered ECU employees).

- d. Tissues collected from other employees, students, patients, or autopsy cases of unknown pathogen status are assumed to be infected and should be handled with standard precautions.

H. Oncogenic Agents

Tumorigenic/oncogenic human cells are potential hazards if self-inoculated. Human isolates from malignant tissues or tissues likely to harbor mammalian oncogenic viruses should be considered as moderate risk agents. Oncogenic viruses of moderate risk are handled at BSL-2 minimum.

I. Specific Viruses/Viral Vectors

Adenovirus

This fact sheet applies to any work with adenovirus, adenoviral vectors, and Adeno-Associated Virus (AAV). Work with these agents requires at minimum of BSL-2 practices, and possibly BL-2 plus or BL-3 depending upon planned manipulation.

Approval

Experiments using *Adenoviral* Vectors require the approval of the IBC before initiation.

BACKGROUND

There are approximately 50 different serotypes of human adenoviruses, inducing a spectrum of illnesses including acute, self-limiting pharyngitis (a common cold), keratoconjunctivitis (pink eye), and diarrhea. In rare cases, human adenovirus may cause hepatitis (inflammation of the liver), or inflammation of other organs. Immunocompromised individuals are at increased risk.

The risk of exposure to recombinant adenoviral vectors is unknown; the potential risk of exposure to different recombinant adenoviral vectors may not be the same. It is believed that some vectors may have minimum risk (e.g., null and LacZ vectors) while other (e.g., interleukin, TNF, or immune effector vectors) may pose a higher risk.

Adenoviral vectors can infect a wide variety of cell types, including nondividing cells such as hepatocytes, and can be grown to high titers. *Adenoviral* vectors have been modified to provide a safer version of the Adenovirus in which the viral gene coding sequences have been deleted. Both replication competent and deficient vectors can cause corneal and conjunctival damage. In addition, the replication-deficient virus may be complemented in vivo thereby causing the vector to become replication competent.

Summary

- Adenovirus is a pathogen of respiratory and gastrointestinal mucous and eye membranes.
- Adenovirus (replication deficient and replication competent) can cause corneal and conjunctival damage. Eye protection (goggles) must be worn when working with this agent/vector.

- Adenovirus (unlike HIV or herpes) is quite stable. After having been extracted with ether and/or chloroform, it can still be infective.
- The replication-defective virus may be complemented in vivo thereby causing the vector to become replication competent.

MODE OF TRANSMISSION

Adenovirus may be transmitted by:

- Droplet
- Aerosol
- Injection

SYMPTOMS OF EXPOSURE

Any of these symptoms may occur following adenovirus exposure:

- Acute respiratory illness (cold like symptoms)
- Pneumonia
- Conjunctival infection (red eye)
- Corneal inflammation leading up to scarification

Personal Protective Equipment

The following personal protective equipment **MUST** be worn when working with Adenoviral vectors:

- Gloves
- Lab coat for tissue culture manipulation
- Wrap around gown for introducing vector into animals or necropsies
- Goggles (not eye shield)
- Face shield
- Respiratory protection (fitted N095 mask or powered air-purifying respirator) for work that is conducted outside of the biological safety cabinet.

LABORATORY PRACTICES

Training

Personnel must have prior experience with adenovirus or must be provided with suitable and sufficient information, instruction and training prior to initiating work. Work with *Adenoviral* vectors should only be carried out by trained personnel and all personnel must be directed by a competent scientist. All staff involved with the handling and administration of adenoviral vectors should receive Biosafety training that covers safety procedures.

Engineering Controls

1. Access to the laboratory must be limited when the agent is in use.
2. Laboratory will be under negative air pressure relative to the hallway (Confirmed during initial lab inspection). Anteroom on lab if possible.
3. No work with Adenovirus is permitted on the open bench.
4. A certified Biosafety cabinet is used for all manipulations including (but not limited to):
 - a. Pipetting
 - b. harvesting infected cells for RNA
 - c. Loading and opening containers
5. All centrifugation must be done in closed containers using sealed rotors.
6. the most effective germicides (with a minimum 15 min. contact time) are:

- a. Phenol (5%)
 - b. Sodium hypochlorite (household bleach diluted to 200 ppm or 10%)
 - c. **NOTE:** Alcohol is not effective against this virus.
7. If experiment parameters make it impossible to work with adenovirus within a Biosafety cabinet, N-95 fit-tested respirators must be worn. Contact the Biological Safety Officer for more information.
8. All cultures, stocks, or materials used to manipulate or otherwise exposed to adenovirus must be autoclaved prior to disposal. Autoclave conditions to be met: 1 hour at 121°F (15 lbs per square inch of steam pressure). The outside of sharps buckets must be decontaminated after removal from the Biosafety Cabinet and before pick-up for incineration.

The following safety equipment **MUST** be used when working with *Adenoviral* vectors:

- Certified Class II Biological Safety Cabinets
- Sealed centrifuge rotors and/or safety cups
- Vacuum lines equipped with an in-line HEPA filter as well as a primary and secondary vacuum flask containing a 10% bleach solution

ANIMAL USE

1. Concurrent approvals are needed from Institutional Biosafety Committee and Institutional animal Care and Use Committee prior to commencing animal work with adenovirus.
2. Animals must be handled and housed in a BSL-2+ area designated and approved for adenoviral work using microisolator or other cage ventilation system during the first 72 hours after infection.
3. Infected animals may excrete (shed) adenovirus, especially the first 72 hours after infection; virus may be shed in feces for up to ten days at lesser titer.
4. Precautions must be taken not to create aerosols when emptying animal waste material, washing cages, or cleaning the room. After three to ten days, the animals may be changed to a clean cage. Bedding changes and cage cleaning will be performed under a hood or changing station by personnel wearing face mask and eye protection.
5. Special training must be given to all animal husbandry personnel on adenovirus. This training must address the hazards associated with the work, required practices and procedures and proper handling of bedding, cage washing, and all other husbandry materials associated with the experiment.
6. All necropsy must be performed in a necropsy room using Animal BSL-2+ precautionary practices and procedures.
7. Only lab personnel or animal husbandry workers trained to handle animals infected with adenovirus should be responsible for animal husbandry practices during the first 72 hours following infection of the animal.

EMPLOYEE EXPOSURE

Eye exposure from splash or aerosol:

Rinse a minimum of 15 minutes in eye wash or flush with water. Notify the Principal Investigator or Laboratory Supervisor, who will immediately contact **Prospective Health at 744-2070** for medical treatment, and to report the incident.

Needlestick, sharps exposure or non-intact skin exposure

Contaminated skin should be scrubbed for approximately 20 minutes using a 10% povidone iodine solution (such as Betadine®) and copious amounts of water. Notify the Principal Investigator or Laboratory Manager, who will immediately contact **Prospective health at 744-2070** for medical treatment, and to report the incident.

Spill Response Procedures

The following steps must be taken when cleaning up a spill:

1. Stop, notify others and isolate the area!
2. Put on appropriate PPE (lab coat, gloves, eye and face protection)
3. Remove glass/lumps with forceps or scoop if applicable and place into a rigid, puncture resistant container
4. Small spills-Place paper towels soaked in bleach directly on the spill and let soak for 20 minutes
5. Wipe up area and discard towels in biohazard waste container.
6. Continue wiping area with paper towels soaked in bleach until the spill area is completely cleaned.
7. Discard all materials and PPE in biohazard waste container
8. Wash hands thoroughly

J. Lentiviral Vectors

I. Background

Lentiviruses are a subclass of retroviruses which are able to infect both proliferating and non-proliferating cells. Lentivirus vector systems are based on HIV-1 or similar viruses. *Lentiviral* vectors have been modified to provide a safer version of the HIV virus in which the viral replication genes have been removed. During infection, there is a possibility that the *lentivirus* may convert to a replication competent state. Major risks are the generation of replication competent lentivirus (RCL) and the potential for oncogenesis.

A. Other Lentivirus Vectors:

Non-human lentivirus vectors include FIV, SIV and EIAV. Generally BSL-1 is appropriate for Risk Group 1 organisms. Replication deficient vectors in which a heterologous envelope (VSV-G) is used for packaging may require BSL-2 due to increased tropism and capability of transducing human cells.

B. RCL Testing:

1. The frequency of RCL using later generation vectors is very low, but may not be zero.
2. NIH allows IBCs to make containment assignments without requiring RCL testing after a risk assessment that considers the nature of the specific vector system being used and overall past experience with the system.
3. Vectors used in human trials or other high concern situations must be tested for RCL.

Second generation systems are most common. These separate packaging and gene transfer functions into three distinct plasmids and lack certain viral accessory genes. These viruses frequently are made to express the vesicular stomatitis virus G (VSV-G) protein in place of viral Env to increase cell tropism.

Third generation systems go further by using 3 helper plasmids: a packaging construct, a VSV-G construct and a Rev construct, along with a Tat-independent gene transfer vector, providing 4 separate plasmids in all. The elimination of the accessory gene Tat is an important component as this protein is essential for replication of wild-type HIV-1. Vector systems that use more than 4 plasmids are becoming available with even higher levels of Biosafety. For simplicity vectors that utilize 4 or more plasmids will be referred to here as third generation.

Both 2nd and 3rd generation vectors are generally self-inactivating by virtue of promoter disabling mutations engineered into the U3 region of the 3' long terminal repeat. These deletions provide an additional level of safety as vectors should not be able to generate full-length vector RNA after viral integration.

II. Modes of Transmission

- Skin penetration via puncture or non-intact skin scratches, cuts, abrasions, dermatitis or other lesions
- Mucous membrane exposure of the eyes, nose, and mouth

III. Engineering Controls:

The following safety equipment **MUST** be used when working with Lentiviral vectors:

- Certified Class II Biological Safety Cabinets
- Sealed centrifuge rotors and/or safety cups
- Vacuum lines equipped with an in-line HEPA filter as well as a primary and secondary vacuum flask containing a 10% bleach solution

IV. Administrative Controls

Work with Lentiviral vectors should only be carried out by trained personnel who must be directed by a competent scientist. Access to the laboratory must be limited when the agent is in use.

V. General Containment Considerations – Biosafety Levels:

Typically BSL-2+ practices and procedures; may include safety sharps or N-95 respirator.

VI. Work Practices:

- A. Close laboratory doors; use lab with anteroom if possible
- B. Use certified biological safety cabinet for all manipulations
- C. Load and unload ultracentrifuge buckets in Biosafety cabinet. Used sealed centrifuge for any bench top centrifugation and/or safety cups
- D. Do not use "sharps" (needles, glass Pasteur pipettes) to harvest virus pellet. Plastic aspiration pipettes are a replacement for glass Pasteur pipettes.
- E. Vacuum lines equipped with an in-line HEPA filter as well as a primary and secondary vacuum flask containing a 10% bleach solution.
- F. Cells exposed to lentiviral (or retroviral) vectors are not removed from the lab unless inactivated.

VII. Risk Assessment:

1. The nature of the vector system and the potential for regeneration of replication competent virus from the vector components

2. The nature of the transgene insert (e.g., known oncogenes or genes with high oncogenic potential may merit special care)
 3. The vector titer and the total amount of vector
 4. The potential for HIV positive individuals who's native virus may recombine with the vector (this is more of a problem with lower generation viruses)
 5. the inherent biological containment of the animal host, if relevant, negative RCL testing
- Generation of RCL from HIV-1 based lentivirus vectors depends upon:
1. The number of recombination events necessary to reassemble a replication competent virus genome
 2. The number of essential genes that have been deleted from the vector/packaging system
- Later generation vector systems (i.e., 3rd generation) are recommended for a greater margin of safety.

VIII. Employee Exposure

Eye exposure from splash or aerosol:

Rinse a minimum of 15 minutes in eye wash or flush with water. Notify the Principal Investigator or Laboratory Supervisor, who will immediately contact **Prospective Health at 744-2070** for medical treatment, and to report the incident.

Needlestick, sharps exposure or non-intact skin exposure:

Contaminated skin should be scrubbed for approximately 20 minutes using a 10% povidone iodine solution (such as Betadine®) and copious amounts of water. Notify the Principal Investigator or Laboratory Manager, who will immediately contact **Prospective Health at 744-2070** for medical treatment, and to report the incident.

Spill Response Procedures

The following steps must be taken when cleaning up a spill:

1. Stop, notify others and isolate the area!
2. Put on appropriate PPE (lab coat, gloves, eye and face protection).
3. Remove glass with forceps or scoop if applicable and place into a rigid, puncture resistant container.
4. Small spills-Place paper towels soaked in bleach directly on the spill and let soak for 20 minutes.
5. Wipe up area and discard towels in biohazard waste container.
6. Continue wiping area with paper towels soaked in bleach until the spill area is completely cleaned.
7. Discard all materials and PPE in biohazard waste container.
8. Wash hands thoroughly.

VIII. Animal handling practices:

1. Animal Biosafety Level 2 (ABSL2). Personnel working on the protocols must be trained in the hazards of working with the recombinant lentivirus before handling transfected animals.
2. Animals are housed in filter top cages or microisolator systems.

3. Use a class II Biological Safety Cabinet at all times when performing work on these animals and/or when moving animals from dirty to clean cages. When changing cages, use a standard microisolator technique.
4. Infected animals may shed lentivirus for one to seven days. Highest shedding is 72 hours after treatment; take precautions to avoid the creation of aerosols when changing or washing cages, or cleaning the room.
5. Dead animals must be placed in primary plastic bags, which are then placed in red Biosafety bags for incineration.
6. All surfaces and racks that may be contaminated will be decontaminated with 10% bleach ASAP.

All personnel handling the mice must wear:

1. Gloves (consider double-gloving)
2. animal handling gown
3. Eye goggles
4. N-95 mask covering the mouth and nose when not working in a Class II Biosafety Cabinet (BSC)
5. Surgical mask if no entry into microisolator or after 72 hours post infection

Animal Studies

1. Some animals cannot support replication of species specific lentivirus, so potential for shedding of RCL is very low. Animal husbandry and housing Biosafety level may be considered separate from the inoculation itself, which may pose sharps hazards.
2. NIH recommends that the initial delivery of vector should be performed under BSL-2. Containment level may be reduced following vector delivery; if there is no expectation of infection, the site of inoculation has been thoroughly cleansed, and the bedding changed. This time period is to be determined by the IBC and usually ranges between 1-7 days.
3. Procedures that cannot be performed in a Biosafety cabinet will be performed using N-95 respirators.
4. Animals that have been grafted with human cells permissive for HIV-1 replication require continued higher level of containment.

B. Employee Exposure

1. Eye exposure from splash or aerosol:
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5. Wipe up area and discard towels in biohazard waste container.
6. Continue wiping area with paper towels soaked in bleach until the spill area is completely cleaned.
7. Discard all materials and PPE in biohazard waste container.
8. Wash hands thoroughly.

Chapter 3

Training

The 5th edition of the BMBL, 2007 states that the laboratory supervisor or PI is responsible for ensuring that lab personnel, staff or students, are fully trained in the safety and containment procedure needed for their work as well as the laboratory-specific procedures and equipment operation required to perform their research. The laboratory supervisor must ensure that personnel receive training on duties and precautions initially, annually, and as policy and procedures change for the work conducted at BSL-1 or higher, as part of general microbiologic practice. The review of training and competency may become part of the annual performance review process or otherwise integrated into the existing evaluation processes.

The BMBL is a national standard and serves as a guideline for most labs at ECU; however, any lab in the federal Select agent Program must comply fully with BMBL recommendations, as they will be required compliance verified by inspection by CDC, APHIS, or both.

Work with a BSL-3 agent or in BSL-3 containment may require the PI to document the content and process of training at the satisfaction of the Biological Safety Officer and IBC.

ECU Training Resources

- Bloodborne Pathogen and TB training is provided twice a month for all ECU employees as part of New Employee Orientation at BSOM. All new employees of BSOM are required to attend this orientation, including self-help student workers. Any other ECU employees who will have occupational exposure to human blood, fluid, tissue, or cell culture lines should attend this training; the schedule is posted on One Stop. The Bloodborne Pathogen training presents some basic BSL-2 precautions, which applied to the research setting.
- Awareness training on the Biosafety levels and general containment requirements will be added to the BSOM New Employee Orientation process in 2008. A more in-depth slide show on Biological Safety is available on the Prospective Health website under "Training and Education- Biological Safety". A slide show on Recombinant DNA Training is available on the Prospective Health website under "Training and Education- Biological Safety".
- Training on Shipping of Biological Materials resulting in 2 year IATA Certification is available online. Contact the Biological Safety Officer to sign up.
- Training on respiratory protection from infectious agents is available on the Prospective Health website under "Training and Education- Biological Safety".

Chapter 4

Registration Procedure

East Carolina University has established a formal procedure to review use of biohazardous agents and assign the level of containment appropriate for the level of risk indicated by the agents and procedure(s) performed. Registration is necessary in order to use biohazardous agents of Risk Group 2 or greater; to clarify whether use of Risk Group 1 agents requires BL-2 containment; and if any recombinant DNA molecules or organisms are used. Registration forms can be obtained [online](#) at the Prospective Health website. Contact Biological Safety by phone or email to verify whether submission of a registration is needed. Completed registration forms are submitted to [Yvonne Taylor](#) by Email by or mail (Prospective Health LSB 188).

The Principal Investigator will complete the registration, which serves as the basis for the laboratory Biosafety plan. It describes the agents used, the lab procedures and manipulations planned, any recognized hazards and safety measures, the associated risks of exposure, and lists lab personnel. The Biological Safety Committee (IBC) will review the work and determine the applicable containment level at the next scheduled meeting. The registration should be submitted **at least** seven calendar days prior to the scheduled meeting. An annual calendar will be posted on the [Biosafety section of the Prospective Health website](#). New or modified registration materials should be sent to [Yvonne Taylor](#). Researchers may forward inquiries to the Biological Safety Officer or the Chair of the IBC for specific questions prior to registration completion. After review, the IBC will notify the Principle Investigator in writing of its determination with a copy to the department chair. The Principal Investigator will apply the recommended containment level and associated requirements for the work performed. If a project uses animals or human subjects, Biological Safety approval to begin work will be made contingent on approval by the IRB or IACUC; notice of any IBC action on the work will be communicated in writing to the other committee(s).

The Animal Care and Use Committee (IACUC) oversees the routine use of primates in research which will be conducted at ABL-2 at minimum. Two members of the IBC are represented on the IACUC. If the work on non-human primates involves an unusual or increased laboratory risk or requires an increased level of containment, IACUC will refer the project to the IBC. IBC must also approve work involving: *transplantation or injection of human tissues into animals, or use of nonhuman primate tissue obtained from sources outside ECU.*

The Biological Safety Officer may, at his discretion, choose to grant *provisional approval* for work to be conducted at BL-2 or lower under certain conditions. These include:

- Receipt of a complete and satisfactory registration fully describing the work
- Completion of a laboratory inspection of all sites of planned work and correction of any deficiencies noted.
- Concurrence of Director of Prospective Health and Chair of the Institutional Biological Safety Committee.
- Recombinant DNA projects which can be expedited are limited to work at BSL-1 using DNA molecules containing no more than 2/3 the genome of any eukaryotic virus, experiments with whole plants, experiments with transgenic rodents, or Section III-F exempt experiments.

All registrations granted provisional approval will be presented for ratification to the full IBC at the next meeting. The Committee may specify further precautions before the provisional approval can be finalized. The Biosafety Officer will defer provisional approval if the registration is complex or potentially controversial.

Approval for use of a biohazardous agent will consider:

1. The ability and experience of the applicant to handle the hazards involved.
2. The adequacy of the facilities and equipment for the proposed usage.
3. The thoroughness and attention given to the safety precautions by the Principal Investigator in the proposed experimental or clinical procedures.

Biosafety approval is effective for a **three-year period**. The registration may then be renewed if there have been no significant changes to the safety plans or experimental protocols or updated. Biological Safety will contact the principal investigator for the triennial renewal. Due to the constantly changing requirement of NIH and other regulators, a new registration form including Appendix A will be completed for the three year renewal.

During the three year period of approval, if changes occur that could modify the risk level or laboratory hazards, the Principal Investigator will notify the Biological Safety Officer in writing or by e-mail within 30 days. Significant changes include modification of the research protocol, changes in personnel or equipment or laboratory location. If three or more amendments are made to an existing registration, the Biological Safety officer may request submission of a new registration. In addition, if an existing registration is modified by adding a new organism, toxin or use of recombinant material, or by the deliberate transfer of a drug resistance trait to an organism, a new registration may be requested and will be submitted.

The Biological Safety Officer serves as a liaison between the Committee and the individual projects, researchers, and monitors that safety precautions are implemented and practiced.

Transgenic Plants/Plant Symbionts or Recombinant DNA in Plants. The use of these materials triggers the NIH Guidelines requirements in Chapter 10. The process for approval of plants begins with completion of the Registration Form for Plants. A plant expert will be added to the committee as an ad hoc member who will attend when the committee reviews plant projects.

Chapter 5

ENGINEERING AND WORK PRACTICE CONTROLS

Warning Signs and Postings

The universal biohazard warning symbol (shown below) shall be used throughout the institution to notify workers about the presence of biohazardous agents. The symbol will be printed either in red or on a red or orange background and posted on the door/entrance to any laboratory where biohazardous agents are used; **including BL-1 labs**. The sign will list the Biosafety Level, name of the agent, the name of the principal investigator or responsible faculty and daytime and evening/weekend contact numbers and any special precaution to be taken. In rare cases, if posting of the agent name may create undue concerns or a security problem; posting the agent name may be waived after review by Biological Safety. The Principal Investigator will ensure that all necessary postings are installed and properly maintained and updated. The warning symbol must be removed or modified when the hazardous agent is no longer in use or present.



Universal biohazard labels must be affixed to containers of regulated waste, refrigerators and freezers containing blood or other infectious materials, and to containers used to store, transport, or ship blood or other potentially infectious materials. All individual containers of biohazardous agents should also be labeled to identify the contents and any special precautionary measures that should be taken. Use of red bags, red containers, or labeled secondary containers may be substituted for labeling of individual containers such as specimen tubes.

Laboratory Safety Plans

A written Laboratory Biosafety Plan is required for each research and teaching laboratory where employees and students may be exposed to biohazardous agents, including exposures that could result from work with infectious microorganisms, recombinant DNA molecules, human tissues or body fluids, or their use in experimental animals. The Biosafety Plan will address routine safety precautions for the laboratory, and specify responses to possible accidents spills or exposures. The Biosafety registration may form the basis of the laboratory safety plan, if supplemented with laboratory-and project-specific SOPs.

If a spill occurs, soak up the contaminated material with an absorbent material (such as paper towel), then disinfect the surface, wearing gloves during cleanup.

Any spills and other accidents, which create overt or potential exposure to personnel or release of biohazardous agents to the general environment, will be reported immediately to the laboratory

supervisor and to the Biological Safety Officer. A written record of such incidents must be maintained in the laboratory.

All employees and students working in a research or teaching laboratory who have potential exposure to biohazardous agents will be appropriately trained at initial employment and annually thereafter. The Principal Investigator is responsible for providing this training, or to ensuring the worker attends other appropriate safety training. The Principal Investigator will document training and maintain records of attendance.

When preparing the overall Biosafety Plan, the Principal Investigator may consult with:

1. The Biological Safety Officer or Biological Safety Committee Chair.
2. The Office of Prospective Health for information on employee health monitoring, tests, immunizations. Employees who work in laboratories with level 3 or 4 infectious materials may be enrolled in an occupational health program.
3. Office of Environmental Health and Safety for use and disposal of Chemicals.
4. Comparative Medicine or the Animal Care and Use Committee for vertebrate animal use, exposure, and safety.
5. Radiation Safety if radioactive materials or radiation will be used.

Biological Safety Cabinets

Biological Safety Cabinets serve two purposes 1) to protect the work from airborne contamination 2) to protect the operator from the material worked on. In contrast to chemical fume hoods which vent air out, biological safety cabinets direct air through a HEPA filter to remove particles before venting or recirculation.

Biological Safety Cabinet selection should be based on:

1. Hazard classification
2. Amount of protection needed for research products or personnel
3. Amount of hazardous aerosols generated

There are three types of Biological Safety Cabinets: Class I, II and III.

Class I: A ventilated cabinet for personnel and environmental protection, inward air flows away from the operator and all air exhausts to the atmosphere after filtration through a HEPA filter. Class I cabinets are suitable for work where ***no product protection*** is required.

Class II: A ventilated cabinet for **personnel, product, and environmental protection** having an open front with inward airflow for personnel protection, downward HEPA filtered laminar airflow for product protection, and HEPA filtered exhausted air for environmental protection.

Class III

Class III cabinets provide the highest level of personnel protection; the entire unit is enclosed, preventing agent from contacting the worker. Class III cabinets are self-contained, closed front, , and are operated at negative pressure. Manipulations are performed with *arm length rubber gloves sealed to the front* of the unit. *Highly infectious agents* can be manipulated safely in Class III cabinets, e.g. BSL-4.

NOTE: Clean air or horizontal laminar flow workbenches do not operate like biological safety cabinets. The primary purpose of a clean air workbench is to protect the material in the air stream, not the worker. Filtered air is blown horizontally across the material in the hood, towards the worker. Clean air (laminar flow) hoods should never be used to manipulate pathogenic microorganisms, or pathogenic human or animal tissues.

When biological safety cabinets are used for containment, the Principal Investigator will ensure that the cabinet chosen provides the level of protection needed, functions properly with sash in place and airflow is not impeded. Appropriate BSC selection can be verified with Biological Safety; operation will be verified during inspection. **Cabinets must be certified annually and following relocation to verify effective performance.**

Types of Class II Biological Safety Cabinets

There are four subtypes of Class II cabinets, type A1, type A2, type B1, and type B2. The characteristics of these subtypes are outlined below: **Class II cabinets protect both the product and the operator.** Class A cabinets recirculate some filtered air back into the lab for energy efficiency and are not suitable for use with volatile or toxic chemicals or radionucleotides. Class B exhausts **all** air through a HEPA filter and to the atmosphere. Type B2 are used with volatile toxic chemicals or radionucleotides.

Table 6

Class II, type A1 (formerly designated Type A)	Class II, type A2 (formerly designated as Type B3)	Class II, type B1	Class II, type B2 (sometimes referred to as "total exhaust")
Have HEPA filtered down flow air that is a portion of the mixed down flow and inflow air from a common plenum	Have HEPA filtered down flow air that is a portion of the mixed down flow and inflow air from a common exhaust plenum	Have HEPA filtered down flow air composed largely of uncontaminated recirculated inflow air	Have a HEPA filtered down flow air drawn from the laboratory or the outside air
May exhaust HEPA filtered air back in to the laboratory or to the environment through an exhaust canopy	May exhaust HEPA filtered air back in to the laboratory or to the environment through an exhaust canopy	Exhaust most of the contaminated down flow air through a dedicated duct exhausted to the atmosphere after passing through a HEPA filter	Exhaust all inflow and down flow air to the atmosphere after filtration through a HEPA filter without recirculation in the cabinet or return to the laboratory.
May have positive pressure contaminated ducts and plenums that are not surrounded by negative pressure plenums	Have all biologically contaminated ducts and plenums under negative pressure or surrounded by negative pressure ducts and plenums.	Have all biologically contaminated ducts and plenums under negative pressure or surrounded by negative pressure ducts and plenums.	Have all contaminated ducts and plenums under negative pressure or surrounded by directly exhausted negative pressure ducts and plenums
Type A1 cabinets are not suitable for with	Type A2 cabinets used for work with	Type B1 cabinets may be used for work	Type B2 cabinets may be used for work with

<i>volatile toxic chemicals and volatile radionuclides.</i>	<i>minute quantities of volatile toxic chemicals and tracer amounts of radionuclides required as an adjunct to microbiological studies must be exhausted through properly functioning exhaust canopies.</i>	<i>treated with minute quantities of volatile toxic chemicals and tracer amounts of radionuclides required as an adjunct to microbiological studies if work is done in the direct exhausted portion of the cabinet, or if the chemicals or radionuclides will not interfere with the work when recirculated in the down flow air.</i>	<i>volatile toxic chemicals and radionuclides required as adjuncts to microbiological studies.</i>
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See Appendix D for more information on Biological Safety Cabinets.

WORK PRACTICES AND PROCEDURES FOR USE OF BIOLOGICAL SAFETY CABINET

1. Prepare a written checklist of materials necessary for a particular activity and place necessary materials in the BSC before beginning work. This minimizes the number and extent of air curtain disruption compromising the fragile air barrier of the cabinet. Extra supplies (e.g., additional gloves, culture plates or flasks, culture media) should be stored outside the cabinet. Move arms in and out slowly, perpendicular to the face opening of the cabinet to reduce this risk.
2. Laboratory coats or long-sleeved gown should be worn over street clothing. Latex, vinyl, Nitrile or other suitable gloves are worn to provide hand protection. Increasing levels of PPE can be included as determined by an individual risk assessment.
3. Delay manipulation of materials for approximately one minute after placing the hands/arms inside the cabinet. The front grille must not be blocked with toweling, research notes, discarded plastic wrappers, pipetting devices, etc. All operations should be performed on the work surface at least **four inches** in from the front grille. If there is a drain valve under the work surface, it should be closed prior to beginning work in the BSC.
4. BSCs are designed for 24 hours per day operation and continuous operation may help control the laboratory's level of dust and other airborne particulates. If the cabinet has been shut down, the blowers should be operated at least four minutes before beginning work to allow the cabinet to "purge."
5. The work surface, the interior walls (*except* the supply filter diffuser), and the interior surface of the window should be wiped with 1:100 dilution of household bleach (i.e., 0.05% sodium hypochlorite), or other disinfectant as determined by the investigator to meet the requirements of the particular activity. Many disinfectants, such as bleach, require a second wiping with sterile water to remove the residual chlorine, which may eventually corrode stainless steel surfaces. Warning, bleach + alcohol creates chloroform.
6. The surfaces of all materials and containers placed into the cabinet should be wiped with 70% EtOH to reduce the introduction of contaminants to the cabinet environment. Plastic-backed absorbent toweling can be placed on the work surface but *not* on the front or rear grille openings.
7. All materials should be placed as far back in the cabinet as practical, toward the rear edge of the work surface and away from the front grille of the cabinet. Similarly, aerosol-generating equipment (e.g., vortex mixers, tabletop centrifuges) should be placed toward

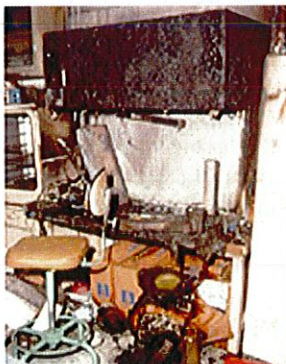
the rear of the cabinet to take advantage of the air split. Bulky items such as biohazard bags, discard pipette trays and vacuum collection flasks should be placed to one side of the interior of the cabinet.

8. The biohazard collection **bag should not be taped to the outside** of the cabinet. Upright pipette collection containers should not be used in BSCs nor placed on the floor outside the cabinet. The frequent inward/outward movement needed to place objects in these containers is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection. Only horizontal pipette discard trays containing an appropriate chemical disinfectant should be used within the cabinet.
9. Potentially contaminated materials should not be brought out of the cabinet until they have been surface decontaminated. Alternatively, contaminated materials can be placed into a closable container for transfer to an incubator, autoclave or another part of the laboratory.

Guidelines for Operation within a Class II BSC

The following are offered as a baseline; work with more hazardous agents or aerosols may require more stringent containment or additional procedures.

1. Good microbiological techniques should always be used when working in a BSC. Techniques used to reduce splatter and aerosol generation will minimize the potential for personnel exposure to infectious materials. As a general rule of thumb, keeping clean materials at least one foot away from aerosol-generating activities will minimize the potential for cross-contamination.
2. The work flow should be from "clean to dirty". Materials and supplies should be placed in the cabinet in such a way as to limit the movement of "dirty" items over "clean" ones.
3. Opened tubes or bottles should not be held in a vertical position. Investigators working with Petri dishes and tissue culture plates should hold the lid above the open sterile surface to minimize direct impaction of downward air. Bottle or tube caps should not be placed on the toweling. Items should be recapped or covered as soon as possible.
4. Open flames are not required and should not be used in the near microbe-free environment of a biological safety cabinet. **Due to risk of fire, explosion, and heat damage to HEPA filter Bunsen burners should never be used in a biological safety cabinet (See photo below).** 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 being supplied to the work surface. Touch-plate microburners equipped with a pilot light to provide a flame on demand may be used. The burner must be turned off when work is completed. Disposable or recyclable sterile loops should be used whenever possible.



**Do not let this be Your
Biosafety Cabinet!**

5. Disinfection

- a. To disinfect pipettes or other small items, suitable liquid disinfectant should be placed into the discard pan before work begins. Items should be introduced into the pan with minimum splatter and allowed appropriate contact time. Nonvolatile liquids can be autoclaved prior to disposal.
- b. Contaminated items should be placed into a biohazard bag, discard tray, or other suitable container prior to removal from the BSC.
- c. Small electric "furnaces" are available for decontaminating bacteriological loops and needles.
- d. Aspirated materials can be decontaminated by placing sufficient chemical disinfectant into the flask to inactivate the microorganisms as they are collected. Aspirator bottles or suction flasks should be connected to an overflow collection flask containing appropriate disinfectant, and to an in-line HEPA or equivalent filter. Once inactivation occurs, liquid materials can be disposed of as noninfectious waste.
- e. With the cabinet blower running, all containers and equipment should be surface decontaminated and removed from the cabinet when work is completed. At the end of the work day, the final surface decontamination of the cabinet should include a wipe-down of the work surface, the cabinet's sides and back and the interior of the glass. The cabinet blower may be turned off after these operations are completed, or left on.(preferred)
- f. When a steam autoclave is to be used, disinfected materials should be placed into a biohazard bag or discard pan containing enough water to ensure steam generation during the autoclave cycle. The bag should be closed or the discard pan should be covered in the BSC. The exterior surface of bags and pans are contaminated just prior to removal from the cabinet. The bag or pan should be transported and autoclaved in a leak proof tray or pan. If airborne spread is a route of infection use of secondary containment is indicated.

6. Spills

- a. Small spills contained within the operating BSC can be handled immediately by removing the contaminated absorbent paper toweling and placing it into the biohazard bag or receptacle. Any splatter onto items within the cabinet, as well as the cabinet interior, should be immediately cleaned up with a towel dampened with an appropriate decontaminating solution. Gloves are changed after the work surface is decontaminated and before placing clean absorbent toweling in the cabinet. Hands are washed whenever gloves are changed or removed.
- b. Spills large enough to result in liquids flowing through the front or rear grilles require more extensive decontamination. All items within the cabinet should be surface decontaminated and removed. After ensuring that the drain valve is closed, decontaminating solution can be poured onto the work surface and through the grille(s) into the drain pan.
- c. Spills outside the BSC may require Biological Safety assistance if aerosol is generated or if airborne route of infection is possible.
- d. The spilled fluid and disinfectant solution on the work surface should be absorbed with paper towels and discarded into a biohazard bag. The drain pan should be emptied into a collection vessel containing disinfectant. Periodic removal of the cabinet work surface and/or grilles after the completion of drain pan decontamination may be justified because of dirty drain pan surfaces and grilles, which ultimately could occlude the drain valve or block airflow. Use extreme caution to avoid injury from broken glass that may be present and sharp metal edges.

See Appendix C for more information about standard procedures for BSC use.

Centrifuge precautions:

Centrifuge may generate aerosols during normal operation; especially if a malfunction occurs such as tube breakage or leaks. Even though organisms routinely manipulated at BSL-2 may not be known to be transmissible by the aerosol route, procedures with aerosol or high splash potential may increase the risk of such personnel exposure and must be conducted in primary containment equipment, such as within a BSC, using safety centrifuge cups. (BMBL 2007)

1. BL-2 Organisms which are recognized to be spread by the respiratory route must be centrifuged in a BSC or using a safety centrifuge sealed screw top rotor and cups which are opened only under a BSC.
2. Strict centrifuge precautions are required for BSL-3 work. Specimens containing infectious materials to be centrifuged will use a unit designed for use within a Biosafety cabinet. If a bench top centrifuge is used, a sealed safety centrifuge will be used. A sealed safety centrifuge combines features such as:
 - a. The specimen tube will have an o-ringed screw cap or a cap that fits over the rim of the centrifuge tube.
 - b. The centrifuge rotor is lidded, preferably with a screw-top cover, so that if tubes leak or break, only the rotor must be disinfected, not the entire centrifuge interior.
 - c. The trunnion cup which holds the specimen tube has a lid or cap which prevents aerosolization or spillage outside the trunnion cup.
 - d. Disposable sealed rotor centrifuge units are available and preclude the need for decontamination of the rotors. The rotor is discarded after use.
3. After centrifugation, all vials and/or safety cups are opened within a biological safety cabinet.
4. A policy will be developed to address the appropriate response to a spill or breakage within the centrifuge, the aerosol generated and how the spill will be cleaned in any laboratory using a centrifuge.

See Appendix M for more information about types of centrifuges and safety features.

Sterilization and Disinfection

An item is considered sterile when it is completely free of all living microorganisms and viruses or spores. The definition is categorical and absolute (i.e. an item is either sterile or it is not). A sterilization *procedure* is one that kills all microorganisms, including high numbers of bacterial endospores. Sterilization can be accomplished by heat, ethylene oxide gas, hydrogen peroxide gas, plasma, ozone, and radiation (in industry). Sterilization is a process, after which the probability of a microorganism surviving on an item subjected to treatment is less than one in one million. This is referred to as the "sterility assurance level".

Disinfection

Disinfection reduces the level of microbial contamination; it is a less lethal process than sterilization. It eliminates nearly all recognized pathogenic microorganisms but not necessarily all microbial forms (e.g., bacterial spores) on inanimate objects. Disinfection does not ensure an "overkill" and therefore lacks the margin of safety achieved by sterilization procedures. The effectiveness of a disinfection procedure depends on:

- the nature and number of contaminating microorganisms (especially the presence of bacterial spores);
- the amount of organic matter present (e.g., soil, feces, and blood);
- the type and condition of instruments, devices, and materials to be disinfected;
- the temperature.

"High-level" disinfection approaches chemicals sterilization but, lacks sporicidal power. A few chemical germicides used as disinfectants have the capability to kill spores but high concentrations and several hours of exposure may be required.

Disinfectants vary in their capacity to disinfect or decontaminate. Some rapidly kill ordinary vegetative forms of bacteria such as staphylococci and streptococci, some forms of fungi, and lipid containing viruses. Others are effective against such relatively resistant organisms as *Mycobacterium tuberculosis* var. *bovis*, non-lipid viruses, and most forms of fungi. It is important to follow the manufacturer's recommendation for contact time to ensure disinfection occurs.

Spaulding Classification

The Spaulding system uses three categories of risk for infection if surfaces are contaminated at time of use. From the laboratory perspective, these categories are:

- Critical – instruments or devices that are exposed to normally sterile areas of the body and require sterilization
- Semi-critical – instruments or devices that touch mucous membranes may be either sterilized or disinfected;
- Non-critical – instruments or devices that touch intact skin. There can be either cleaned and then disinfected with an intermediate-level disinfectant, sanitized with a low-level disinfectant, or simply cleaned with soap and water.

An additional category "environmental surfaces" includes floors, walls, and other "housekeeping surfaces".

Spaulding also classified chemical germicides by activity level:

High-Level Disinfection Kills vegetative microorganisms including *Mycobacterium* and *Tuberculosis* and inactivates viruses, but not high numbers of bacterial spores. Such disinfectants are capable of sterilization when the contact time is long (e.g., 6 to 10 hours). As high-level disinfectants, they are used for shorter periods of time (e.g., 10 to 30 minutes). They are formulated for use on semi-critical medical devices, but not on environmental surfaces.

Intermediate-Level Disinfection kills vegetative microorganisms, including *Mycobacterium tuberculosis*, all fungi, and inactivates most viruses. Chemical germicides used at this level correspond to "Environmental Protection Agency (EPA)-approved hospital disinfectants" that are also "tuberculocidal". They are used commonly in laboratories for disinfection of laboratory benches and as detergent-germicide combinations used for housekeeping purposes.

Low-Level Disinfection kills most vegetative bacteria except *M. tuberculosis*, some fungi, and inactivates some viruses, and are also known as "EPA disinfectant/sanitizers", and are typically used on environmental surfaces.

Decontamination vs. Cleaning

Decontamination reduces the level of microbial or toxin contamination so that the likelihood of infection or hazard is reduced. Decontamination of items, spent laboratory materials, and regulated laboratory wastes may be accomplished by a sterilization procedure such as steam autoclaving, or by chemical disinfection, such as soaking in bleach solution.

If the item or area is not precleaned, the presence of any organic matter necessitates longer a contact time for any decontamination method. For example, a steam cycle used to sterilize precleaned items is 20 minutes at 121°C; for used items with no pre-cleaning and a high bioburden, (i.e., infectious waste) a longer cycle would be needed.

See Appendix N for more information on sterilization and disinfection and available from the Medical Storeroom.

ACTIVITY LEVELS OF SELECTED LIQUID GERMICIDES ^a

PROCEDURE/PRODUCT	AQUEOUS CONCENTRATION	ACTIVITY LEVEL
STERILIZATION		
glutaraldehyde	variable	
hydrogen peroxide	6-30%	
formaldehyde	6-8% ^b	
chlorine dioxide	variable	
peracetic acid	variable	
DISINFECTION		
glutaraldehyde	variable	High to intermediate
ortho-phthalaldehyde	0.5%	High
hydrogen peroxide	3-6%	High to intermediate
formaldehyde	1-8%	High to low
chlorine dioxide	variable	High
peracetic acid	variable	High
chlorine compounds ^c	500 to 5000 mg/L free available chlorine	Intermediate
alcohols (ethyl, isopropyl) ^c	70%	Intermediate
phenolic compounds	0.5 to 3%	Intermediate to low
iodophor compounds ^c	30-50 mg/L free iodine up to 10,000 mg/L available iodine	Intermediate to low
quaternary ammonium compounds	0.1 - 0.2%	Low

A large number of commercial products used these generic components. Users should ensure that commercial formulations are registered with EPA or FDA.

Because formaldehyde is a potential human occupational carcinogen, its use is limited to certain specific circumstances under carefully controlled conditions, e.g., for the disinfection of certain hemodialysis equipment. There are no FDA cleared liquid chemical sterilant/disinfectants that contain formaldehyde.

Generic disinfectants containing chlorine are available in liquid or solid form (e.g., sodium or calcium hypochlorite) are rapid acting and broad-spectrum (tuberculocidal, bactericidal, fungicidal, and virucidal). No proprietary hypochlorite formulations are formally registered with EPA or cleared by FDA. Common household bleach is an excellent and inexpensive source of sodium hypochlorite (5.25% hypochlorite). Concentrations between 500 and 1000 mg/L chlorine are appropriate for the vast majority of uses as intermediate level of germicide (1:5 or 1:10 dilution). Higher concentrations are extremely corrosive as well as irritating to personnel, and their use should be limited to situations where there is an excessive amount of organic material or unusually high concentrations of microorganisms (e.g., spills of cultured material in the laboratory).

The effectiveness of alcohols as intermediate level germicides is limited by rapid evaporation, resulting in short contact times and in ability to penetrate residual organic material. Alcohols are rapidly tuberculocidal, bactericidal and fungicidal, but vary in spectrum of virucidal activity. Items to be disinfected with alcohols should be carefully pre-cleaned then totally submerged for an appropriate exposure time (e.g., 10 minutes).

Only those iodophors registered with EPA as hard-surface disinfectants should be used, following the manufacturer's instructions for proper dilution and product stability. Antiseptic iodophors are not suitable to disinfect devices, environmental surfaces, or medical instruments.

There are many chemical disinfectants on the market, with the main constituent being one of the following: chlorine, quaternary ammonium compounds, alcohol, iodine, phenolics, or glutaraldehyde.

<p>Chlorine Compounds</p> <ul style="list-style-type: none"> • 5.25% sodium hypochlorite • 1:5 dilution is effective on mycobacterial in sputum • 5000 ppm concentration needed • Contact time – 20-30 minutes for HIV • Wide spectrum germicidal • Sanitizing properties • No residue • Low toxicity • Bronchial irritant and skin irritation from extended contact • Good for disinfecting surfaces • Solution must be made fresh since chlorine will off-gas over a period of time. 1:10 dilution lasts for about one day in a closed bottle. • Light contact which accelerates decomposition, can be prevented by using opaque containers 	<p>Quaternary Ammonium Compounds</p> <ul style="list-style-type: none"> • 0.1% to 2% concentration of active ingredient needed • Soluble in water • Excellent for vegetative bacteria (gram +), lipo viruses, and HIV • Good detergent properties • Relatively non-toxic • Not effective with spores and poor response with pseudomonas • Best for routine disinfecting of surfaces
<p>Alcohol (ethyl or isopropyl)</p> <ul style="list-style-type: none"> • 60-95% concentration needed • Effective on vegetative bacteria • Contact time seconds • No residue formation • Non-toxic (generally) • Inexpensive 	<p>Iodophor Compounds</p> <ul style="list-style-type: none"> • 0.47% concentration of iodine needed • Contact time – 10 to 30 minutes • Relatively non-toxic • Wide spectrum germicide • Fairly safe to use • Not stable above 54°C
<p>Glutaraldehyde</p> <ul style="list-style-type: none"> • Commonly referred to as "Cidex" • Mode of action is alkylation • Used in a 2% dilution • Chemosterilizer and high level disinfectant • Typically used for medical equipment such as endoscopes 	<p>Phenolics</p> <ul style="list-style-type: none"> • 0.2 to 3.0% concentration effective • Wide spectrum antimicrobial agent • Excellent sanitizing agent • Toxic compound and a poor sporicidal agent

The susceptibility of microorganisms to disinfectants is broad and variable. The vegetative bacteria, fungi, and lipid containing viruses are highly susceptible to inactivating agents. Non-lipid containing viruses are moderately resistant to these agents, especially to alcohol; spore forms are the most resistant. The effectiveness of alcohols is limited because they evaporate rapidly, resulting in short contact time; they also cannot penetrate residual organic material. Antibiotic iodophors are not suitable to disinfect devices, environmental surfaces, or medical instruments. (NOTE: the range of contact times for different agents, disinfection is not instantaneous.)

Disinfection of Equipment Before Moving or Repair

Equipment which has been contaminated and is labeled with the universal biohazard symbol must be thoroughly disinfected with a 1:10 dilution of bleach or other EPA approved disinfectant before sending it offsite for surplus or repair. The biohazard symbol must be removed after disinfecting and before sending equipment to surplus; a sticker indicating that disinfection has been performed is applied by the individual who performs the disinfection process. (See Appendix I for policy and stickers)

Biological Safety Cabinets must be disinfected before repair and before moving/warehousing. This will consist of routine disinfectant wipe down of all surfaces by the users. Biological Safety is contacted to perform a formaldehyde vapor disinfection of the interior HEPA filter(s) if the cabinet has been used for infectious microorganisms. Facilities Services will disconnect the air, electrical, and gas connections before the cabinet is moved. (See protocol in Appendix H for moving Biosafety Cabinets)

Autoclave Use

Autoclaving is one of the most dependable methods for decontaminating laboratory materials or waste. Autoclaves use saturated steam under pressure to achieve high temperatures to kill microorganisms. Attaining the proper temperature for the proper length of time is essential for an effective kill.

Items for autoclaving

CAN

Cultures & Stocks
Culture Dishes & Related devices
Discarded live & Attenuated Vaccines
Contaminated soiled items (petri dishes, pipettes, gloves, paper towels)
Items for sterilization such as glassware, media, water, equipment
Infected small animal carcasses

CANNOT

Materials containing solvents, volatile or corrosive chemicals
Material contaminated with chemotherapeutic agents
Radioactive material
Heat sensitive material, lab equipment or reagents to be re-used

Packaging

- Use **only APPROVED AUTOCLAVE BAGS**. Autoclave bags (orange) are available from the Medical Storeroom. (Red Biohazard bags are designed for incineration, and not suitable for autoclave use)
- Containers must be heat resistant
- Do not overfill autoclave bags or containers (do not fill beyond 75 % of holding capacity)
- Do not compress material – sufficient space is required to ensure steam penetration
- Dry material should be separated from liquid material
- If outside bag is contaminated, apply a second autoclave bag before removal from lab



(Orange Autoclave bag in plastic container; autoclave labeling not visible)

Transportation

- Surface decontaminate prior to transport
- Use cart (metal or hard plastic) with guard rails

- Use secondary containers
- Flasks must be capped with at least aluminum foil
- Use direct but not heavily populated route
- Bags should be closed for transport and loosely sealed or taped. Do not seal airtight. This prevents spillage of contents but prevents “popping” of tightly sealed bags.

Cycle Instructions

- Operate at a minimum of 250°F (121°C) for a minimum of 30 minutes.
 - Cycles will vary depending on the biological hazard and size of load.

Loading Autoclave

- Ensure material is acceptable for autoclaving
- Separate Similar Loads
- Use Secondary Containers
- Load material to ensure steam penetration
- Ensure all containers & bags are well vented

Unloading Autoclave

- Wait until chamber pressure gauge reaches 0 before opening the door
- Open door slightly to allow any remaining steam to dissipate
- Remove waste safely wearing personal protective equipment
- Verify heat sensitive tape has changed color. This indicates that proper temperature has been achieved on the package surface. It does not indicate sterility of the inner contents.

Safety Considerations

- Wear personal protective equipment (heat resistant gloves, goggles, and a lab coat)
- Use caution when opening the autoclave door. Allow superheated steam to exit
- Use caution when handling a bag in case sharp objects have been inadvertently placed in the bag. Never grab from the bottom of the bag to load the chamber. Handle the bag from the top.
- Watch out for pressurized containers. Super heated liquids may spurt from sealed containers. Never seal a container of liquid with a cork which may cause a pressurized explosion.
- Agar plates will melt and the agar will become liquefied. Avoid contact with this molten liquid.
- Glassware may crack or shatter if cold liquid comes in contact with this superheated glassware. If glass breaks in the autoclave, use tongs, forceps, and other mechanical means to recover fragments.

Autoclave Testing

- All autoclaves used for infected waste decontamination should be regularly monitored for effectiveness in three areas: mechanical, thermal, and biological performance
- Autoclave indicator tape indicates that the outside of the container came to temperature, it does not reflect time or conditions inside the load and does not prove effective decontamination
- General mechanical maintenance will be conducted on an annual basis by Facilities Services or as recommended by the manufacture
- Research autoclaves *should be tested quarterly with a spore indicator for biological effectiveness* and the results documented by user (See Autoclave Validation Policy).
 - Spore indicators should be placed at the slowest point of heating (e.g. the center of the load)
 - For researchers who autoclave infectious waste, it is suggested that a *simulated load* of typically autoclaved materials which is *not infectious/contaminated* with a spore indicator be run each quarter as a validity check. This load can be safely

disassembled after autoclaving to check the spore indicator without risk of infectious exposure.

Safety Maintenance

- A safety check should be completed prior to each use. (e.g. door closes and seals properly, rack is in place, correct setting are being used, the interior is clean)

Record Keeping

- Daily Autoclave Log
 - The use of an autoclave logbook is recommended for each autoclave. Prior to autoclaving any items, users fill in all required information. (Users Name, cycle time, cycle setting, time in/out, verification of results)

AUTOCLAVED WASTE GUIDELINES

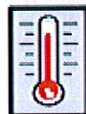
(Treating Infectious or Potentially Infectious Waste)

What is an autoclave?

An autoclave, or steam sterilizer, is an insulated pressure chamber in which saturated steam is used to elevate the temperature. Autoclaves are found in research, diagnostic and microbiology laboratories, health centers and other places that require high-level disinfection.

How does an autoclave work?

An autoclave uses pressurized steam to Laboratory autoclaves normally operate at a pressure of 15 pounds per square inch (psi) The effectiveness of an autoclave depends steam contact with infectious agents. Other factors that influence treatment efficiency include waste density, physical state and size and organic content.



decontaminate infectious waste. temperature of 250°F (121°C), a and a minimum cycle time of 30 minutes. on the time, temperature and direct

How do I use the autoclave?

- Autoclaves come in many different styles. Therefore, always follow the manufacturer's instructions when using the autoclave and ask your supervisor if you have questions.
- There are different autoclave and exhaust cycles for liquids and solids.
- Loosely close the bag or vessel; do not tie the bag or seal the vessel tightly before autoclaving. **Sealed containers can explode.**

(NOTE: All autoclaves should be validated by spore testing: Weekly for healthcare use, quarterly for research use.)

What must be autoclaved?

The following human and animal infectious waste products *must* be autoclaved:

- Cultures and stocks of infectious agents.
- Laboratory wastes that were exposed to infectious agents from medical, pathological, or pharmaceutical laboratories.
- Pathological wastes, including tissues, and biopsy specimens.
- Research animal tissues/carcasses infected with pathogenic organisms, or biotoxins weighing less than five pounds. (All research animals must be disposed according to ECU ACUC and Department of Comparative Medicine procedures. Return to coolers in appropriate containers designating hazard status as instructed.)
- Biological waste and discarded materials contaminated with excretion, exudates, or secretion from humans or animals.
- Discarded equipment and utensils which have been in contact with pathogenic infectious agents concentrated cultures of any organisms or biotoxin.
- Preparations made from genetically altered living organisms and their products.



(See Infectious Waste flow sheet pg. 46 for additional detail)

What cannot be autoclaved?

Types of waste that should not be autoclaved include cancer drugs, toxic chemicals, radioisotopes, volatile chemicals or any other harmful material that can be vaporized and disseminated with heat. In general, do not autoclave **flammable, reactive, corrosive, toxic or radioactive materials**.



Preserved specimens (i.e. formaldehyde) must not be autoclaved; they can be put into the biohazard waste stream (if the excess liquid preservative has been removed). (Contact Environmental Health and Safety for liquid formaldehyde/formalin disposal). Use sharps containers for disposal of stained or preserved specimens on glass slides

How should I collect and dispose the waste?

Liquid infectious waste (excluding the chemicals listed above) may be autoclaved or decontaminated and then disposed via the laboratory drainage system. The leak-proof, labeled container may also be autoclaved, sealed and placed into a waste cart for pickup. (Do not pour melted agar into sink or floor drains. Allow it to cool and solidify for disposal as a solid waste.) Liquid waste may also be decontaminated by treating with an appropriate disinfectant. The amount of contact time will depend on the chemical used and the material.

Follow these disposal procedures for **solid infectious waste**: (See flowchart to determine if autoclaving is needed)

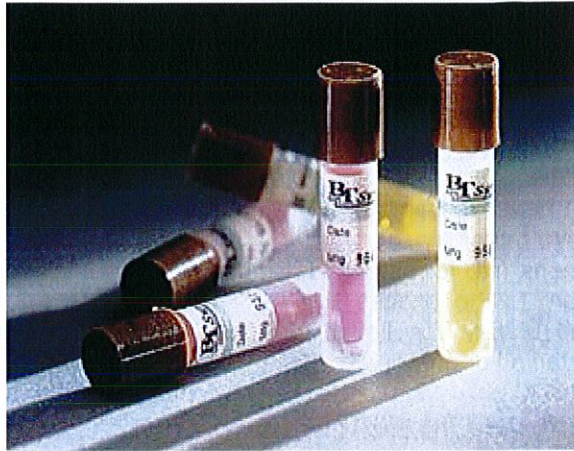
1. For sharps (like needles, scalpels, pipette tips or breakable glass materials like slides, glass tubing or vials): collect the infectious waste in a puncture-resistant Red Biohazard plastic box or bin. *Cardboard boxes may be used for disposal of sharps which are dry (no associated liquid) and which do not require autoclaving.*
2. Use plastic red biohazard bag for collection of soft or non-sharp materials (used gloves, paper towels or plastic ware). Bags should be stored inside a plastic or metal receptacle labeled and lidded when in use in the lab.
3. Waste that does not need to be autoclaved should be placed directly in a red bag and sealed for pickup.
4. Soft solid waste to be autoclaved should be collected in orange autoclave bags.
 - a) The orange bag should be placed in an autoclave-resistant tub and autoclaved by the user, cooled and then placed into a new red outer bag and sealed for pickup.
 - b) The ECU waste technicians have been trained to recognize orange autoclave bags placed into a red bag as "autoclaved". (They will not pick up orange bags unless placed into a red bag.)
5. Ultimately only sealed sharps containers and waste in sealed red biohazard bags should be placed into the biomedical waste cart. Contact hazardous waste technician for information regarding pick up process at 744-DUMP.



Questions?

Contact Biological Safety at 744-2237 or 744-3437.





Biological Indicator Spore Testing Procedure

- Biological indicator spore testing is performed quarterly on all research autoclaves used for biological waste, sterilizing glassware or any other research related uses as outlined in the ECU Biological Safety Manual (Chapter 5, page 49).
- Without biological indicator testing, adequacy/ efficacy of the sterilization process cannot be assumed.
- Ampules containing *Bacillus stearothermophilus* are used for this testing, due to its resistance to heat, to measure biological performance.
- These ampules may be purchased from Comparative Medicine (Courtney Silliman, 744-2452 sillimanc@ecu.edu).
- These ampules may also be purchased directly from Medical Storeroom or from different vendors such as Fisher Scientific, VWR, or 3M. Make sure you are purchasing *Bacillus stearothermophilus* biological spore indicators. You can search "biological indicator" on these given sites and find results.
- OPTIONS:
 - If you decide to buy your own ampules, and then have Comparative Medicine incubate, read and record the results their charge to you is \$2.
 - The cost is \$5 if you purchase the ampules from Comparative Medicine AND have Comparative Medicine incubate, read and record the results.
- QUESTION: How many ampules will be needed per year? Most ampules have a shelf life of 48 months. You will need one ampule per autoclave per quarter as well as one control. If a researcher has one autoclave they will need 8 ampules total per year (1 ampule per quarter + 1 control ampule x 4(quarterly) =8).
If a lab has 3 autoclaves they will need 16 ampules for the year (1 ampule per autoclave per quarter (3x4 = 12) +1 control used for all 3 autoclaves once quarterly (conducted at the same time) 12+4=16).
- A log should be created and kept by the lab to record the results (see example below). This log will be checked during yearly biosafety lab inspections to ensure that this biological indicator testing is being conducted quarterly.

- If you purchase your own ampules and do all the incubating, reading and recording yourself, there should be a set of instructions included with the ampules, similar to these (follow protocol that comes with your specific ampules):
1. Every quarter perform the autoclave QC test.
 2. Place *Bacillus stearothermophilus* vial in the center of the bag, or autoclave (if autoclave strictly used for sterilizing glassware/labware/instruments). (Do not place vial into concentrated culture materials being inactivated or other waste material that should not be re-handled).
 3. Process the load using normal operating procedures.
 4. Compress plastic vial to break glass ampule inside (this releases a bacterial growth media).
 5. Incubate *B. stearothermophilus* at 55-60°C for 24-48 hours (see specific protocol included with ampules).
 6. Incubate a control vial that has not been autoclaved, media should turn yellow to indicate growth. If the control vial remains purple, there may be a problem with the batch of indicator vials and the test may not be valid. Repeat the run, and if the result is the same obtain a new set of test vials. Return the waste to the bin as in step 8 below and re-run with a new batch of vials.
 7. If the test vial media is purple after incubating 72 hours, sterilization is successful, there was no growth.
 8. If the media turns yellow the bacteria grew, sterilization failed.
 - a. Review the run chart to see if the physical conditions (time, temperature) were met. If they were, discontinue using the autoclave, return the waste to a biological waste bin, and contact the service provider to get the autoclave repaired.
 - b. If the conditions were not met, repeat the run with a new test vial.
 - c. Retest after the repairs are completed.
 9. Record all results and retain in the Autoclave logbook (see example below).

**EAST CAROLINA UNIVERSITY
DEPARTMENT OF COMPARATIVE MEDICINE
AUTOCLAVE/STERILIZER
MONITORING PROGRAM**

MONTH/YEAR _____

LOCATION	CONTACT PERSON	DATE RECEIVED	CONTROL	DATE OF RESULTS	RESULTS	COMMENTS
LSB 2nd Floor Surgery	Peggy Pittman					
LSB Ground Bar Primus	Ryan Blum					
LSB Ground Bar Steris	Ryan Blum					
LSB020 Hall Quarantine	Tammy Barnes					
GW-43	Aaron Hinkle					
Rawl 209 - Ragsdale Annex	Tuan Tran					
3S28	Cindy Kukoly					
AMSSN63	Teresa Roebuck					
AMSSN88	Teresa Roebuck					
GET5N67	Teresa Roebuck					
VERN5N67	Teresa Roebuck					
5S29	Meg Shaver					
6S25	Jackie Masterson					
6S07	Jackie Masterson					
6S28	Jackie Masterson					
7N49	Joannie Zary					
	Dr Virag's lab					
16 total						

Personal Protective Equipment

Personal protective clothing supplements engineering controls, but does not substitute for them. Individuals will be provided and instructed on use of appropriate personal protective equipment by the Principal Investigator. Personal protective equipment is provided at no cost to the employee, is readily accessible and includes items such as: gloves, gowns, laboratory coats, face shields or masks, and eye protection. If an individual is determined to be unable to use certain protective devices, an alternate will be identified or they will be prohibited from performing the hazardous activity. It is the responsibility of the Principal Investigator to ensure that the protective equipment is being used consistently appropriately.

Gloves must be worn when handling blood, other potentially infectious, or biohazardous material, and when handling or touching contaminated items or surfaces. Disposable single use gloves are replaced as soon as possible when visibly soiled, torn, punctured, or when their function as a barrier is compromised. Disposable gloves are never washed or disinfected for reuse. Utility gloves may be disinfected for reuse if the integrity of the glove is not compromised; they must be discarded if they are cracked, peeling, discolored, torn, punctured, or exhibit any sign of deterioration.

Disposable, well-fitting gloves made of Latex, Nitrile or other material will be worn. Double gloving should be used when working with sharp instruments and infected animals or with materials known to be infected with HIV. If a glove is torn/cut or has a leak, the individual must

immediately remove the gloves and wash his/her hands with a disinfectant solution or soap. Gloves should be removed so as to avoid contamination of the hands turning exposure side inward. Used gloves are handled as contaminated waste and autoclaved with other laboratory wastes before disposal (BSL-2 or BSL-3). Nitrile gloves are more resistant to chemicals and solvents than latex. Use of latex may lead to allergic sensitization and should be avoided when possible.

Laboratory employees with open cuts or abraded skin are required to keep these surfaces covered with a dressing. Employees with wounds that are weeping, purulent (pus-exuding) or who have open wounds on the hand which require a dressing and preclude hand washing, will not work in the laboratory, animal, or patient care settings until healed.

Masks and eye protection are worn whenever procedures generate splashes, spray, droplets, or aerosols or there is a potential for eye, nose, or mouth contamination. Mask and eye protection may be discarded after use (disposable) or disinfected (reusable) after each use.

Laboratory coats, gowns, aprons, clinic jackets, or similar outer garment must be worn where there is a potential for biohazard exposure to the arms and body. All personal protective equipment is removed before leaving the work area, and/or placed in an appropriately designated area or into a container for storage, washing, decontamination, or disposal. Reusable protective clothing, such as laboratory coats or gowns must be laundered by a professional laundry service or on the premises by trained individuals. Employees are not to take home potentially contaminated personal protective clothing to launder at home. Disposable protective clothing is discarded after each use treated as biohazardous waste.

Eyewash

Eyewash facilities are required where procedures creating splashes or aerosols are likely, especially if concentrated cultures of high hazard biological materials are used.

Personal eyewash water bottles using deionized water must be changed and automatic spigots must be flushed on at least a monthly basis. Personal eyewash water bottles should be disinfected and rinsed at least annually. The date of cleaning and disinfection is recorded on the bottle. If a plumbed eyewash is installed, it will be flushed and checked regularly.

Housekeeping

All equipment and working surfaces will be cleaned and decontaminated upon completion of procedures, after contact with blood or other potentially infectious materials, after any spills or **at least daily**. Initial cleaning with detergent for physical removal of any concentrated organic material is followed by wiping with an approved disinfectant for the indicated contact time per the manufacturer's label. Protective coverings, such as absorbent paper, are removed and replaced when overtly contaminated or at the completion of daily procedures, whichever occurs first. All receptacles such as bins, pails, or cans intended for reuse, are inspected and cleaned/decontaminated on a regular basis.

Broken glassware will be cleaned up using mechanical means, such as brush, broom, dustpans, tongs, forceps, etc, not by hand. Equipment which may become contaminated with blood or other biohazard infectious materials shall be checked and decontaminated on a routine basis and prior to servicing or shipping.

Areas using biohazardous agents will be cleaned by laboratory personnel on a regular basis using soap or detergents followed by an approved disinfectant. Cleaning schedules will be established by the Principal Investigator.

Housekeeping and Facilities Services personnel should only enter laboratories using BSL-1 or 2 agents at times when these materials are not in use and have been safely stored out of their way to avoid contact or mishaps. The PI will communicate any access restrictions to their housekeeping personnel. Laboratory entry doors should be kept closed during work with BSL-2 agents; housekeeping personnel will be trained to knock if a laboratory door is closed and not to enter unless the lab staff verifies that the agent is not in use.

Biohazardous Waste

Sharps

Sharps will be disposed of in puncture-resistant, closable, leak proof, biohazard labeled or red/orange color-coded containers. Sharps containers will be easily accessible to personnel and located close to the work area. Containers should remain upright and not be overfilled. Close and seal the container when 2/3-3/4 full and transport to the designated biohazard waste pickup site. Used needles shall not be recapped, bent, or sheared, and will be deposited whole into the appropriate sharps containers. Use of safety sharps is required unless justified in writing in the Biological Safety Registration as incompatible with the procedure to be performed.

The following items should always be placed in approved sharps containers, **not** placed into biohazard waste bags/or soft-sided bags or bag-lined receptacles.

- Glass Ampules
- Butterfly Units
- Capillary Pipettes
- Capillary Tubes
- Cover Slips
- Microscopy slides
- Microtome Blades
- Needles
- Pasteur Pipettes
- Razor Blades
- Scalpels
- Vacutainer-needle combo

Biohazard Waste

All contaminated or potentially contaminated materials will be placed into red biohazard bags/containers, which are closable, leak proof, labeled "biohazard", the bag container will be closed prior to removal. If the outer surface of the container becomes contaminated, it shall be placed in a secondary container meeting the same standards and secured before removal. Remove the sealed red bags or boxes from the lab to the designated site for pickup by the Biohazard Waste Techs.

If biohazardous waste is to be autoclaved, it is placed into an **orange autoclave bag**, and autoclaved as described. The orange autoclave bag is then placed into a red biohazard bag, and taken to the designated waste pickup site. This red bag over orange indicates completion of autoclaving to ECU Hazmat Techs.

Call the Biohazard collection technician at 744-DUMP for pickup or to replenish red biohazard bags at the pickup site.

Waste Always Considered Biohazardous

- Any waste that is grossly soiled with biohazardous agents (culture plates, culture media, gram stain slides) or other concentrated infectious or biohazardous material (used pipette tips, specimen vials, pipettes, or cell cultures), human blood or other potentially infectious materials (Serous, plural, joint, pericardial, peritoneal and vaginal fluids, semen, unfixed human tissue or human cell cultures) or primate blood, tissue or fluid.

Specific examples of biohazardous waste items generated in research

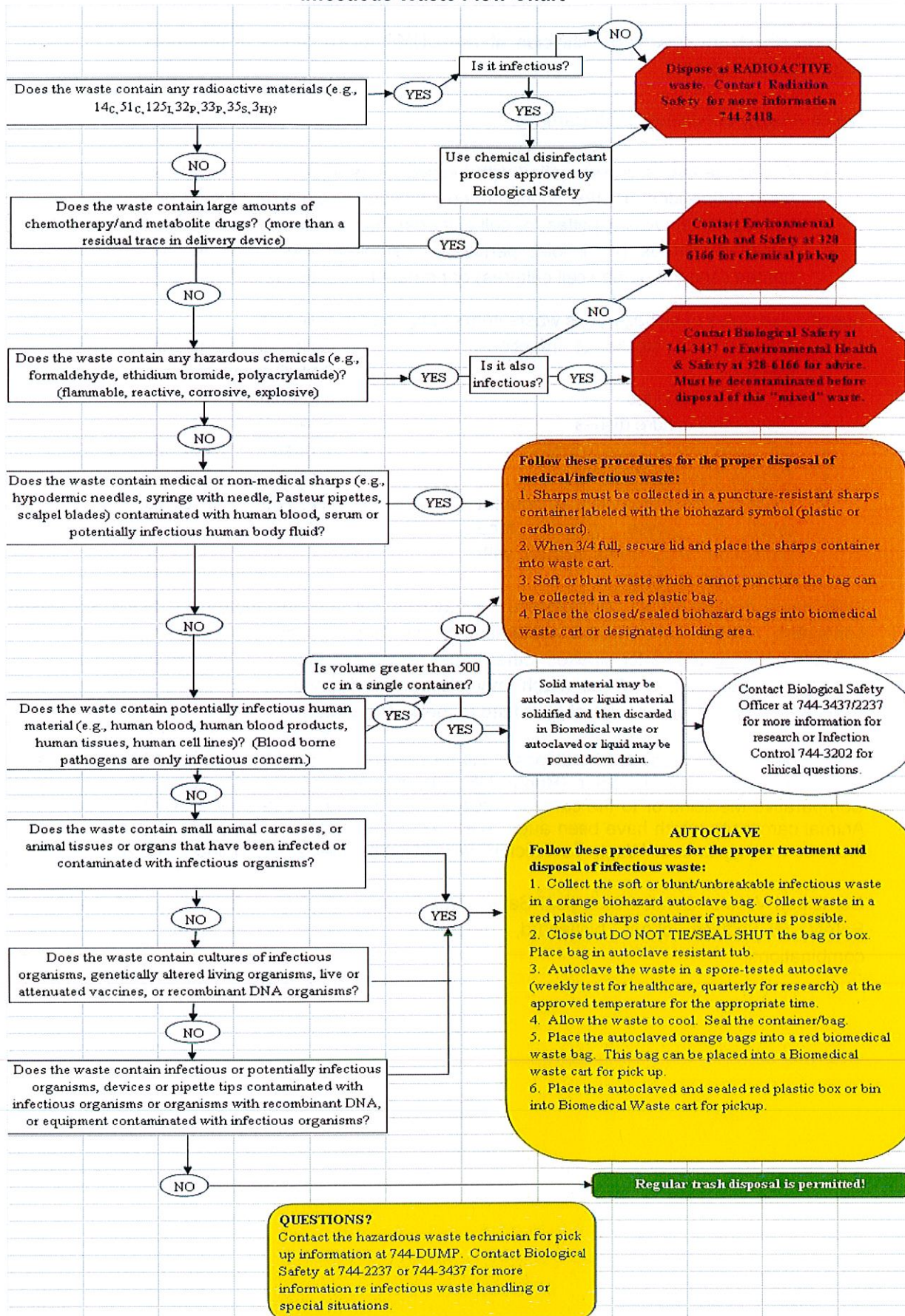
- | | |
|---|--|
| • Cultures and stocks of infectious agents | • Soiled dressings and sponges |
| • Discarded vaccines | • Soiled tissue papers used to wipe pipette tips |
| • Disposable culture dishes | • Specimens |
| • Drainage sets or Lavage tubes | • Transfer and inoculation devices |
| • Organs/Tissues of animals | • Tissue culture-human or animal |
| • Used personal protective equipment (such as gloves, gowns, face protection, etc.) contaminated with biohazardous agents | • Used specimen containers |

Only waste designated as “biohazardous” should be put into designated red biohazard waste containers. Items not biohazard contaminated should be discarded in the regular trash; this includes paper wrappers for pipettes, other paper refuse and other non-biologic material which is handled without gloves. (It is much more costly to dispose of biohazard waste, so non-contaminated materials should not be added to the waste stream.

Uninfected animal carcasses for disposal will be placed into durable, leak proof containers and delivered to the walk-in cooler (Room GE 23) in the School of Medicine, Department of Comparative Medicine or to the carcass freezer in the Biology Department Animal Facility room. Animal carcasses which have been autoclaved to inactivate agent or toxin are stored for disposal there as well. Hazardous Waste Technicians will collect the carcasses for incineration.

Combined Waste: Consult Biological Safety &/or Radiation Safety or Environmental Health and Safety for advice about biohazard, hazardous chemical, and/or radioactive materials combinations.

Infectious Waste Flow Chart



Laboratory Inspections

Biological Safety will conduct routine surveys of all work areas using biohazardous agents, to verify the practices, procedures, and equipment are in place and in use and personnel are trained and prepared. The frequency of these surveys is based on the level of hazard and the quantity of material being used and typically occurs annually. New laboratory or work areas or those previously found to be non-compliant with the measures set forth in this manual may be evaluated more frequently in order to verify that safety measures have been implemented. Safety violations discovered during routine surveys will be addressed according to the following procedure.

Violations immediately dangerous to life, health or environment. Any operation causing a biological hazard (situation immediately dangerous to life or health) will be suspended immediately by Biological Safety. The situation will be promptly reviewed by the Biological Safety Officer and the Biological Safety Committee Chair with the Principal Investigator and Department Chair. Work may not resume until the hazard is satisfactorily addressed. The dean and/or VC for Health Sciences will be notified if the situation is not remedied.

Violations not immediately dangerous to life or health.

Verbal Warning: If, during a routine inspection, a problem involving biological safety procedures is observed, a verbal warning will be issued to the individual and a record of the verbal warning will be noted by the Office of Prospective Health/Biological Safety. Upon receipt of this verbal warning, the laboratory staff should take immediate steps to correct the problem. No verbal or written is required from the laboratory.

Step Two: If, within a six-month period of the verbal warning, the same biological safety problem is observed, written notification of the problem will be sent to the Principal Investigator responsible for the laboratory. The Principal Investigator will then be charged with correcting the situation. Written response is required.

Step Three: If the problem is observed for a third time, both the Principal Investigator and the Department Chair will be given a written notice of the situation. The situation will also be presented to the Biological Safety Committee to consider further consequences or other measures.

Biosecurity

The need for a Biosecurity program and extent of the security provisions will be based on the possible impact of the theft, loss, diversion, or intentional misuse of the materials. Posting the name of the agent used on the laboratory door may be forgone upon request of the Principal Investigator with concurrence of the Biological safety Officer or IBC if posting is likely to draw attention to its presence or create a security hazard. (See Select Agents for more on Biosecurity).

Chapter 6

SPECIFIC BIOHAZARDOUS AGENTS ISSUES

Visitors: Anyone who is not an ECU employee or registered student in a curricular experience is considered a visitor. See the BSOM policy regarding visitors and allowable activities/ exposures for one school's approach.

- a. *Short-term students and non-essential visitors* to the laboratory will not be exposed to potentially hazardous material unless they are fully trained in safe procedures, equipped and immunized per the safety plan of the laboratory. All personnel, including short-term students and non-essential visitors, will be enrolled in the medical surveillance program as indicated by the level of hazard in the laboratory, as recommended by the Biological Safety Committee.
- b. *Essential visitors* include visiting faculty, visiting scientists or other professionals who will present evidence of relevant training or immunization from their home institution, which may be supplemented with site-specific training.
- c. *A student participating in a formal ECU enrichment program* who is under age 18 may observe the use of infectious agents after training and immunization and may perform low hazard procedures under supervision, but should not perform high hazard procedures. Use of BSL-1 is recommended.
- d. *Non-essential visitors and children under age 18* should not be allowed access to a laboratory where infectious or other biohazardous agents may be present. Non-essential visitors include casual visitors, friends, acquaintances and family, not associated with a specific curricular or other formal program.

NOTE: Minors may visit a laboratory in an organized tour if: 1) it is department sanctioned; 2) parental consent is obtained; 3) the tour is well supervised; 4) there is not potential for hazardous exposure by direct contact or aerosol.

Visitors who are Minors

- Minors, who participate in formal enrichment programs at ECU and have some high school education, may enter labs using biohazardous agent if special conditions are met (See Visitors Above). Principal Investigators must be aware of the following special provisions established for the safety of individuals less than 18 years of age, and minimize their exposure to hazardous agents by selective assignment of minors to activities with low risk potential.
- Written parental consent is required for individuals under 18 years of age to participate in such organized enrichment programs at ECU, such as in teaching laboratories or advanced placement or summer science learning programs. It is strongly recommended that minors' direct, hands-on work be limited to BSL-1. Work at BL-2 requiring training or immunization will not occur until all training and immunization has been provided by the PI and full comprehension is verified and documented.
- No minor shall be permitted to work with open containers or dispersible forms of biohazardous agents.
- No minor shall work in the vicinity of any source of biohazardous agents without the immediate and constant supervision of an adult who is familiar with all applicable safety practices and ensures these practices are strictly followed (This allows for observation of BSL-2 work in a controlled and supervised setting).
- Work in labs at Level BL-3 or 4 is never appropriate for a minor.

Animal Use

The Animal Care and Use Committee oversees the use of vertebrate animals in research or teaching at ECU. This includes laboratory and field collected animals. Natural infections will be controlled by use of pathogen free animals in the laboratory, whenever possible, and by a broad program of optimum veterinary care for all animal subjects. Exposure to zoonotic infections will be controlled by appropriate experimental design, techniques, containment equipment, and building engineering systems. Employee and student exposure to infectious or other hazards associated with animal and animal tissue contact will be minimized by following the prescribed animal care and use SOP's and training. Occupational Health and employee training is an integral part of the prevention program.

The Animal Care and Use Committee and Biological Safety communicate formally and informally. Biological Safety is represented on the Animal Care and Use Committee and Comparative Medicine is represented on the Biological Safety Committee.

The Office of Prospective Health provides health reviews, physical examinations, medical surveillance, or immunizations to individuals exposed to experimental animals as part of the ECU Animal Care and Use Committee's program of education and Occupational Health monitoring.

Pregnancy

Exposure to teratogenic biological agents may be of concern to fertile employees of either sex, or such exposure should be minimized. The concerns of the pregnant employee will be evaluated and addressed on a case by case basis.

Any female (or male) working with biohazardous agents who knows, suspects, or is trying to become pregnant or to father a child should contact Prospective Health as soon as possible to obtain information on pregnancy and any known risks from the biohazardous agents used. A confidential meeting will be scheduled to inform the employee/student of the special risks associated with reproduction and their specific biohazardous agent exposure. If the agent has adverse effects, modification of job duties to avoid exposure may be recommended. Human Resources may be consulted if restrictions during pregnancy cannot be accommodated by the Lab director or supervisor.

SPECIAL INFECTIOUS AGENT ISSUES

Transmissible Spongiform Encephalopathy Agents (Prions)

Prions are the causative agent of CJD and a variety of transmissible spongiform encephalopathies of the human and animal central nervous system. Prions are resistant to conventional uses of heat and/or chemical germicides for sterilization of instruments or equipment. Concentrated NaOH (1N) solutions are used for disinfection. Consult Biological Safety directly if use of prions is planned.

Chapter 7

Spills, Exposures and Emergencies

Detailed Cleanup Guidelines: *The cleanup procedure for a spill is dependent on the nature of the biohazardous agent, the location of the spill and the exposure route for infection. The following general guidelines are offered.*

Small Biohazardous Spill

1. At minimum gloves and a lab coat, will be worn when cleaning up biohazardous spills. Additional personal protective clothing may be indicated, such as goggles, fluid resistant body protection, face shield, or possible respiratory protection.
2. Paper towels or other absorbent materials should be placed over liquid spills, to absorb any excess fluid or remove organic materials. Detergent or soap may be used for this step. Discard these materials into a biohazardous waste container.
3. Pour an appropriate disinfectant solution (e.g. 1:10 dilution of household bleach) slowly to the outer margin of the area and allow it to flow in, using care not to cause spatter. This method helps to minimize aerosolization.
4. Let bleach solution stand for 20 to 30 minutes to allow adequate contact time for disinfection (or follow manufacturer's specific directions for commercial product) then clean up with more paper towels.
5. Discard all contaminated materials (paper towels, gloves, and other wastes from clean-up) into an autoclave bag and autoclave.
6. Wash hands for at least ten seconds with soap and water after removal of gloves at completion of cleanup; use mild antibacterial soap if available

Large Biohazardous Spill

("Large" based upon material volume virulence or possibility of airborne infection)

1. If airborne infection is possible, and respiratory protection with N-95 masks or powered air-purifying respirator is not being worn, leave the room immediately, and close the door.
2. Notify supervisor and warn others not to enter the contaminated area. Post a temporary warning sign on the door. If the spill exceeds the capacity of the lab to handle in the supervisor's assessment due to lack of training or PPE, occurrence outside BSC, or risk of aerosol transmission, Biological Safety assistance in cleanup may be needed. Notify the Office of Prospective Health/Biological Safety.
3. Biological Safety will determine whether laboratory staff or Biological Safety will perform the cleanup. If Biological Safety directs lab personnel to perform the cleanup, wait at least 30-60 minutes to re-enter the lab to allow dissipation of spill-created aerosols based on the room ventilation and air changes per hour.
4. Appropriate personal protective equipment, such as lab coats, masks, and double gloves, should be worn before reentering the room. Additional personal protective clothing, such as splash goggles, fluid resistant body protection, face shield, and respiratory protection may be needed, depending on the Biosafety level and nature and source of the material.
5. Paper towels or other absorbent materials soaked with an appropriate soap or detergent disinfectant should be placed over liquid spills, to absorb any excess fluid and remove organic waste. Discard these absorbent materials into a biohazard waste container.
6. Pour an appropriate disinfectant solution (e.g. 1:10 dilution of household bleach) slowly to the outer margin of the spill and allow it to flow in. To minimize aerosolization, avoid pouring the disinfectant solution directly onto the spill.

7. Let bleach solution stand for 20 to 30 minutes to allow an adequate contact time for disinfection (or follow manufacturer's specific directions for commercial product). Then clean up the waste with more paper.
8. Disinfect any equipment which has been contaminated using appropriate method or discard contaminated equipment which cannot be disinfected.
9. Use an autoclavable dustpan, squeegee, or forceps to remove any sharp materials. Transfer all contaminated materials (paper towels, glass, liquid, etc.) into an autoclave bag or sharps box and autoclave according to standard directions.
10. Wash and mop adjacent area and spill area with an appropriate disinfectant-detergent solution.
11. Remove and discard protective clothing and gloves and place in biohazard bag.
12. Remove any contaminated garments and place into a container for autoclaving. Thoroughly wash your hands and face with soap and water. Shower using soap and water if agent penetrated clothing or contacted the body.

Biohazardous Spill Inside a Biological Safety Cabinet

Chemical decontamination procedures should be initiated at once while the cabinet continues to operate to prevent escape of contaminants from the cabinet. Be careful with paper towels, which can be sucked into the blower fan or HEPA filters. For concentrated culture material or a spill greater than 1-5 ml or if spill spreads outside the BSC, Consult the Biological Safety Officer before cleanup in case respiratory protection may be needed.

1. Wearing gloves, spray or wipe walls, work surfaces, and equipment with an appropriate disinfectant-detergent combination to remove organic substances
2. If cabinet is Class II, flood the top work surface tray and the drain pan below the work surface with a disinfectant and allow to stand for at least 20 minutes.
3. Wipe excess disinfectant from the tray. For Class II cabinets, drain the tray into the cabinet drain pan, lift out tray and removable exhaust grillwork, and wipe off top and bottom (underside) surfaces with a disinfectant. Replace grillwork and drain disinfectant from the drain pan into an appropriate container and autoclave according to standard procedures. Gloves, cloths or sponges are discarded into biohazardous waste containers and autoclaved.
4. Wash hands for at least ten seconds with soap and water after glove removal; use antibacterial soap if available

Emergency Response to Human Blood & Body Fluid Exposures or to other potentially infectious material

1. For cutaneous exposures to *intact* skin the area should be cleaned immediately with antibacterial soap and water.
2. For cutaneous exposures involving *broken skin* (either chapped, abraded, afflicted with dermatitis), *needle sticks or cuts*, Wash the area thoroughly with an antimicrobial soap and water, dress the area with bandages and/or gauze if needed.
3. *For Mucous membrane exposure*
 - For exposure to the eyes, flush immediately with water for twenty minutes.
 - For mouth exposures, rinse with water and spit out several times (e.g. 3 or more).

Follow-up Treatment and/or Monitoring

Report the exposure ASAP to Prospective Health for follow-up treatments and monitoring based on nature of exposure and agent. Prospective Health will provide post exposure prophylaxis for HIV exposure during the workday. If exposure occurs after-hours to HIV infected material, prophylactic antiviral medication may be indicated and should be initiated ASAP. This can be obtained from VIDANT MEDICAL HOSPITAL Emergency Department during evenings and weekends Prospective Health will provide long-term follow-up to the emergency treatment. Medical surveillance will be provided for the duration of the incubation period of the agent.

Animal Exposures

Bite or Scratch by Dogs, Cats, and Wild Captured Animals

Rabies is enzootic in wild animals in North Carolina. In 2006, ECU Department of Comparative Medicine adopted a policy of using only "purpose bred" animals at ECU and suspended use of pound source animals which might carry incubating rabies infection. As a result, users of dogs, cats, ferrets at ECU are no longer required to be immunized routinely

Rabies vaccination is recommended for long-term field research personnel. Who should avoid skin contact with dead animals or live carnivores, and use standard precautions for all animal contact.

Rabies infection should be considered after exposure to a feral dog or cat, or to any wild caught animal especially carnivores. This is a particular hazard for investigators exposed to body fluids or neural tissue. If an injury or other animal exposure occurs, the exposed worker should be evaluated ASAP by Prospective Health or VIDANT MEDICAL HOSPITAL Emergency Department. Bats have transmitted rabies without a history of recognized bite incident. The Comparative Medicine veterinarian, may be consulted to ensure that the animal is quarantined for rabies observation or the brain examined by the North Carolina Public Health Laboratory.

Rabies Protection Policy

1. If wild animals or pound source dogs/cats are used for research, or contacted in field work, all workers, including those handling tissues in the laboratory, must be immunized against rabies and have laboratory confirmation of protective antibody titer.
2. Bites or scratches from a wild animal or a feral or pound source dog/cat must be immediately reported to a staff veterinarian or principal investigator and the exposed person must be evaluated by Prospective Health.
3. Wild animals susceptible to rabies (skunks, raccoons, foxes, etc.) or carcasses must be handled, in the laboratory or the wild, as if infected.
4. In wild animals or feral dogs or cats, any abnormal behavior could be a sign of rabies, and should be immediately reported to a staff veterinarian.
5. If a worker is exposed to animal blood or body fluids- especially saliva, or other tissue- especially neural tissue, via a bite, scratch, cut, needle stick, or splash onto broken skin, the site should be washed thoroughly with disinfectant soap and the worker should report ASAP to the Prospective Health or the VIDANT MEDICAL HOSPITAL Emergency Department. If body fluid contacts a mucosal surface, rinse thoroughly with saline or clear water and then report to Prospective Health or VIDANT MEDICAL HOSPITAL Emergency Department.

Bite or Scratch by Laboratory Animal of Any Species

Animals frequently carry significant pathogens as normal flora of the oral cavity: *Streptobacillus moniliformis* in rats, *Pasteurella multocida* in cats and dogs, and *Capnocytophaga canimorsus* in dogs. These agents are capable of causing cellulitis and regional lymphadenitis in normal individuals or septicemia and death in immunocompromised workers. If bite or scratch occurs, consult Prospective Health. Prospective Health will ascertain the employee's immune status, provide appropriate instructions for the injured employee or administer antibiotics as needed.

Exposure to Macaques:

Herpes B virus naturally infects macaque monkeys resulting in a disease similar to herpes simplex B virus in humans. B virus infection is usually asymptomatic or mild in macaques but can be fatal to humans. Simian B virus may be spread by animal bite, splashes of body fluid, or percutaneous inoculations with infected material. Diffuse encephalomyelitis may result.

The ECU Animal Use SOP for macaques includes strict use of PPE (minimum ABL-2) for all contact with live monkeys and tissues and a detailed protocol to be followed for exposure events. Contact the PI, staff veterinarian and Prospective Health ASAP per the SOP if an exposure event occurs. Non-human primate tissues are handled with BL-2 protections.

EXPOSURES AND EMERGENCIES

Emergency Phone Numbers

Emergency/All Hazards (**ECU Police will handle the call and interface with community Police, fire and rescue.**) All after hour calls are directed to ECU Police who will who contact Biological Safety or Radiation Safety.

911

For non-emergencies @ BSOM

744-2246

ECU Office of Prospective Health (8am-5pm)
(For blood/body fluid or other infectious exposures)

744-2070

Exposure Hotline (5pm-8am) Vidant Medical Center
(For recorded advice on human blood/body fluid exposures after-hours procedure)

847-8500

Infection Control

744-3202

Biological Safety

744-2237/3437

Radiation Safety

744-2070/2418

ECU Environmental Health & Safety
(Chemical Exposures/Workers Compensation)

328-6166

City of Greenville Fire & Rescue *

329-4390

City of Greenville Police *

329-4315

NC Highway Patrol *

752-6118

Pitt County Emergency Management *

902-3950

- Will be contacted by ECU Police if needed

Chapter 8

OCCUPATIONAL HEALTH SERVICES

Prospective Health provides occupational health services as a component of the ECU Biological Safety and Employee Health Programs.

Initial Exams

1. All new BSOM faculty, and employees complete a basic Health History and immunization review
2. Animal users complete a health history when trained at the Animal Use course and every 3 years. They are sent an annual reminder to notify Prospective Health if interim changes in health status have occurred. Authorized visitors will complete a health history form as well.
3. The Prospective Health reviews the Health History Forms. If there any problems related to occupational duties or exposures are identified a visit with the physician/ extender will be scheduled.
4. Persons with a history of an immune suppressing condition or who are taking chronic immunosuppressive medication will be advised of the potential health risks of exposure to infectious material, such as experimental animals, human tissues, or known pathogens. Recommendations for modification of job assignment and accommodations are made if necessary.

Immunizations

Employees, students and authorized visitors will be given immunizations and/or skin tests as required based on their occupational risks. These may include:

1. Tuberculosis skin testing (PPD), repeated annually (i.e. work exposure to patients or nonhuman primates). Individuals with a prior history of positive TB skin tests will have a baseline chest radiograph. Individuals with a history of BCG immunization will be given skin tests unless they have a history of severe positive reactions. Persons with past positive skin tests will be monitored for symptoms.
2. Tetanus vaccine is administered every 10 years to laboratory staff and animal users and a booster administered post-injury if needed.
3. Rabies vaccine will be no longer required for all animal users (dogs, cats, ferrets) as purpose bred animals are used exclusively at ECU. Selected employees who may have contact with wild animals or animals at other institutions will be continued in a rabies immunity surveillance program.
4. Hepatitis B vaccine if indicated by exposure to human cells, tissues or body fluids or animals injected with human materials (provided to all Comparative Medicine animal staff).
5. Measles/Mumps/Rubella and Varicella as indicated by exposure to agent or susceptible animals like non-human primates (marmosets are susceptible to mumps and Biosafety). Varicella as indicated per department policy or nature of research.

Periodic Monitoring

Periodic monitoring evaluations are designed to:

- To detect changes in an individual's health that might indicate the need for a change in job placement or in the work process.

- To detect evidence of adverse effects from exposure to biohazardous agents, the presence of sub clinical infections, and/or exposures to chemical toxins or other physical hazards.

Typical periodic monitoring evaluations may include the following:

- Annual request for update of health status by animal users.
- Tri-annual review and update of health histories by animal users.
- Annual review and update of immunizations or TB skin tests for BSOM, Comparative Medicine.
- Physical exams as indicated for job, exposure, or health status change.
- Completion of laboratory tests if indicated for serologic surveillance or post exposure treatment.

Serologic Surveillance

Exposure to high-level infectious agents (BL-3 or 4) or other hazards, may trigger a need for serological surveillance. Historically, serum samples were collected and banked by the P.I. and maintained frozen for the duration of the individual's stay, and for a period of 10 years thereafter and for use as a baseline specimen if an exposure event occurs.

The use of serologic surveillance is controversial and fraught with concerns about the possession, use, maintenance, and disposal of frozen specimens. Prospective Health does not have serum banking capability.

An alternative approach to serologic surveillance is the collection of baseline and period specimens for routine serologic surveillance and collection of baseline and interval specimens post exposure. This approach is the preferred approach at ECU at present.

Exit evaluations may be performed at the termination of employment, or authorized visitation. Based on specific hazard exposure. Exit evaluations might include:

- Updating of health history.
- Completion of a physical exam.
- Collection of a serology specimen
- Exit TB Skin test

Employee Records

Health records obtained from employees, and authorized visitors as a result of occupational evaluation, illness, or injury, will be kept confidential and maintained by the Office of Prospective Health. Access to records will be limited to authorized personnel per state and federal law. Records will not be released to anyone without the individual's written consent, except in situations required by law. All medical and exposure records will be maintained for the duration of the individual's employment, plus 30 years. Employee training records, will be maintained by Prospective Health for 5 years. The Principal Investigator will maintain procedure and agent-specific training records for individuals working in their labs.

Response to Exposures

Exposures can arise from splashes of liquid onto mucous membranes or broken skin, needle sticks other cuts or scrapes which break the skin or animal bites. First aid:

1. Any bleeding should be controlled;

2. The wound(s) should be thoroughly cleaned with soap and water or a disinfectant soap if available.
3. Eyes, mouth, or nose should be rinsed with saline or tap water for 10-15 minutes

The incident should be reported to Prospective Health immediately. If exposure occurs after work hours, the employee should go to Pitt County Memorial Hospital Emergency Department for urgent wound care, taking treatment information sheet from lab if available. Report to Prospective Health on next working day to arrange for follow-up.

Students who are BSOM medical or graduate students are treated by Prospective Health for illness or injury related to specific curricular exposures. Student Health Service provides treatment for all other ECU students' curricular illness or injury. Prospective Health will provide consultation/advice for uncommon conditions related to biohazard exposures. Prospective Health forwards information sheets to VIDANT MEDICAL HOSPITAL ED regarding the emergency treatment of usual laboratory exposures, but the employee is also encouraged to bring a copy of this communication with them when using the ED after-hours.

Other Special Medical Considerations

Allergies

Individuals with histories of allergies relevant to work exposures, for example animal allergies in animal users or latex allergy in healthcare workers, will be evaluated individually re occupational risk, and ways to reduce exposures by Prospective Health when signs, symptom as diagnosis of allergy is noted on the health history.

Individuals with histories of animal allergies will be evaluated by ECU Prospective Health Medical Director to determine proper PPE and respiratory protection prior to entering the animal facilities in Comparative Medicine (Brody Ground and Edward Warren Life Sciences Building). If respiratory protection is required, Prospective Health staff will teach individuals on how to properly don/test the respirators or PAPR's (Powered Air Purifying Respirators) and proper care of the PPE and equipment.

Disease Outbreak Evaluations

In the event of a disease outbreak, the following may occur:

1. Exposure/ containment practice/failure will be investigated/documented.
2. Physical examinations will be done.
3. Serum samples will be collected and stored.
4. Lab/ diagnostic tests will be done.
5. Appropriate treatment will be provided or referral made as needed.
6. Report to the Institutional Biological Safety Committee or other appropriate authority.
7. Report to Vice Chancellor for Health Sciences, ECU Workers Compensation Program, and Director of Pitt County Health Department as indicated.

Chapter 9

OCCUPATIONAL HEALTH SERVICES BY AGENT CATEGORIES

The Prospective Health services required for any employee, student or authorized visitor are

- **Preplacement evaluation:** Completion of Basic Health History with further evaluation as needed.
- **Immunizations, screenings, and serum banking:** Tetanus immunization, Hepatitis B vaccine as indicated, TB screening or other immunizations or serology as indicated.
- **Periodic monitoring evaluations:** Annual update of Health History (BSOM)
- **Employee health records:** Maintained Prospective Health.
- **Response to exposures:** Report to Prospective Health for evaluation in the event of work related illness/ injury.
- **Other considerations:** Significant changes in health status should be reported to Prospective Health ASAP.

Exposure to Human Tissues and Body Fluids

- **Preplacement evaluation:** Basic Health History with further evaluation as needed.
- **Immunizations, screenings, and serum banking:** Hepatitis B vaccine, tetanus immunization if indicated. TB screening or other immunizations if indicated.
- **Periodic monitoring evaluations:** TB test if exposure to patients or primates
- **Employee health records:** Maintained in Prospective Health.
- **Response to exposures:** Provide counseling, treatment and follow-up according to protocol in the Blood/Body Fluid Exposure section of this manual. Evaluate other work related illness/ injury.
- **Other considerations:** Significant changes in health status should be reported to Prospective Health.

Exposure to Experimental Animals

Department of Comparative Medicine animal care employees

- **Preplacement evaluation:** Completion of Basic Health History AND Animal User History with further evaluation if indicated.
- **Immunizations and serum banking:** Tetanus for all; measles, rubella, seasonal influenza vaccine, TB test and hepatitis B.
- **Periodic monitoring evaluations:** Annual update of Health History. Tuberculin test annually for nonhuman primate contacts.
- **Employee health records:** Maintained at Prospective Health.

Rabies immunity will be maintained for staff with potential off-campus exposure. (Rabies status review via titer every 2 years and booster as needed).

University employees, including student workers, and students in contact with dogs, cats, sheep, rabbits, rodents, and other non primate vertebrate animals, or wild carnivores.

- **Preplacement evaluations:** Animal Users Health History at the time of employment/ onset of work with animals with further evaluation as needed.
- **Immunizations and serum banking:** Rabies (specified feral animal or wild carnivores contact only) and tetanus immunizations. Others based on specific exposures.

- **Periodic monitoring evaluations:** Health History every 3 years.
- **Employee health records:** Maintained in Prospective Health.
- **Response to exposures:** Report to Prospective Health. See Animal Exposures section for additional details on specific pathogens of concern.
- **Other considerations:** Significant changes in health status should be reported to Prospective Health ASAP.
- **Response to exposures:** Report to Prospective Health. Evaluation as indicated by employee illness or exposure. See Animal Exposures section for additional details on specific pathogens of concern.
- **Other considerations:** Significant changes in health status should be reported to Prospective Health.

University employees, student workers or visitors in contact with non-human primates.

- **Immunizations:** Rubella required; seasonal influenza recommended.
- **Periodic monitoring evaluation:** Tuberculosis test annually, semi-annually for regular contact, at minimum for sporadic contact.

Exposure to Recombinant DNA Molecules in Research Approved by NIH/OBA in Addition to the Institutional Biological Safety Committee

- **Preplacement evaluation:** Completion of Health History Form.
- **Immunizations, screenings, and serum banking:** If indicated.
- **Periodic monitoring evaluations:** Annual update of Health History at BSOM.
- **Employee health records:** Maintained in Prospective Health.
- **Response to exposures:** Report to Prospective Health in the event of suspected work related illness injury.
- **Other considerations:** Health History Form should also be updated to reflect significant changes in health status.

Appendices

A-Z



Appendix A

SELECT AGENT ADDENDUM TO BIOLOGICAL SAFETY POLICY 8/05/10

Special Biological Safety requirements exist for users of Select Agents. The following is offered as a general guideline. Please consult the Select Agent Program information online at Biological Safety or www.selectagents.gov for the most up to date information.

Security

1. Develop and implement a written security plan establishing policy and procedures to ensure the security of areas containing select agents and toxins (Appendix A). Systematically define potential threats and examine vulnerabilities. Mitigate the risks associated with those vulnerabilities with a security systems approach.
2. The plan must describe/contain:
 - A. Inventory control procedures, minimal education and experience criteria for those individuals with access to select agents or toxins, physical security, and cyber security. *(No undergraduate students will have access select agents. No hands on work with select agent until security clearance is received).*
 - B. Provisions for routine cleaning, maintenance, and repairs, *(e.g., no housekeeping or maintenance personnel access without supervision by lab personnel. See Appendix D, suggested signage)*. Provisions for training personnel in security procedures; provisions for securing the area *(e.g., card access, key pads, locks)* and protocols for changing access numbers or locks following staff changes. The Principal Investigator will orient personnel to the organism *(characteristics and health risks)* needed containment level and micro-biological techniques as well as security and emergency response plans.
 - C. Procedures for loss or compromise of keys, passwords, combinations, etc. *(access system for BL-3 or 4 laboratories is strongly recommended. The card reader system will record entries, can delete personnel who leave and can be reconfigured if breached).*
 - D. Procedures for reporting suspicious persons or activities, loss or theft of listed agents or toxins, release of listed agents or toxins, or alteration of inventory records. *(Report suspicious person to Laboratory Director or Principal Investigator or ECU Police; loss, theft or altered records to ECU Police and Biological Safety Officer; release to Biological Safety or employee health for subsequent report to CDC, when verified).*
 - E. Provisions for the control of access to containers where listed agents and toxins are stored *(e.g. lock box for freezer keys)*.
 - F. Provisions for ensuring that all individuals with access, including workers and visitors, understand security requirements and are trained and equipped to follow established procedures.
 - G. Procedures for reporting and removing unauthorized persons. *(Report to the principal investigator or ECU Police. The special security needs of these labs have been discussed with ECU Police and closer surveillance patrols are in place).*
 - H. Procedures for securing the area when individuals approved *(after a background check)* by U.S. Department of Justice are not present *(e.g., card)*. Protocols for changing access numbers or locks following staff changes will be described. All labs with select agents should have doors closed and locked at all times. Officers will report any suspicious events to Biological Safety as soon as investigated.
3. The security plan will be reviewed at least annually by the Biological Safety Officer and/or by the Biological Safety Committee. It will be reviewed after any incident or adverse event occurs. Amendments or additional measures may be requested.
4. Approved Persons
 - A. Approved persons are ECU employees, graduate students or visiting scientists who have access to select agents for research purposes, who are trained as described below and who have passed a background check has been conducted by the U.S. Department of Justice to exclude restricted persons per Appendix B.
 - B. Background checks will be conducted by the U.S. Department of Justice using the personnel lists generated by the Laboratory Director.

- C. Failure to provide the information required to complete such a check will disqualify an individual from work with or access to select agents.
5. The following security requirements are required as a minimum:
- A. Allow unescorted access only to individuals who have been approved and who are performing a specifically authorized function during hours required to perform the defined job (Packaging and shipping of agent for transportation in commerce should be performed only by an approved person.)
 - B. Allow individuals not so approved to conduct routine cleaning, maintenance, repairs, and other non-laboratory functions ONLY when escorted and continually monitored by individuals who have been approved. (Set out trash in red bags for Prospective Health if collection from central repository; autoclave first if required by lab SOP.)
 - C. Provide for the control of access to containers where select agents and toxins are stored by requiring freezers, refrigerators, cabinets, and other containers where stocks of select agents and toxins are stored to be LOCKED (e.g., card access system, lock boxes) when they are not in the direct view of approved staff, and by using other monitoring measures as needed, such as video surveillance.
 - D. Require the inspection of all packages upon entry to and exit from the lab.
 - E. Establish a protocol for intra-entity transfers, including provisions for ensuring that the packaging, and movement from a laboratory to another laboratory or from a laboratory to a shipping place, is conducted by an approved individual.
 - F. Require that each approved individual not share with any other person, his or her keycards or passwords access to the area or agent.
 - G. Require that each approved individual report any of the following immediately to the Responsible Official (Director of Prospective Health):
 - a. Any loss or compromise of their keys, passwords, combinations, etc.
 - b. Any suspicious persons or activities
 - c. Any loss or theft of select agents or toxins
 - d. Any release of select agents or toxins; Note: occupational exposure is considered as a release by the CDC and must be reported to them on Form 3. If a recombinant organism is involved, report to NIH is required as well.
 - e. Any sign that inventory and use records of select agents or toxins have been altered or otherwise compromised.
 - f. The Responsible Official will report these incidents to the Biological Safety Officer (Associate Responsible Official). The ECU Police will also be notified, e.g. items b and c.
 - H. Areas where select agents and toxins are stored or used will be separate from the public areas of the buildings
 - I. Upon termination of the use, a select agent or toxin must be:
 - J. Securely stored in accordance with the requirements of this section; or
 - K. Transferred to another registered facility; or
 - L. Destroyed on-site by autoclaving, incineration, or another recognized sterilization or neutralization process after notification of Biological Safety Officer and HHS.

Emergency response

- A. Develop and implement an emergency response plan that meets the requirements of OSHA Hazardous waste operations and emergency response standard at 29 CFR 1910.120.
- B. The emergency response plan should interface with the ECU Emergency Response Plan (see www.ecu.edu/oehs). It must address such events as bomb threats, severe weather (hurricanes, floods), earthquakes, power outages, and other natural disasters or emergencies and what hazards might result related to the agent or research.
- C. The emergency response plan must address the following 14 issues (if not applicable, please mark as such and why).
 - a. The hazards associated with the use of the select agents or toxin;
 - b. Any hazards associated with response actions that could lead to a spread of a select agent or toxin (such as use of fire hose to spray water);
 - c. Planning and coordination with outside parties such as ECU police, or Greenville Police or Fire Departments;

- d. Personnel roles, lines of authority (laboratory contact list or phone tree), training, and communication;
- e. Emergency recognition and prevention;
- f. Safe distances and places of refuge;
- g. Site security and control;
- h. Evacuation routes and procedures;
- i. Decontamination;
- j. Emergency medical treatment and first aid;
- k. Emergency alerting and response procedures;
- l. Critique of response and follow-up;
- m. Personal protective and emergency equipment; and
- n. Special procedures needed to address the hazards of specific agents.
- o. The lab- and agent-specific incident response plan will be developed by the principal investigator with Biological Safety assistance.

Training

1. Training on safety, security and emergency response for working with and handling or maintaining select agents and toxins must be provided to each approved individual prior to being granted independent access. The information and training must meet the requirements of this section and must ensure that all individuals who work in, or visit, the areas understand the hazards of the select agents or toxins present in the area. The Training and evidence of Understanding must be provided and documented yearly.
2. Training must also be provided to each unapproved individual who enters to work, or visiting, areas where select agents and toxins are handled or stored (under supervision). (This training will be limited to what is required for this individual to safely complete the housekeeping or repair work required in the lab and will be given at the time of the visit.)
3. This information and training will be provided by the supervisor at the time of an individual's initial assignment to a work area where select agents or toxins are present and prior to assignments involving new exposure situations. Refresher training will be provided annually by or with Biological Safety.
4. The Biological Safety Officer must ensure that appropriate general training in safety, containment, security and emergency response is provided to all individuals with regular access to areas where select agents and toxins are handled or stored. The supervisor will provide procedure –and agent- specific training.
5. Prospective Health/Biological Safety must ensure that each individual with access to areas where select agents or toxins are handled or stored received and understood the training required by this section. Training records must contain the identity of the individual trained, the date of training, the means used to verify that the employee understood the training (either by post-test, or written attestation statement). Verification of understanding is the responsibility of the trainer. Training records are maintained for three years.

Transfers

1. A select agent or toxin may not be transferred from one entity to another entity within the United States (regardless of whether the transfer is interstate or intrastate), or received by an entity in the United States from an entity outside the United States, unless the sender has a certificate of registration that covers the transfer of the particular select agent or toxin to be transferred. Exceptions are:
 - A. If the sender meets the exemption requirement, or
 - B. Is transferring the select agent or toxin from outside the United States (and all import requirements have been met); the recipient must have a certificate of registration that includes the particular select agent or toxin to be transferred.
2. Transfer Process
 - A. Prior to the transfer, the recipient and sender will each complete a CDC Form 2. At ECU, this paperwork will be completed by the RO or ARO Biological Safety. CDC will

- then authorize the transfer based on the finding that the recipient has a certificate of registration covering the transfer of the select agent or toxin.
- B. Any select agents requested from commercial sources by ECU may be ordered by the laboratory through Materials Management but the request must specify the package be sent to the following address, to serve as a **central receiving site: Warren Life Sciences Building, Room 186, Attn: Biological Safety Officer.**
 - C. When the package arrives, Biological Safety will document receipt and the condition of the package. Intact packages will be delivered to the requesting lab. An intra-entity transfer form will be completed.
 - D. No one may receive or handle the package unless they are a Select Agent approved person.
3. Transfer completed: Biological Safety provides a completed paper copy or facsimile transmission of CDC Form 2 to the sender and to the HHS Secretary within 2 business days of receipt of the select agent or toxin; and
 4. The sender will comply with all applicable laws concerning packaging and shipping following DOT, FAA rules. (Any ECU individual packaging a select agent must be certified to ship biological materials by the Biological Safety Officer after completing the required 2-hour training course.)
 5. Transfer Problems:
 - A. When the requesting lab or the Biological Safety Officer observes that the select agent or toxin has not been received within 48 hours after the expected delivery time, or if the package received containing select agents or toxins has been leaking or was otherwise damaged, the Biological Safety Officer will report this to the HHS Secretary.
 - B. When the select agents or toxins are consumed or destroyed in a transfer, the recipient must report such fact to the HHS Secretary within five business days. The Biological Safety Officer will file CDC Form 2 upon recognition of this event.

Records

Complete records will be maintained relating to these activities.

1. The Biological Safety Officer will maintain an up-to-date, accurate list of the individuals approved for access to select agents and toxins. The laboratory will maintain an accurate, current inventory of each select agent and toxin held. The laboratory director or principal investigator must immediately inform the Biological Safety Officer in writing of any changes in personnel or significant changes in inventory.
2. The laboratory director or principal investigator must keep inventory records in a bound permanent logbook or password protected, non-networked computer database including the following information for each select agent and toxin:
 - A. The name, characteristics, and source data;
 - B. The quantity held on the date of the first inventory (toxins only);
 - C. The quantity acquired, the source, and date of acquisition;
 - D. The quantity, volume, or mass destroyed or otherwise disposed of and the date of each such action;
 - E. The quantity used and date(s) of the use (toxins only);
 - F. The quantity transferred, the date of transfer, and individual to whom it was transferred (this includes transfers within an entity when the sender and the recipient are covered by the same certificate of registration);
 - G. The current quantity held (e.g. number of vials and size of vials);
 - H. Any select agent or toxin lost, stolen, or otherwise unaccounted for; and
 - I. A written explanation of any discrepancies.
3. The laboratory must also maintain the following records:
 - A. For access to the select agents or toxins;
 - B. The name of each individual who has accessed any select agent or toxin;
 - C. The select agent or toxin used;
 - D. The date when the select agent or toxin was removed, if removed from long-term storage or holdings for stock cultures;
 - E. The quantity removed (if measurable);
 - F. The date the select agent or toxin was returned to the long-term storage or holdings for stock cultures; and
 - G. The quantity returned (e.g. number of vials. Size of vial);
4. For access to the area where select agents are used or stored:

- A. The name of each individual who has accessed the area;
 - B. The date and time the individual entered the area;
 - C. The date and time the individual left the area; and
 - D. For individuals not approved by the Department of Justice, the approved individual who accompanied the unapproved individual into the area.
 - E. The education provided to the visitor by the escort.
5. The laboratory director or principal investigator must implement a system to ensure that all such records and databases created are accurate, and that the authenticity of records may be verified. These records will be reviewed by the Biological Safety Officer during laboratory inspections.
 6. The Biological Safety Officer will create and maintain a record concerning each inspections conducted. The functioning of the mechanical system will be verified at least annually. A written report reviewing the mechanical systems functioning will be made annually and sent to the RO.
 7. Safety, security, and emergency response plans will be maintained by the laboratory director and updated when needed based on changes in research protocols, records keeping, mechanisms, etc. The Biological Safety Officer will receive copies of any changes or updates to these plans. ECU Police will be informed of changes as indicated.
 8. Initial training records will be maintained by the PI, Comparative Medicine and copies sent to Prospective Health/Biological Safety.
 9. Transfer documents (CDC Form 2) and permits will be maintained by Biological Safety.
 10. Safety and security incident reports will be maintained by Biological Safety. A copy of the completed investigation report performed by ECU Police affecting a laboratory with a select agent will be provided to the Biological Safety Officer. All records created will be maintained for a minimum of three years.

Inspections

The HHS Secretary, without prior notification and with or without cause, shall be allowed to inspect any site at which activities regulated by this part are conducted and shall be allowed to inspect and copy any records relating to the activities covered by this part. If an inspection results in the assessment of fines, these will be paid by the laboratory or department found to have the deficiency.

Notification for theft, loss, or release

1. Upon discovery of a theft, loss, or release or occupational exposure of a select agent or toxin, the laboratory director or PI must immediately notify the Responsible Official, or Alternatively Biological Safety Officer, who will notify ECU Police and the HHS Secretary (by either telephone, facsimile, or e-mail). If the ECU Police discover the theft, the Responsible Official or Biological Safety Officer will be notified.
2. Thefts or losses must be reported to HHS whether the select agent or toxin is subsequently recovered or the responsible parties are identified.
3. The report will include:
 - A. The name of the select agent or toxin and any identifying information (e.g., strain or other characterization information);
 - B. An estimate of the quantity lost or stolen;
 - C. An estimate of the time during which the theft or loss occurred; and
 - D. The location (building, room) from which the theft or loss occurred.
4. The laboratory director or PI shall immediately notify the Responsible Official or Alternatively Biological Safety Officer of any release of a select agent or toxin causing occupational exposure or release outside of the primary containment barriers. (If the release triggers the ECU Emergency Response Plan, the ECU Police and the Office of Environmental Health and Safety, which administers the ECU Hazmat team, will be notified.) The Responsible Official or Alternative ARO will report this release to the HHS Secretary and State and possibly to local public health agencies. The reporting of a release must provide the following information:
 - A. The name of the select agent or toxin and any identifying information (e.g., strain or other characterization information);
 - B. An estimate of the quantity released;
 - C. The time and duration of the release;

- D. The environment into which the release occurred (e.g., in building or outside of building, waste system);
 - E. The location (building, room) from which the release occurred;
 - F. The number of individuals potentially exposed at the facility;
 - G. Actions taken to respond to the release; and
 - H. Hazards posed by the release. **Note:** Occupational exposures are considered to be releases and must be reported on Form 3.
5. Within seven calendar days of theft, loss, or release, the Biological Safety Officer must submit a follow-up report in writing to the HHS Secretary on CDC Form 3.

Select Agent Appendix A
Consult Select Agent Website www.selectagents.gov for updated list

- I. HHS Select Agents and Toxins (24CFR Part 73)
 - A. Virus
 - 1. Crimean-Congo haemorrhagic fever virus
 - 2. Ebola Viruses
 - 3. Cercopithecine herpes virus 1 (Herpes B virus)
 - 4. Lassa fever virus
 - 5. Marburg virus
 - 6. Monkeypox virus
 - 7. South American Haemorrhagic Fever viruses (Junin, Machupo, Sabia, Flexal, Guanarito)
 - 8. Tick-borne encephalitis complex (flavi) viruses (Central European Tick-borne encephalitis, Far Eastern Tick-borne encephalitis [Russian Spring and Summer encephalitis, Kyasanur Forest disease, Omsk Hemorrhagic Fever])
 - 9. Variola major virus (Smallpox virus) and Variola minor virus (Alastrim)
 - B. Bacteria
 - 1. Rickettsia prowazekii
 - 2. Rickettsia rickettsii
 - 3. Yersinia pestis
 - C. Fungi
 - 1. Coccidioides posadasii
 - D. Toxins
 - 1. Abrin
 - 2. Conotoxins
 - 3. Diacetoxyscirpenol
 - 4. Ricin
 - 5. Saxitoxin
 - 6. Tetrodotoxin
 - 7. Shiga-like ribosome inactivating proteins
 - E. Genetic elements, recombinant nucleic acids, and recombinant organisms
 - 1. Select agent viral nucleic acids
 - 2. Nucleic acids that encode for the functional form(s) of any of the toxins listed that are transmissible and/or expressible
 - 3. Viruses, bacteria, fungi, and toxins listed that have been genetically modified
- II. Overlap Agents
 - A. Viruses
 - 1. Eastern Equine Encephalitis virus
 - 2. Nipah and Hendra Complex viruses
 - 3. Rift Valley fever virus
 - 4. Venezuelan Equine Encephalitis virus
 - B. Bacteria
 - 1. Bacillus anthracis
 - 2. Brucella abortus
 - 3. Brucella melitensis
 - 4. Brucella suis
 - 5. Burkholderia mallei (formerly Pseudomonas mallei)
 - 6. Burkholderia pseudomallei (formerly Pseudomonas pseudomallei)
 - 7. Botulinum neurotoxin producing species of Clostridium
 - 8. Coxiella burnetii
 - 9. Francisella tularensis
 - C. Fungi: Coccidioides immitis
 - D. Toxins
 - 1. Botulinum neurotoxins
 - 2. Clostridium perfringens epsilon toxin
 - 3. Shigatoxin

4. Staphylococcal enterotoxins
 5. T-2 toxin
- E. Genetic elements, recombinant nucleic acids, and recombinant organisms
1. Select agent viral nucleic acids
 2. Nucleic acids that encode for the functional form(s) of any of the toxins listed that are transmissible and/or expressible
 3. Viruses, bacteria, fungi, and toxins listed that have been genetically modified

Exclusions

Entities that **do not** at any time **have more** than the following aggregate amounts (in the purified form or in combinations of pure and impure forms) under the control of a principal investigator are excluded from requirements of the regulation:

Abrin	100 mg
Botulinum neurotoxin	0.5 mg
<i>Clostridium perfringens</i> epsilon toxin	100 mg
Conotoxins	100 mg
Diacetoxyscirpenol	1000 mg
Ricin	100 mg
Saxitoxin	100 mg
Shiga-like ribosome inactivating proteins	100 mg
Shigatoxin	100 mg
Staphylococcal enterotoxin	5 mg
Tetrodotoxin	100 mg
T-2	1000 mg

Select Agent Appendix B

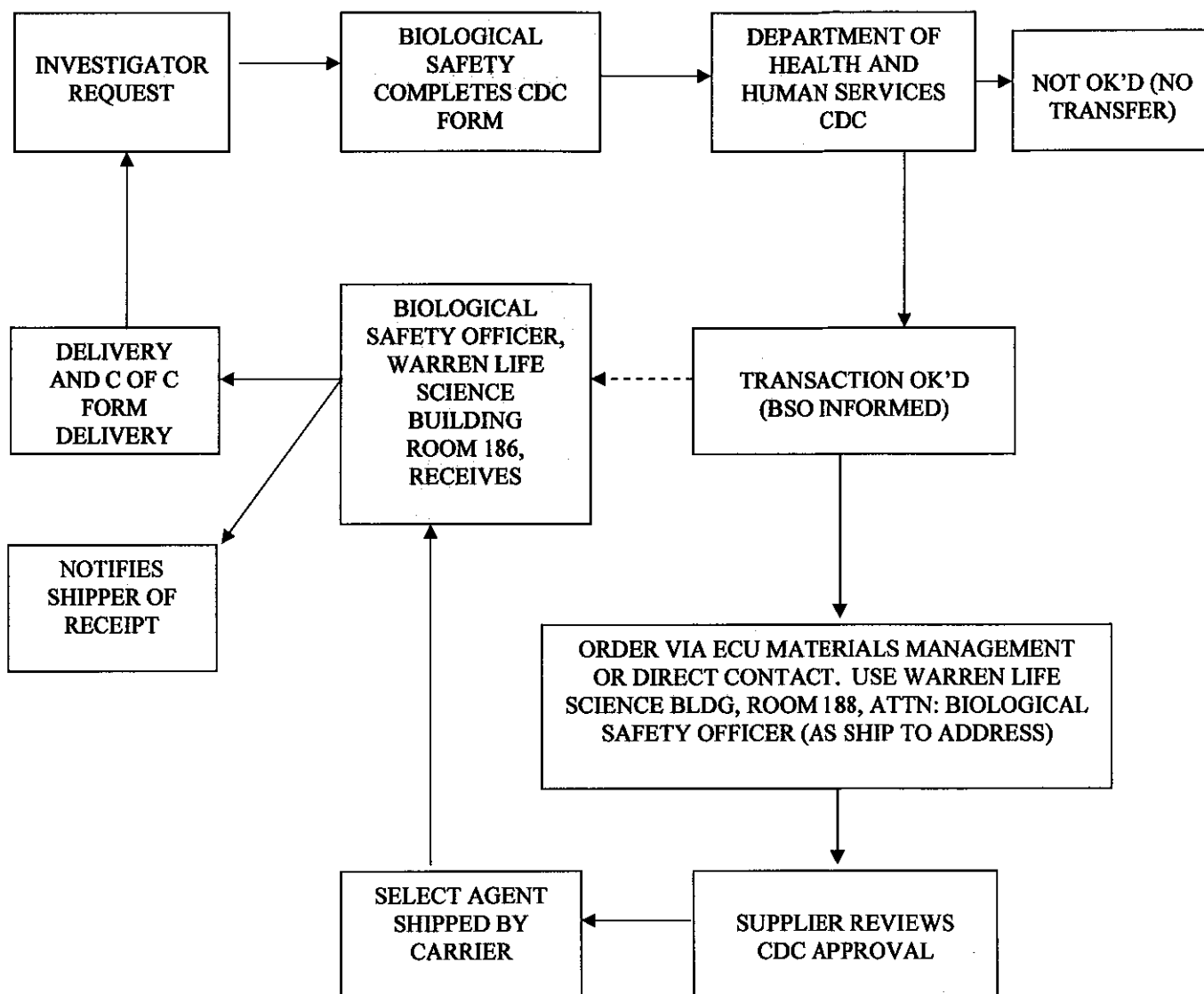
The term "restricted person" means an individual who:

- A. Is under indictment for a crime punishable by imprisonment for a term exceeding 1 year
- B. Has been convicted in any court of a crime punishable by imprisonment for a term exceeding 1 year
- C. Is a fugitive from justice
- D. Is an unlawful user of any controlled substance
- E. Is an alien illegally or unlawfully in the United States
- F. Has been adjudicated as a mental defective or has been committed to any mental institution
- G. Is an alien (other than an alien lawfully admitted for permanent residence) who is a national of a country as to which the Secretary of State
- H. Has been discharged from the Armed Services of the United States under dishonorable conditions

OR

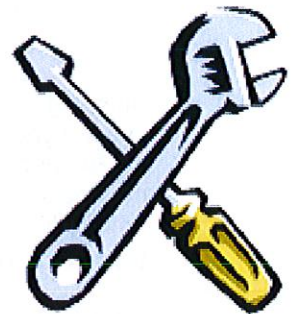
- 1. Reasonably suspected by any Federal law enforcement or intelligence agency of
 - i. Committing a crime specified in 18 U.S.C. 2332b(g)(5)
 - ii. Having a knowing involvement with an organization that engages in domestic or international terrorism (as defined 18 U.S.C. 2331) or with any other organization that engages in intentional crimes of violence
 - iii. Being an agent of a foreign power (as defined in 50 U.S.C. 1801)

Select Agent Appendix C



DO NOT ENTER

Without an Escort from this Lab Present



This includes **Housekeeping, Maintenance, Computer and Telephone Services**

personnel. Call _____ at _____ for emergency access after hours. ECU Police for Emergency Call Back List.

Appendix B

Transportation and Shipment of Biological Materials

Each ECU Principal Investigator is responsible for shipping out or transferring in stock, cultures, and specimens. Biological Safety provides web-based training for the individuals responsible for shipping; this training results in a 2 year certification by IATA. Some departments designate a single individual to be the trained to package and ship specimens under the IATA regulations. Biological Safety will maintain records of the IATA training.

The investigator or department is responsible for maintaining records of shipping and receiving diagnostic or research specimens for FAA or DOT.

Biological Safety completes the paperwork when a CDC or USDA permit is required for the materials or if a Select Agent is involved, and may assist with questions about shipping biological materials, and import/export issues.

Shipped biological specimens, infectious agents and other biological materials are regulated by governmental and non-governmental, consensus development organizations. Penalties or non-compliance with the rules are significant and could result in the following fines.

- Up to \$200,000 and up to a year jail sentence for individuals
- Up to \$500,000 per incident for organizations

Several agencies regulate the shipment of biological materials including:

- International Air Transport Association (IATA)
 - Dangerous Goods Regulations
- US Department of Transportation (DOT)
 - 49 CFR Parts 171-178
- US Public Health Service (PHS)
 - 42 CFR Part 72
- Occupational Health and Safety Administration (OSHA)
 - 29 CFR Part 1910.1030

Shipping regulations change frequently so it is necessary to repeat training certification at least every 2 years. Training sessions reviewing the material are available by appointment in the Office of Prospective Health/Biological Safety. To ask questions regarding the shipment of biological materials please call 744-3437 or 744-2237, or call to schedule an appointment for the class at 744-2070

Shipment Types

For shipment purposes, biological materials are characterized into four classes:

- Diagnostic Specimens
- Infectious Substances
- Biological Products
- Genetically Modified organisms and micro organisms

Diagnostic Specimens

A diagnostic specimen is any human or animal material including, but not limited to, excreta, secretions, blood and its components, tissue and tissue fluids, being transported for diagnostic or investigational purposes, but excluding live infected animals. Diagnostic specimens resulting from medical practice and research are not considered a threat to public health.

Infectious Substances

Infectious substances are substances known to contain, or reasonably expected to contain pathogens. Pathogens are microorganisms (including bacteria, viruses, rickettsia, parasites, fungi) or recombinant microorganism (hybrid or mutant) that are known or reasonably expected to cause infectious disease in humans or animals.

Biological Products

Biological Products are those products derived from living organisms that are manufactured and distributed in accordance with the requirements of national governmental authorities that may have special licensing requirements. They are used for prevention, treatment, or diagnosis of disease in humans or animals, or for development, experimental or investigational purposes related thereto. They include, but are not limited to, finished or unfinished products such as vaccines and diagnostic products.

Genetically Modified organisms and microorganisms

Genetically modified organisms or microorganisms that are dangerous, infectious, or are carried by an animal host are regulated for transportation. For the following guidelines, make sure to distinguish those that apply to *organisms* vs. *microorganisms*.

A genetically modified *microorganism*, which meets the definition of an infectious substance, must be classified as an infectious substance for transportation. For these materials, follow instructions for shipping an infectious substance.

Genetically modified *microorganisms* which are not infectious substances but which are capable of altering animals, plants or microbiological substances in a way that is not normally the result of natural reproduction can be transported when classified as a Miscellaneous Hazard (Class 9). These materials are packed for shipment in the same way as infectious substances, except there are no specific testing requirements for the packaging; this packaging variation is IATA Packing Instruction 913. You may not be able to purchase packages designed for Packing Instruction 913. In this case, use packages designed for infectious substances (Packing Instruction 605) and use a Class 9 label. These materials are shipped with the proper shipping name, "Genetically modified micro-organisms" and UN 3245. The maximum allowable quantity per primary receptacle is 100 mL or 100 g. There is no maximum net quantity per package.

Genetically modified *organisms* that are known or suspected to be dangerous to humans, animals or the environment cannot be transported by air. Animals, which contain, or are contaminated with, genetically modified *microorganisms* or *organisms* that meet the definition of an infectious substance cannot be shipped by air.

Classification of Shipment

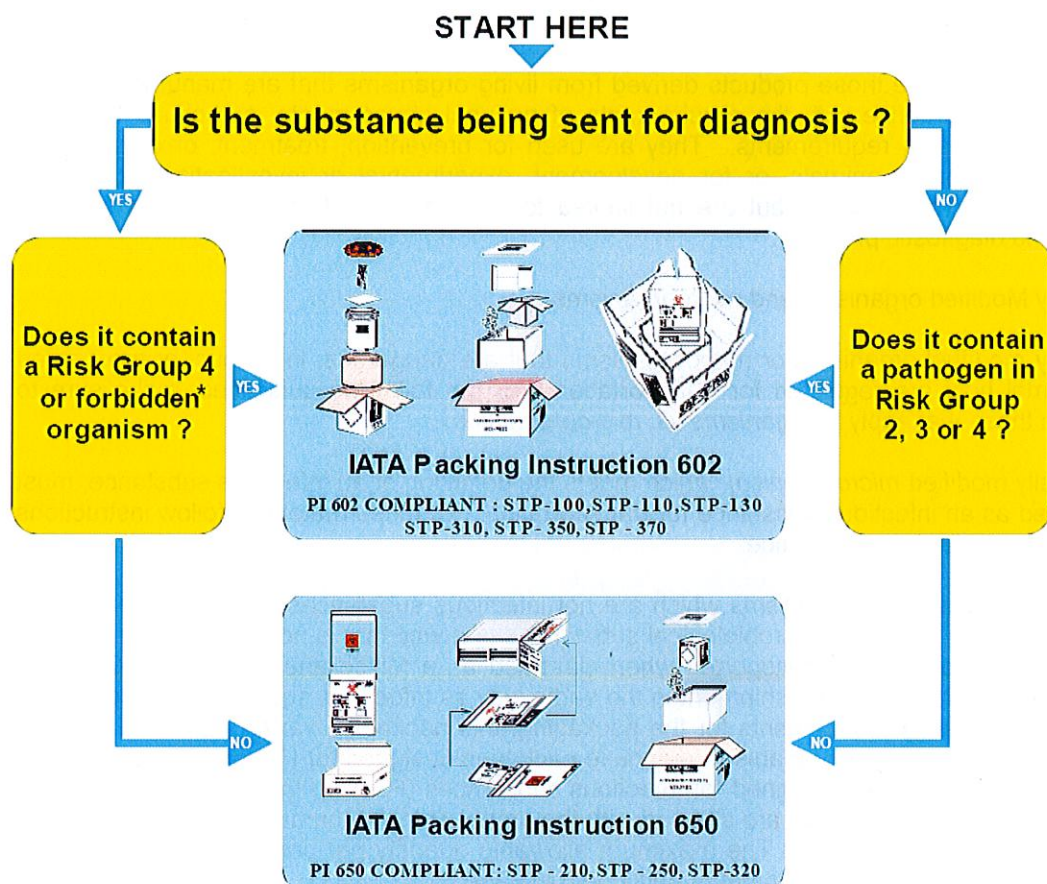
The shipper must determine if the material is considered an infectious substance affecting humans (or animals). The CDC/NIH list of Etiologic Agents on the basis of Hazard classifies human pathogens on the basis of risk. Agents are classified into 4 basic risk groups. If you are shipping any agents classified in Risk Groups II – IV, you must conform to the dangerous goods regulations. Any quantity of an infectious substance (Risk Group II – IV agents) is considered hazardous and must be shipped according to the dangerous goods regulations. There are no limited quantity exemptions with these materials.

If you are unable to classify the material before shipment, it may not be transferred. The term "potentially infectious" does not apply to shipping regulations. A substance is an infectious substance, a clinical or diagnostic specimen, or a biological product. UN approved packaging and the infectious substance label must be used for all shipments of infectious substances or select agents. Diagnostic specimens and infectious substances cannot be consolidated into one shipping package. They must be shipped separately.

Dangerous Goods Classification Flow Chart

INFECTIOUS SUBSTANCES • DIAGNOSTIC SPECIMENS • BIOLOGICAL PRODUCTS

What packaging do I need to ship safely?



Packaging

Hazardous biological materials must be packed according to the regulations. They must be packaged to withstand leakage of contents, shocks, temperature and pressure changes and other conditions that can occur during ordinary handling in transportation. Certified containers purchased from commercial suppliers must be used, regardless of the mode of transportation.

Figure 1. depicts the principal of triple packaging (primary receptacle, water tight secondary packaging, durable outer packaging) upon which all regulations are built. Infectious substances, diagnostic specimens, and genetically modified microorganisms that fall under the category of hazardous material must be packaged in this way.

The Primary receptacle contains the material and must be watertight to prevent leakage. The primary receptacle must be labeled with the name of the specimen. Primary receptacle may be made of glass, metal, or plastic and include screw cap tubes, flame sealed glass Biosafety, or rubber stopped vials fitted with metal seals. Petri plates cannot be used as

Secondary Packaging. One or more primary receptacles are placed in watertight secondary packaging. The primary receptacle or the secondary packaging must withstand, without leakage, an internal pressure differential and temperature range as described in the United Nations (UN) packaging specifications and performance tests. The secondary packaging should also bear a label with the name, address, and telephone number of the shipper, as well as the name of the specimen.

Absorbent Material. Absorbent material must be placed between the primary receptacle and secondary packaging. Multiple primary receptacles must be individually wrapped to prevent contact between them. The absorbent material must be sufficient to absorb the entire contents of the primary container(s).

Itemized List. An itemized list of contents must be enclosed between the secondary packaging and the outer packaging.

Outer Packaging. Outer packages must be at least 100 mm (4 in) in the smallest overall external dimension in order to bear the required markings and labels. Outer packaging must be of adequate strength for its capacity, mass, and intended use, and must be capable of meeting the rigorous performance tests and be marked with a UN specification mark.

Other Packaging Requirements

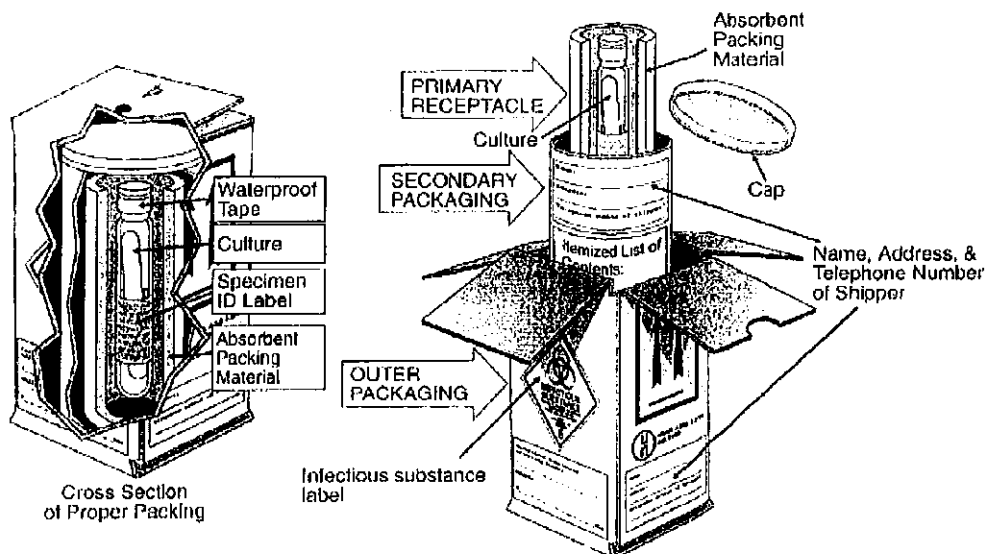
Overpacks. An overpack can be used to combine several triple packages into one large package. This may be done to save freight charges when shipping multiple samples. Each triple package inside the overpack must be properly marked and labeled. The outside of the overpack must bear the same markings and labels as the triple packages within. If packed with dry ice, the total net quantity of dry ice must be listed on the outer container. The overpack must also be marked with the statement:

“Inner Packages Comply with Prescribed Specifications.”

Ice and Dry Ice. If a shipment includes ice or dry ice, special packaging must be purchased. If shipping with ice, the packaging must be leak-proof. If dry ice is used, the outer packaging must allow for the release of carbon dioxide gas when the solid sublimates. Ice or dry ice must be placed outside the secondary packaging. Interior supports must be provided to secure the secondary container as the refrigerant melts/sublimates. Dry ice is considered a miscellaneous hazard (Class 9) by IATA. Packages containing dry ice must bear a Class 9 label and be marked with the proper shipping name, UN number and net quantity, e.g., Dry Ice, UN1845, 3 KG. Certified packages for dry ice most likely will be pre-labeled and marked. A Declaration for Dangerous Goods is not required for shipments in which dry ice is the only hazardous material. Dry ice is included on the Declarations for shipments, which include other hazardous materials such as infectious substances.

Liquid Nitrogen. Biological materials can be shipped in liquid nitrogen or dry shippers, which are insulated packages containing refrigerated liquid nitrogen fully absorbed in a porous material. Special packing regulations apply to shipments containing nitrogen. Contact EHS if you need to ship materials with liquid nitrogen

Figure 1



Labeling

The sender and recipient's addresses must be printed and clearly displayed. The container should be labeled with the name and telephone number of a person responsible for the shipment. If packaged with dry ice, a Class 9 diamond label (Figure 2) must be placed on one side of the outer package. The container should be labeled with an infectious substance label (Figure 3)). When shipping over 50 ml or 50 g of infectious substance, you must also put a Cargo Aircraft Label on the outer container (Figure 4). When shipping infectious substances by air, you must make advance arrangements with the consignee and the operator to ensure that the shipment can be transported and delivered without delay. In the "Additional Handling Information" section of the Shipper's Declaration for Dangerous Goods, include the following statement: "Prior arrangements as required by the IATA Dangerous Goods Regulations 1.3.3.1 have been made." There are two proper shipping names for infectious substances: • Infectious substance, affecting humans (UN 2814); and • Infectious substance, affecting animals (UN 2900).

If you have any reason to believe the infectious material could affect humans you should ship your material as UN 2814. Materials that can affect humans and animals should be shipped as UN 2814.

Figure 2

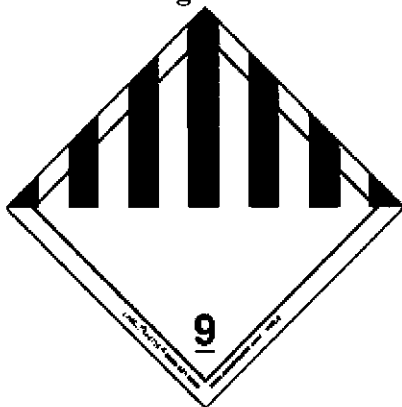


Figure 3

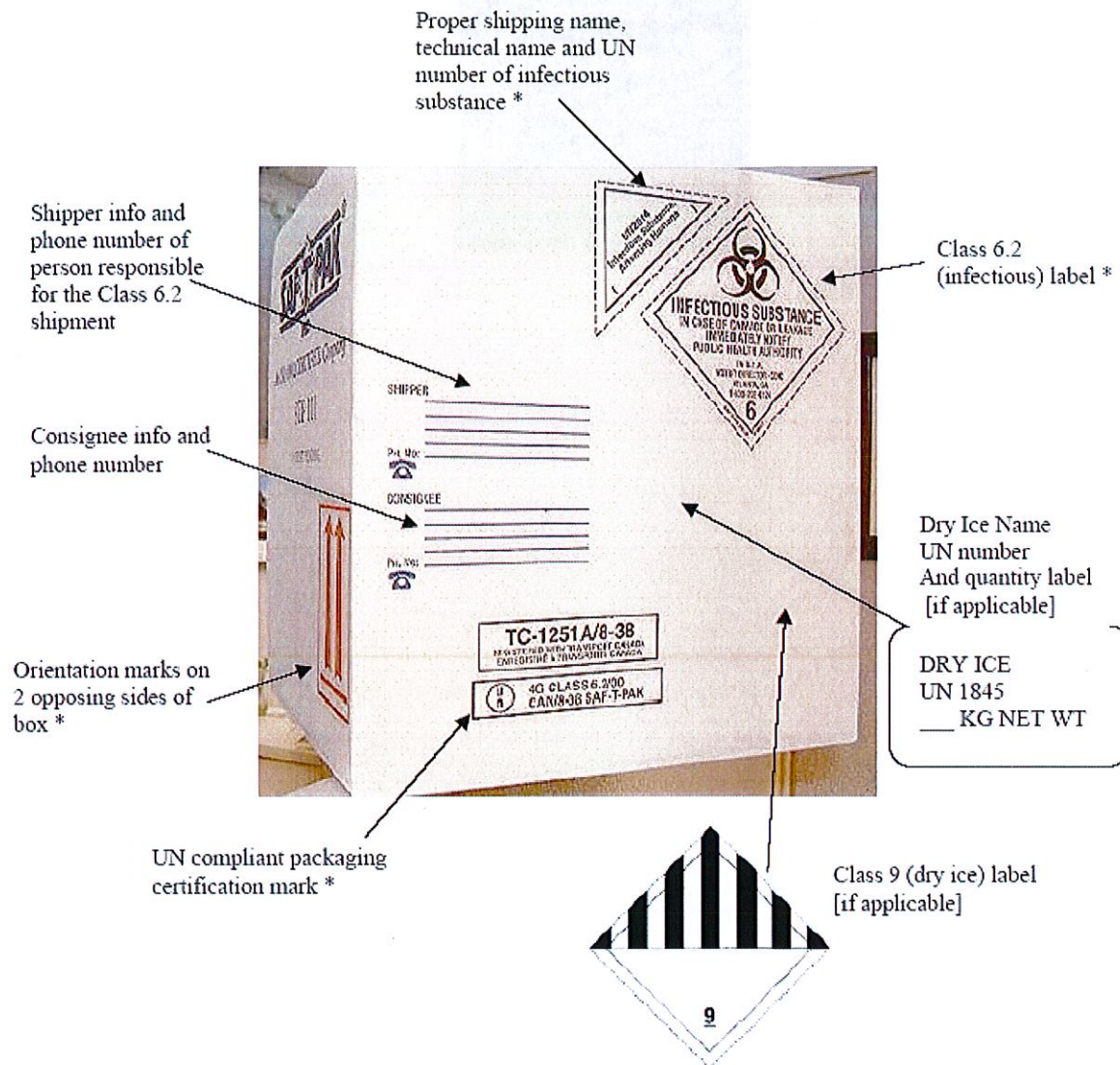


Figure 4



Example of Proper Labeling and Marking of Infectious Substance or Diagnostic Specimen Package

[Items marked with {*} are not required for diagnostic specimen package]



Note: Biohazard symbol required on diagnostic specimen package

Shippers Declaration of Dangerous Goods

A shippers declaration of dangerous goods must be completed when shipping infectious substances or genetically modified microorganisms. A declaration is not required for shipments in which dry ice is the only hazardous material. Dry ice should be listed on declarations for shipments containing infectious substances or genetically modified microorganisms. A declaration is not required if you are only shipping diagnostic specimens.

Improperly completed declarations are the most common cause of package refusal.

Refer to the Shippers Declaration for Dangerous Goods in Figure 5 for a location of each section explained below.

- A. Shipper – Enter your full name, address, and telephone number.
- B. Consignee – Enter full name and address of the recipient. When shipping infectious substances, include the text, "Person responsible for the shipment" followed by your name and telephone number.
- C. Transport Details – Indicate here if your shipment is restricted to cargo aircraft only (if it is more than 50 ml or 50g of an infectious substance). Airport of departure and airport of destination will be filled out by the carrier, leave blank.
- D. Shipment Type – Cross out radioactive to indicate you are shipping a non-radioactive substance
- E. Proper Shipping Name – Enter the proper shipping name exactly as it appears in Figure 6.
- F. Class or Division – Enter appropriate hazard class as found in Figure 6.
- G. UN or ID Number – Enter appropriate UN number as found in Figure 6.
- H. Packing Group – For Dry Ice, enter III in this column, Biological materials are not assigned packaging groups.
- I. Subsidiary Risk – Leave this column blank
- J. Quantity and Type of Packaging – Enter the net quantity for each material here. Use only metric units. At the bottom of the column, indicate the number and type of packages (usually, "All packed in one fiberboard box"). Do not spell like fiberboard. If using an overpack, indicate here with overpack used.
- K. Packing Instructions – Enter appropriate packing instruction number. (See figure 6)
- L. Authorization- Leave this column blank
- M. Additional Handling Instructions
- N. Signature and Date

Figure 5

SHIPPER'S DECLARATION FOR DANGEROUS GOODS

Shipper A					Air Waybill No Page of Pages Shipper's Reference Number <i>(optional)</i>		
Consignee B					<i>For optional use for Company logo name and address</i>		
Two completed and signed copies of this Declaration must be handed to the operator.					WARNING Failure to comply in all respects with the applicable Dangerous Goods Regulations may be in breach of the applicable law, subject to legal penalties. This Declaration must not, in any circumstances, be completed and/or signed by a consolidator, a forwarder or an IATA cargo agent.		
TRANSPORT DETAILS C							
This shipment is within the limitations prescribed for <i>(delete non-applicable)</i>			Airport of Departure				
PASSENGER AND CARGO AIRCRAFT		CARGO AIRCRAFT ONLY					
Airport of Destination					Shipment type: <i>(delete non-applicable)</i> NON-RADIOACTIVE RADIOACTIVE D		
NATURE AND QUANTITY OF DANGEROUS GOODS							
Dangerous Goods Identification							
Proper Shipping Name	Class or Division	UN or ID No.	Pack-ing Group	Subsidiary Risk	Quantity and Type of packing	Packing Inst.	Authorization
E	F	G	H	I	J	K	L
Additional Handling Information M							
Emergency Telephone Number							
I hereby declare that the contents of this consignment are fully and accurately described above by the proper shipping name, and are classified, packaged, marked, and labelled/placarded, and are in all respects in proper condition for transport according to applicable international and national governmental regulations.					Name/Title of Signatory Place and Date N Signature		

Note: -Handwritten alterations/amendments are acceptable, provided each alteration is legible and signed with the same signature used to sign the Shipper's Declaration (IATA 8.1.2.6).

-Shaded area must be computer generated or typewritten for FedEx.

Figure 6
Summary of Shipping Information

SHIPMENT TYPE	PROPER SHIPPING NAME	UN NUMBER	HAZARD CLASS	PACKING GROUP (PG)	PACKING INSTRUCTION (PI)	MAX NET QTY/PKG FOR PASSENGER AIRCRAFT	MAX NET QTY/PKG FOR CARGO AIRCRAFT
Infectious substance, affecting humans and possibly animals	Infectious substance, affecting humans (technical name)	UN 2814	6.2	-	602	50 ml or 50 g	4L or 4 kg
Infectious substance, affecting only animals (not humans)	Infectious substance, affecting animals (technical name)	UN 2900	6.2	-	602	50 ml or 50 g	4L or 4 kg
Diagnostic or clinical specimen	Diagnostic specimens	UN 3373	-	-	650	4L or 4 kg	4L or 4 kg
Dry Ice	Dry Ice or Carbon Dioxide, solid	UN 1845	9	III	904	200 kg	200 kg
Non Infectious, transducing genetically modified micro-organisms	Genetically modified micro-organisms	UN 3245	9	-	913	No Limit	No Limit

Appendix C

BSC Use by the Investigator: Work Practices and Procedures

Preparing for Work Within a Class II BSC

Preparing a written checklist of materials necessary for a particular activity and placing necessary materials in the BSC before beginning work serves to minimize the number of arm-movement disruptions across the fragile air barrier of the cabinet. The rapid movement of a worker's arms in a sweeping motion into and out of the cabinet will disrupt the air curtain and may compromise the partial barrier containment provided by the BSC. Moving arms in and out slowly, perpendicular to the face opening of the cabinet, will reduce this risk. Other personnel activities in the room (e.g., rapid movement, open/closing room doors, etc.) may also disrupt the cabinet air barrier.⁵

Laboratory coats should be worn buttoned over street clothing; latex/nitrile gloves are worn to provide hand protection. A solid front, back-closing lab gown provides better protection of personal clothing than a traditional lab coat. Gloves should be pulled over the knitted wrists of the gown, rather than worn inside. Elasticized sleeves can also be worn to protect the investigator's wrists.

Before beginning work, the investigator should adjust the stool height so that his/her face is above the front opening. Manipulation of materials should be delayed for approximately one minute after placing the hands/arms inside the cabinet. This allows the cabinet to stabilize and to "air sweep" the hands and arms to remove surface microbial contaminants. When the user's arms rest flatly across the front grille, room air may flow directly into the work area, rather than being drawn through the front grille. Raising the arms slightly will alleviate this problem. The front grille must not be blocked with research notes, discarded plastic wrappers, pipetting devices, etc. All operations should be performed on the work surface at least four (4) inches from the inside edge of the front grille.

Closure of the drain valve under the work surface should be done prior to beginning work so that all contaminated materials are contained within the cabinet should a large spill occur.

Materials or equipment placed inside the cabinet may cause disruption to the airflow, resulting in turbulence, possible cross-contamination, and/or breach of containment. Extra supplies (e.g., additional gloves, culture plates or flasks, culture media) should be stored outside the cabinet. Only the materials and equipment required for the immediate work should be placed in the BSC.

BSCs are designed to be operated 24 hours per day, and some investigators find that continuous operation helps to control the laboratory's level of dust and other airborne particulates. Although energy conservation may suggest BSC operation only when needed, especially if the cabinet is not used routinely, room air balance is an overriding consideration. In some instances, room exhaust is balanced to include air discharged through ducted BSCs.

Cabinet blowers should be operated at least three to five minutes before beginning work to allow the cabinet to "purge". This purge will remove any particulates in the cabinet. The work surface, the interior walls (not including the supply filter diffuser), and the interior surface of the window should be wiped with 70% ethanol (EtOH), a 1:100 dilution of household bleach (i.e., 0.05% sodium hypochlorite), or other disinfectant as determined by the investigator to meet the requirements of the particular activity. When bleach is used, a second wiping with sterile water is needed to remove the residual chlorine, which may eventually corrode stainless steel surfaces. Wiping with non-sterile water may recontaminate cabinet surfaces, a critical issue when sterility is essential (e.g., maintenance of cell cultures).

Similarly, the surfaces of all materials and containers placed into the cabinet should be wiped with 70 % EtOH to reduce the introduction of contaminants to the cabinet environment. This simple step will reduce introduction of mold spores and thereby minimize contamination of cultures. Further reduction of microbial load on materials to be placed or used in BSCs may be achieved by periodic decontamination of incubators and refrigerators.

Material Placement Inside the BSC

Plastic-backed absorbent toweling can be placed on the work surface (but not on the front or rear grille openings). This toweling facilitates routine cleanup and reduces splatter and aerosol formation³ during an overt spill. It can be folded and placed in an autoclavable biohazard bag when work is completed.

All materials should be placed as far back in the cabinet as practical, toward the rear edge of the work surface and away from the front grille of the cabinet (Figure 11). Similarly, aerosol-generating equipment (e.g., vortex mixers, tabletop centrifuges) should be placed toward the rear of the cabinet to take advantage of the air split described in Section III. Active work should flow from the clean to contaminated area across the work surface. Bulky items such as biohazard bags, discard pipette trays and suction collection flasks should be placed to one side of the interior of the cabinet.

Certain common practices interfere with the operation of the BSC. The autoclavable biohazard collection bag should not be taped to the outside of the cabinet. Upright pipette collection containers should not be used in BSCs nor placed on the floor outside the cabinet. The frequent inward/outward movement needed to place objects in these containers is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection. Only horizontal pipette discard trays containing an appropriate chemical disinfectant should be used within the cabinet. Furthermore, potentially contaminated materials should not be brought out of the cabinet until they have been surface decontaminated. Alternatively, contaminated materials can be placed into a closable container for transfer to an incubator, autoclave or for other decontamination treatment.

Operations Within a Class II BSC

Laboratory Hazards

Many procedures conducted in BSCs may create splatter or aerosols. Good microbiological techniques should always be used when working in a biological safety cabinet. For example, techniques to reduce splatter and aerosol generation will minimize the potential for personnel exposure to infectious materials manipulated within the cabinet. Class II cabinets are designed so that horizontally nebulized spores introduced into the cabinet will be captured by the downward flowing cabinet air within fourteen inches²⁴ of travel. Therefore, as a general rule of thumb, keeping clean materials at least one foot away from aerosol-generating activities will minimize the potential for cross-contamination.

The work flow should be from "clean to contaminated (dirty)" (see Figure 11). Materials and supplies should be placed in such a way as to limit the movement of "dirty" items over "clean" ones.

Several measures can be taken to reduce the chance for cross-contamination when working in a BSC. Opened tubes or bottles should not be held in a vertical position. Investigators working with Petri dishes and tissue culture plates should hold the lid above the open sterile surface to minimize direct impaction of downward air. Bottle or tube caps should not be placed on the toweling. Items should be recapped or covered as soon as possible.

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. When deemed absolutely necessary, touch-plate microburners 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.

Aspirator bottles or suction flasks should be connected to an overflow collection flask containing appropriate disinfectant, and to an in-line HEPA or equivalent filter (see Figure 12). This combination will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment. Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution into the flask to kill the

microorganisms as they are collected. Once inactivation occurs, liquid materials can be disposed of as noninfectious waste.

Investigators must determine the appropriate method of decontaminating materials that will be removed from the BSC at the conclusion of the work. When chemical means are appropriate, suitable liquid disinfectant should be placed into the discard pan before work begins. Items should be introduced into the pan with minimum splatter, and allowed appropriate contact time as per manufacturer's instructions. Alternatively, liquids can be autoclaved prior to disposal. Contaminated items should be placed into a biohazard bag or discard tray inside the BSC. Water should be added to the bag or tray prior to autoclaving.

When a steam autoclave is to be used, contaminated materials should be placed into a biohazard bag or discard pan containing enough water to ensure steam generation during the autoclave cycle. The bag should be taped shut or the discard pan should be covered in the BSC prior to removal to the autoclave. The bag should be transported and autoclaved in a leak proof tray or pan. It is a prudent practice to decontaminate the exterior surface of bags and pans just prior to removal from the cabinet.

Decontamination

Surface Decontamination

All containers and equipment should be surface decontaminated and removed from the cabinet when work is completed. At the end of the work day, the final surface decontamination of the cabinet should include a wipe-down of the work surface, the cabinet's sides and back, and the interior of the glass. If necessary, the cabinet should also be monitored for radioactivity and decontaminated when necessary. Investigators should remove their gloves and gowns in a manner to prevent contamination of unprotected skin and aerosol generation and wash their hands as the final step in safe microbiological practices.

Small spills within the BSC can be handled immediately by removing the contaminated absorbent paper toweling and placing it into the biohazard bag. Any splatter onto items within the cabinet, as well as the cabinet interior, should be immediately wiped with a towel dampened with decontaminating solution. Gloves should be changed after the work surface is decontaminated and before placing clean absorbent toweling in the cabinet. Hands should be washed whenever gloves are changed or removed.

Spills large enough to result in liquids flowing through the front or rear grilles require more extensive decontamination. All items within the cabinet should be surface decontaminated and removed. Decontaminating solution can be poured onto the work surface and through the grille(s) into the drain pan.

Twenty to thirty minutes is generally considered an appropriate contact time for decontamination, but this varies with the disinfectant and the microbiological agent. Manufacturer's directions should be followed. The spilled fluid and disinfectant solution on the work surface should be absorbed with paper towels and discarded into a biohazard bag. The drain pan should be emptied into a collection vessel containing disinfectant. A flexible tube should be attached to the drain valve and be of sufficient length to allow the open end to be submerged in the disinfectant within the collection vessel. This procedure serves to minimize aerosol generation. The drain pan should be flushed with water and the drain tube removed.

Should the spilled liquid contain radioactive material, a similar procedure can be followed. Radiation safety personnel should be contacted for specific instructions.

Gas Decontamination BSCs that have been used for work involving infectious materials must be decontaminated before HEPA filters are changed or internal repair work is done.^{4,9-12} Before a BSC is relocated, a risk assessment which considers the agents manipulated within the BSC must be done to determine the need for decontamination. The most common decontamination method uses formaldehyde gas, although more recently hydrogen peroxide vapor¹⁰ has been used successfully. This environmentally benign vapor is useful in decontaminating HEPA filters, isolation chambers and centrifuge enclosures.

Appendix D

Primary Containment for Biohazards (From CDC Office of Health and Safety)

SECTION I

A. Preparing for work within a Class II BSC

1. Prepare a written checklist of materials necessary for a particular activity or place necessary materials in the BSC before beginning work serves to minimize the number of arm-movement disruptions across the fragile air barrier of the cabinet.
2. The rapid movement of a worker's arms in a sweeping motion into and out of the cabinet will disrupt the air curtain and may compromise the partial barrier. Moving arms in and out slowly, perpendicular to the face opening of the cabinet, will reduce this risk.
3. Rapid movement, open/closing room doors, etc. may also disrupt the cabinet air barrier.
4. Adjust the stool height so that his/her face is above the front opening.

B. Personal Protective Equipment

1. Laboratory coats should be worn buttoned over street clothing; solid front, back-closing lab gown provides better protection of personal clothing than a traditional lab coat.
2. Gloves should be pulled over the knitted wrists of the gown, rather than worn inside.
3. Elasticized sleeves can also be worn to protect the investigator's wrists.

C. Manipulation

1. Manipulation of materials should be delayed for approximately one minute after placing the hands/arms inside the cabinet. This allows the cabinet to stabilize and to "air sweep" the hands and arms to remove surface microbial contaminants.
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III. Operations within a Class II BSC

Many procedures conducted in BSCs may create splatter or aerosols. Good microbiological techniques should always be used when working in a biological safety cabinet. For example, techniques to reduce splatter and aerosol generation will minimize the potential for personnel

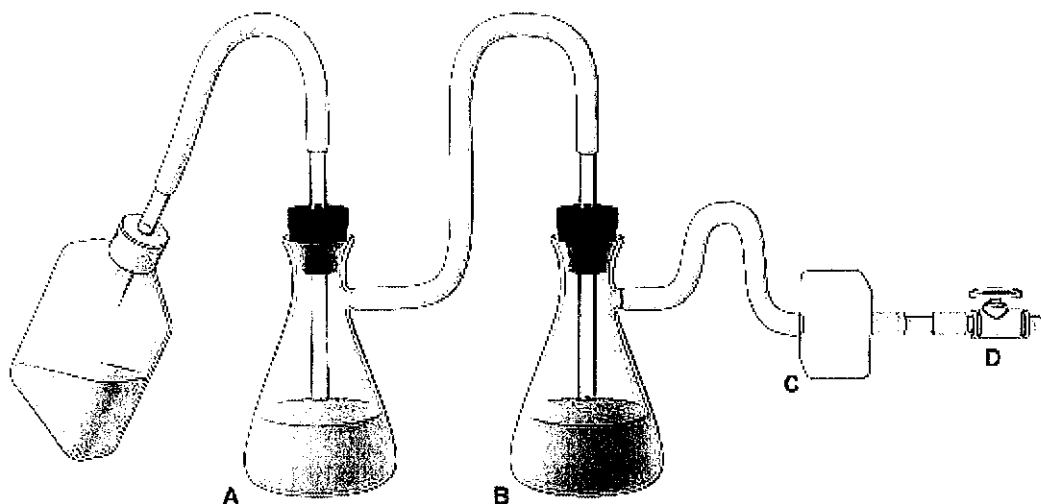
exposure to infectious materials manipulated within the cabinet. Class II cabinets are designed so that horizontally nebulized spores introduced into the cabinet will be captured by the downward flowing cabinet air within fourteen inches²⁴ of travel. Therefore, as a general rule of thumb, keeping clean materials at least one foot away from aerosol-generating activities will minimize the potential for cross-contamination.

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Tissue Culture Flask A. Collection Flask B. Liquid Trap C. HEPA Filter D. Vacuum
(house or pump)

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IV. Decontamination

Surface Decontamination

All containers and equipment should be surface decontaminated and removed from the cabinet when work is completed. At the end of the work day, the final surface decontamination of the cabinet should include a wipe-down of the work surface, the cabinet's sides and back, and the interior of the glass. If necessary, the cabinet should also be monitored for radioactivity and decontaminated when necessary. Investigators should remove their gloves and gowns in a manner to prevent contamination of unprotected skin and aerosol generation and wash their hands as the final step in safe microbiological practices.

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Start-up Work Practices with Biological Safety Cabinets (Sample SOP #2)

1. Turn off ultraviolet (UV) lamp, if one is used. (Most Biological Safety Experts do not require or advocate use of UV light.)
2. Turn on fluorescent light, and remove any obstructions and foreign materials in the front and rear ventilation grills.
3. Adjust view screen to proper height.
4. Turn on blower and allow five minutes to purge the air.
5. Wash hands and arms with mild soap, put on a lab coat and a pair of high quality disposable gloves, pulled over the knitted insert of the gown or back-closing gown.
6. Disinfect the interior surfaces of the biological safety cabinet including the work surface and interior walls (not including the supply filter diffuser) and interior surface of the window by wiping down with disinfectant and place a plastic-backed pad on the work surface

- without covering the air intake/exhaust grills. Do not use sodium hypochlorite (household bleach, Clorox) to disinfect biological safety cabinets due to potential corrosion.
7. Assemble all items for the experiment in the biological safety cabinet before initiating work and keep clean items segregated from dirty items.
 8. Organize the material so that dirty "contaminated" items are not passed over clean items causing cross contamination. Allow air to stabilize for a few minutes before starting work.
 9. Work from "clean" to "dirty" areas. Work at least six inches back from the front air intake grill.
 10. Assemble all materials to be used in experiment before initiating work. Wipe surfaces of all materials and containers placed into the cabinet with 70% ETOH to reduce introduction of contaminants.
 11. Do not use biological safety cabinets to store excess laboratory equipment.
 12. Do not block front or rear ventilation grills.
 13. Avoid sudden movements and remove arms slowly from biological safety cabinets. Delay manipulating materials for one minute after placing hands/arms flatly on front grill; raise arms slightly.
 14. Operators should keep their face above the opening of the biological safety cabinet.
 15. Use a horizontal pipette discard tray. Do not use upright pipette collection containers or containers placed on the floor outside of the cabinet; these can disrupt the air barrier and compromise your protection.
 16. Avoid flaming; an open flame creates air turbulence. Use of Bunsen burners in the cabinet may cause fire or explosion.
 17. Biological safety cabinets should be recertified annually, after changing HEPA filters, or after moving to a new location.

Appendix E

NIH GENERAL GUIDELINES Chart 1

The purpose of the *NIH Guidelines* is to specify the practices for constructing and handling: (i) recombinant nucleic acid molecules, (ii) synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, and (iii) cells, organisms, and viruses containing such molecules.* Recombinant DNA work is defined as: (1) Molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell or the DNA molecules that result from the replication of these molecules or (2) organisms and viruses containing recombinant DNA.

There are three potential levels of review of such work: (1) The ECU Biosafety Committee (IBC), the local recombinant DNA review board of the university. (2) Office of Recombinant DNA Activities (ORDA), an office within NIH; (3) Recombinant DNA Advisory Committee (RAC), a national committee that advises the Secretary, Assistant Secretary for Health, and the Director of NIH, on recombinant DNA research.

The [NIH Guidelines](#) must be followed at ECU in order for ECU to remain eligible for any NIH grant funding. All recombinant work must follow NIH rules whether it is funded by the NIH or not.

NIH defines six categories of experiments:

- (III-A) Experiments that require specific RAC review and NIH/ORDA and IBC approval before initiation of the experiment.
- (III-B) Experiments that require IBC and NIH/ORDA approval before initiation of the experiment.
- (III-C) Experiments that require IBC approval and NIH/ORDA registration before initiation.
- (III-D) Experiments that require IBC approval before initiation of experiments.
- (III-E) Experiments that require IBC notice simultaneously with initiation.
- (III-F) Experiments that are exempt from NIH Guidelines. However, registration with the IBC is required or status verified by Biological Safety from Animal use Protocol.

III-A. Experiments that require IBC approval, RAC review, and NIH director approval before initiation.

1. Deliberate release into the environment of any organisms containing recombinant DNA.
2. Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire it naturally.
3. Deliberate transfer of certain recombinant DNA molecules into a human subject that are deemed Major Actions by the NIH (see Appendix D of the NIH Guidelines for examples).

III-B. Experiments that require NIH/ORDA and IBC approval before initiation.

Experiments involving the cloning of toxin molecules with LD50 of less than 100 nanograms per kilogram body weight. Examples: botulinum, tetanus, and diphtheria toxins; *S. dysenteriae* neurotoxin.

III-C. Experiments that require IBC approval and NIH/ORDA registration before initiation.

Experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into a human subject.

Note: IBC approval must be obtained from each institution at which recombinant DNA material will be administered to human subjects (as opposed to each institution involved in the production of vectors for human application and each institution at which there is ex vivo transduction of recombinant DNA material into target cells for human application).

III-D. Experiments that require IBC approval before initiation of the experiment (generally BL2 or higher containment required).

1. Work using human or animal pathogens (risk groups 2, 3, 4 or restricted agents) as host-vector systems.
 - a. introduction of recombinant DNA into risk group 2 agents can be carried out at BL2.
 - b. introduction of recombinant DNA into risk group 3 agents can be carried out at BL3.
 - c. introduction of recombinant DNA into risk group 4 agents can be carried out at BL4.
 - d. introduction of recombinant DNA into restricted agents is a case-by-case situation to be decided after NIH/ORDA review.
 - e. in all cases, whole animal experiments will require containment levels equivalent to the risk group.
2. Work in which DNA from risk group 2, 3, 4, or restricted human or animal pathogens is cloned in nonpathogenic prokaryotic or lower eukaryotic host-vector systems.
 - a. cloning of DNA from risk group 2 or 3 agents can be carried out at BL2.
 - b. cloning of DNA from risk group 4 agents can be carried out at BL4 unless a totally and irreversibly defective fraction of the genome was cloned (BL2).
 - c. cloning of DNA from restricted agents is a case-by-case situation.
 - d. specific lowering of containment to BL1 for particular experiments can be approved by IBC.
3. Work involving the use of infectious viruses or defective viruses in the presence of helper virus in tissue culture systems.
 - a. risk group 2 agent work can be carried out at BL2.
 - b. risk group 3 agent work can be carried out at BL3.
 - c. risk group 4 agent work can be carried out at BL4.
 - d. restricted agent work is a case-by-case situation.

Experiments involving the generation of transgenic animals and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals (not lower than BL2 containment).

Introduction of recombinant DNA (ie, naked DNA injections) into a non-human vertebrate or invertebrate organism (BL1), unless the DNA represents greater than two-thirds of a eukaryotic viral genome.

Work involving more than 10 liters of culture (IBC will determine containment level).

III-E. Experiments that require IBC notification at the time of initiation. (BL1 containment required).

Work involving no more than two-thirds of any eukaryotic viral genome (except risk group 3, 4, or restricted agents; see III-D) when performed in tissue culture in the absence of helper virus.

Experiments involving the generation of transgenic rodents judged to require only BL1 containment.

III-F. Experiments that are exempt and require no registration with or approval by IBC (BL1 containment suggested).

1. Those experiments involving recombinant DNA molecules that:
 - a. are not in organisms or viruses.
 - b. 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.
 - c. 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), or when transferred to another host by well established physiological means.
 - d. consist entirely of DNA from a eukaryotic host including its mitochondria or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain).
 - e. consist entirely of DNA segments from different species that exchange DNA by known physiological processes.
 - f. contain less than one-half of any eukaryotic viral genome from risk groups 1 or 2, and are propagated and maintained in cells in tissue culture. However, experiments that involve the deliberate introduction of genes coding for the biosynthesis of molecules toxic to vertebrates or whose other aspects warrant a section III-A or III-B designation are not exempt.

Experiments which use *E. coli* K-12 host-vector systems provided that the *E. coli* host contains no conjugation-proficient plasmids or generalized transducing phages, and that lambdoid or Ff phages or non-conjugative plasmids are used as vectors.

Experiments involving *S. cerevisiae* or *S. uvarum* host-vector systems.

Experiments involving any asporogenic *B. subtilis* or asporogenic *B. licheniformis* host-vector system.

Note: Not exempt for any of the above mentioned host-vector systems (items 2-4) are:

- a. experiments whose other aspects warrant a section III-A or III-B designation.
- b. experiments involving DNA from risk groups 3, 4, or restricted organisms (see III-D).
- c. large-scale (more than 10 liters of culture) experiments.
- d. experiments involving the cloning of vertebrate toxin genes.

Experiments involving recombinant DNA molecules derived entirely from extrachromosomal elements and maintained in the natural host from a number of *Bacillus*, *Listeria*, *Pediococcus*, *Staphylococcus*, and *Streptococcus* species (see NIH Guidelines for a specific listing).

The purchase or transfer of transgenic rodents for experiments that require BL1 containment.

Note: In general if an experiment falls into two categories, the rules pertaining to the more stringent category will be followed.

EXAMPLES:

Drug Resistant Microorganism Creation

NIH, Office of Biotechnology Activities (OBA), and Recombinant DNA Advisory Committee (RAC) approval is required for work involving the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if this could compromise the use of the drug to control disease in humans, animals, or agriculture. This federal approval is followed by ECU Institutional Biological Safety Committee review and approval before initiation of the work.

III. Potent Toxin Formation

NIH/OBA will determine containment conditions for experiments involving the deliberate formation of recombinant DNA containing genes for biosynthesis of toxin molecules lethal for vertebrates at an LD of less than 100 nanograms per kg. body weight (e.g. microbial toxins, diphtheria toxins, shigella neurotoxins) ECU IBC approval is required before work is initiated.

IV. Human Gene Transfer: For deliberate transfer of Recombinant DNA or DNA or RNA derived from recombinant DNA into human research participants (Human Gene Therapy), RAC review must be completed, following ECU Institutional Review Board for Human Subjects and ECU Biological Safety Committee Review prior to enrollment of participants.

V. Experiments involving:

- A. The introduction of recombinant DNA into Risk Group 2, 3, or 4 agents or using Restricted Agents as host vector system or
- B. Cloning DNA from Risk Group 2, 3, or 4 or Restricted Agents into nonpathogenic prokaryotic or lower eukaryotic host vector systems or
- C. Using infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper viruses in tissue culture systems or
- D. The formation of recombinant DNA molecules containing more than two-thirds of the genome of any eukaryotic virus or
- E. Whole animals in which the animal genome has been altered by stable introduction of recombinant DNA into the germline (transgenic animals) or viable recombinant DNA-modified microorganisms tested on whole animals or
- F. Genetically engineered plants by recombinant DNA methods or uses plants together with microorganisms or insects containing recombinant DNA or
- G. More than 10 liters of culture material

VI. For experiments not included in sections IIIA, B, C, or D above which may be conducted at BL-1 containment, if the conditions. *Consultation with Biological Safety is required to verify that the conditions are met* and that the work is presented to the committee. (This work is clarified as "initiated simultaneously with IBC notification" in the NIH manual.)

VII. Some Recombinant DNA work which is conducted at BL-1, not conducted in organisms or viruses, does not generate aerosols, and "does not represent a significant risk to health or the environment" may be exempted as outlined on page 20

of the NIH Guidelines. *Consultation with ECU Biological Safety is required to verify that exemption applies.* (NIH Guidelines Appendix C "Exceptions to Exemptions" for specific host-vector systems).

- VIII. No work with recombinant DNA at ECU will begin before Biological Safety has reviewed the registration and assigned a containment level.**

Appendix F

ADDITIONAL NIH GUIDELINES OF PROTOCOLS FOR THE TRANSFER OF RECOMBINANT DNA MOLECULES INTO HUMAN SUBJECTS CHART 2

In addition to submitting a review/approval form to the IBC, those researchers proposing human gene therapy protocols must submit information addressing each of the following points. The submission may be in narrative form, but must address each point in the following order:

1. What is the structure of the cloned DNA that will be used?
 - a. Describe the gene (genomic or cDNA), the bacterial plasmid or phage vector, and the delivery vector (if any). Provide complete nucleotide sequence analysis or a detailed restriction enzyme map of the total construct.
 - b. Describe the regulatory elements the construct contains (e.g., promoters, enhancers, polyadenylation sites, replication origins, etc.). Name the source from which these elements are derived. Summarize what is currently known about the regulatory character of each element.
 - c. Describe the steps used to derive the DNA construct.
2. What is the structure of the material that will be administered to the patient?
 - a. Describe the preparation, structure, and composition of the materials that will be given to the patient or used to treat the patient's cells.
 - i. If DNA, describe the purity (both in terms of being a single DNA species and in terms of other contaminants) and the sensitivity of the assays that will be used to determine this.
 - ii. If a virus, describe any special features of the cell lines, media, or sera used to propagate it. Describe the purification methods and assays with their sensitivity that will be used to detect and eliminate any contaminating materials (including helper virus or other organisms) that may have biological effects.

If co-cultivation is employed, describe the cells used for co-cultivation. Describe the purification methods and assays with their sensitivities that will be used to detect and eliminate any contaminating materials. Specifically, describe the tests used to assess the material to be returned to the patient for the presence of live or killed donor cells or other non-vector materials originating from those cells.
 - iv. If other methods are to be used, describe the purification methods and assays with their sensitivities that will be used to detect and eliminate any contaminating materials. Name the possible sources of contamination.
 - b. Describe any other material to be used in the preparation of the material to be administered to the patient.
 - i. If a viral vector is proposed, describe the nature of the helper virus or cell line.
 - ii. Describe the nature of any carrier particles that are to be used.
3. What cells are the intended target cells of recombinant DNA?
 - a. Describe how ex vivo targeted cells will be characterized before and after treatment.
 - b. Describe the theoretical and practical basis for assuming that only the target cells will incorporate the DNA.
 - c. Provide the percentage of target cells that contain the added DNA.
 - d. Describe whether the added DNA is extrachromosomal or integrated and whether it is unrearranged. Describe the assays with their sensitivities to monitor this.

- e. Provide the number of copies of added DNA present per cell and describe the stability of the added DNA both in terms of its continued presence and its structural stability.
4. How efficient and specific is gene transfer and expression?
- Describe the animal and cultured cell models used to assess the in vivo and in vitro efficacy of the gene transfer system, comparing and contrasting these to the proposed human treatment.
 - Provide the minimal level of gene transfer and/or expression that is estimated to be necessary for the gene transfer protocol to be successful in humans. How was this determined?
 - Explain in detail all results from animal and cultured cell model experiments which assess the effectiveness of the delivery system in achieving the minimally required level of gene transfer and expression.
 - Describe to what extent expression is only from the desired gene (and not from the surrounding DNA). Describe to what extent the insertion modifies the expression of other genes.
 - Provide the percentage of cells that express the desired gene, whether the product is biologically active, and if so, the percentage of normal activity that results from the inserted gene.
 - Describe the extent to which the gene is expressed in cells other than the target cells.
5. Is a retrovirus delivery system being used?
- Describe the cell types that have been infected with the retroviral vector preparation and describe which cells, if any, produce infectious particles.
 - Describe the stability of the retroviral vector and resulting provirus in terms of loss, rearrangement, recombination, or mutation. Describe steps taken in designing the vector to minimize instability or variation and any assays, with their sensitivities, used to measure stability. Provide information on how much rearrangement or recombination with endogenous or other viral sequences is likely to occur in the patient's cells.
 - Describe laboratory evidence that is available concerning potential harmful effects of the transfer (eg, development of neoplasia, harmful mutations, regeneration of infectious particles, or immune responses).
 - Describe steps taken in the design of the vector to minimize its pathogenicity and describe assays with their sensitivities to determine this.
 - Provide any evidence from animal studies that vector DNA has entered untreated cells, particularly germ-line cells.
 - Provide whether a similar protocol has been conducted in non-human primates and/or other animals. If so, describe the results. Specifically, provide any evidence that the retroviral vector recombined with any endogenous or other viral sequences in the animals.
6. Is a non-retrovirus delivery/expression system being used?
- Describe animal studies that have been conducted to determine if there are pathological or other undesirable consequences of the protocol (including insertion of DNA into cells other than those treated, particularly germ-line cells).
 - Provide how long the animals have been studied after treatment.
 - Describe any safety studies that have been conducted, including data about the level of sensitivity of such assays.

Appendix G

Approved Safety Medical Device List Available in Medical Store Room

Category	Product/ Brand	Catalog #
IV connectors/ Needleless tubing	Braun-Ultrasite Horizon pump set	180-26010
	Braun- Primary tubing with safeline injection sites	180-26030
	Braun-Safeline injection cap	180-26230
	Braun Safeline clip-lock cannula	180-26235
Safety Lancets	Lifescan Roach Safe T-Pro	090-22858 060-22128
Sharps Disposal	BD Guardian Nestable 8qt	170-25600
	6gal	170-25620
	BD Mountable 5qt	170-25630
Venous Access Needle	B Braun Introcan 22g 1"	180-26085
Butterfly winged steel needle	BD Vacutainer 25g3/4"	180-26160
	23g	180-26180
	21g3/4"	180-26200
	multisample	180-26185
Blood Collection Tube Holder And Blood Collection Needle	BD Vacutainer one use holder	060-22150
	BD Vacutainer Eclipse 21g1"	060-22200
	BD Vacutainer Eclipse 22g1"	060-22220
Blood Transfer Device	BD Vacutainer	060-22225
Blood Smear Device	DIF Safe	060-22240
Scalpel*	BD self sheathing #10	130-24024
	#11	13024045
	#15	130-24065
Needles and Syringes	1CC INSULIN 29G X1/2"INTEGRA	170-2520
	½MLINSL/29GX1/2 SAFETYGLIDE	170-25265
	1ML 25G X 5/8 DETACH NEEDLE	
	ECLIPSE	170-25375
	1CC TB/25G X 5/8" SAFETY SAFETY	
	GLIDE	170-25380
	1CC TB 26GX3/8 305946 SAFETY GLIDE	
	BD TB 1ML 27G X ½ NEEDLE	170-25395
	SAFETYGLIDE	
	1ML 27G X ½ LUER DETACH	170-25398
	SAFETYGLIDE	
	BD INTEGRA SAFETY3ML 21G X 1	170-25399
	3ML 23G X 1" ECLIPSE	
	3ML 22G X 1 ½ " ECLIPSE	170-25405
	3CC 21G X 1.5 INTEGRA SAFETY	170-25407
	3ML 25G X 5/8 INTEGRA NEEDLE	170-25408
	3ML 25G X 1" INTEGRA SAFETY	170-25410
		170-25412
		170-25415
Huber Needles	DELTEC GRIPPER PLUS 22G X .25	180-26243
	DELTEC GRIPPER PLUS 22G X 5/8	180-26245
	HUBER INFUS SFTY NEED 22GX1	180-26250

	SPECIALIZED	180-26255
	DELTEC GRIPPER PLUS 22G X .75	
	DELTEC GRIPPER PLUS 22G X 1	180-26257
	HUBER INFS SAFTY NDLE 20GX1.5	180-26260
	HUBER INFS SAFTY NDLE 22GX.75	
		180-26270

- Pediatrics evaluated and chose to use Eclipse safety needles and syringes
- Pediatrics evaluated and chose to use Deltec Gripper Plus Huber Needle, Adult Heme Onc is using Specialized Huber Needle

Appendix H

SOP for moving/removing Biological Safety Cabinets (BSC)

1. Department will:
 - A. Submit Work Order to Facilities Services re move request, simultaneously*
 - B. Notify Biological Safety:
Biological Safety will:
Verify that the cabinet has been thoroughly disinfected by the user
 - ii. Decontaminate the cabinet, if needed, based on the organism.Post a certificate marking the cabinet as disinfected.
2. Biological safety will contact the Facilities Services Utilities Director that cabinet is ready for disconnection.
3. Facilities Services will:
 - A. Verify disinfection sticker present
 - B. Disconnect gas and electrical connections
 - C. Schedule air exhaust disconnection if needed
 - D. Inform Biological Safety if other researchers using the same vent trunk will be affected.

If so, Biological Safety will notify other researchers of interruptions in airflow, warn them not to use their cabinets, and expect possible alarms during this process.

After all of these disconnections and notifications are complete, Facilities Services

Utilities Director will contact Moving Services.

- A. Moving Services will:
 - i. Schedule date to relocate the cabinet to its next site, storage, or removal from cam
 - ii. Provide date to the Biological Safety Officer and to department.Biological Safety will notify other researchers on shared ventilation system re-date and potential impact on their work
- C. Cabinet is moved
- D. Biological Safety notifies other researchers when ventilation is re-established and cabinets may be used again.

Appendix I

TRANSFER OF POTENTIALLY CONTAMINATED SURPLUS PROPERTY

Approved by: _____
ENVIRONMENTAL SAFETY COMMITTEE CHAIRPERSON DIRECTOR, ENVIRONMENTAL HEALTH
& SAFETY

Policy

Departments/personnel offering equipment for disposal or surplus must certify that the equipment has been appropriately characterized and is not contaminated with and/or does not contain chemicals, biological materials, radioactive materials or any other type of material that would pose a safety, health or environmental hazard. Items that are potentially contaminated with or contain chemicals, biohazardous materials or radioactive materials must receive appropriate clearance from Environmental Health and Safety (EH&S) and/ or Prospective Health before the equipment is offered for disposal or surplus. The purpose of this policy is to prevent the unintentional exposure of employees, the public or the environment to these hazardous materials.

Equipment meeting this criteria includes but is not limited to: Chemical fume hoods, biological safety cabinets, centrifuges, incubators, cryostats, microfuges, refrigerators, freezers, shakers, chemical storage cabinets, chemical products, thermometers, barometers, gas cylinders, gas chromatographs, germicidal UV lamps, lasers, scintillation counters, and x-ray equipment.

Prohibited Items – The following items cannot be offered for surplus

- **Before the following items are offered for disposal or surplus, contact Prospective Health at 744-2070 for authorization and technical guidance:**
 - Any device which produces ionizing radiation. Ex: x-ray units, x-ray diffraction machines, accelerators, etc.
 - Equipment which may be contaminated with radioactive material. Ex: refrigerators, freezers, centrifuges, etc.
 - Equipment with sealed radioactive source(s). ex: some gas chromatographs, Beta and Gamma scintillation (older models) counters, portable hazardous chemical detectors, etc.
 - Sealed radioactive sources. Ex: small check sources, Cs-137, Am 241, Co-57, etc.
 - Class III and IV LASER devices.
 - Equipment which may be contaminated with biological material.
- **For items that contain or may be contaminated with chemical hazards or other safety and/or environmental hazards contact EH&S at 328-6166 for authorization and technical guidance.**

Responsibility

Equipment Owner

1. When offering equipment for surplus or transfer that contains or is potentially contaminated with chemical, biological or radioactive materials, the equipment owner must contact EH&S (chemical hazards) or Prospective Health (biological and radiological hazards) for authorization and guidance.
2. If contamination levels exceed appropriate release criteria, equipment owners must assure that the equipment is decontaminated in accordance with procedures approved by EH&S and/or Prospective Health; follow the required containment, labeling, and transportation procedures specified by EH&S and/or Prospective Health; and arrange for proper disposal when deemed necessary.
3. The equipment owner must certify that equipment is free of contamination or that hazards have been properly secured or eliminated. The equipment owner must complete the "Surplus Property Hazard Assessment" tag included as appendix A and attach to the property in a conspicuous location and secure manner.
4. Equipment owners retain custody of the equipment until completion of the appropriate transfer procedure.
5. Equipment owners are responsible for any costs associated with required sampling/analysis and/or disposal.

Surplus Property/Moving Services

1. Personnel must not accept equipment for transfer unless a completed "Surplus Property Hazard Assessment" tag is attached to the equipment.
2. Personnel shall inspect all equipment before taking possession to ensure no obvious unsecured hazards exist.
3. Personnel must immediately cease activities if they identify any potential health or safety hazard and then shall contact their supervisor and EH&S or Prospective Health for assistance.
4. Personnel must attend training provided by EH&S and Prospective Health.

EH&S

1. Authorize surplus or transfer of equipment containing or potentially contaminated with chemicals and/or environmental hazards.
2. Survey equipment for chemical contamination and related hazards. Determine the appropriate sampling procedures, collect samples for analysis, and interpret results to characterize the item.
3. Advise equipment owners (departments) of proper decontamination, containment, labeling, transportation and disposal requirements as appropriate.
4. Serve as advisory agent to department and surplus personnel and provide training opportunities for affected personnel.
5. Periodically audit program to assure compliance with policy.



Prospective Health

1. Authorize surplus or transfer of equipment containing or potentially contaminated with biological or radiological hazards.
2. Survey equipment for biological and radiological contamination and related hazards. Determine the appropriate sampling procedures, collect samples for analysis, and interpret results to characterize the item.
3. Advise equipment owners (departments) of proper decontamination, containment, labeling, transportation and disposal requirements as appropriate.
4. Serve as advisory agent to department and surplus personnel and provide training opportunities for affected personnel.
5. Periodically audit program to assure compliance with policy.

Procedure

1. When offering equipment for surplus or transfer that contains or is potentially contaminated with chemical, biological or radiological hazards, the equipment owner must first contact EH&S (chemical) or Prospective Health (biological or radiological) for authorization and guidance.
2. Equipment owners must certify that the equipment does not contain or is not contaminated with hazardous materials.
3. If the property contains or has contained any hazardous material then the property must be properly decontaminated in accordance with procedures approved by EH&S and/or Prospective Health. For biological and/or radiological hazards, contact Prospective Health at 744-2070. For chemical and all other hazards contact EH&S at 328-6166. If it is determined that the equipment is beyond decontamination then the equipment must be offered for proper disposal.
4. The "Surplus Property Hazard Assessment" tag must be completed by the equipment owner and attached to the property in a conspicuous location and secure manner. Tags are available from EH&S and/or Prospective Health.
5. **EH&S and/or Prospective Health must inspect the equipment and authorize the move before the department submits a work order to Moving Services. EH&S and/or Prospective Health staff must check the appropriate authorization box on the "Surplus Property Hazard Assessment" tag and initial to confirm inspection/authorization.**
6. The equipment owner must schedule move of the equipment in accordance with Facilities Services Moving Services Policy and Materials Management standard practice that includes, but is not limited to, submitting a work order via PETS <http://www.ecu.edu/cs-admin/purchasing/surplus/PETS.cfm>. A work order for moving services will also have to be created. **NOTE: A minimum of 10 working days advance notification is required for scheduling moves and longer during busy periods such as Registration, Commencement, Homecoming, etc.**
7. Moving personnel will not accept equipment for transport unless "Surplus Property Hazard Assessment" tag is attached and there is no visible contamination.

Appendix A – “Surplus Property hazard assessment” TAG

	NOTICE	
Surplus Property Hazard Assessment		
<p>I hereby certify that this property (CHECK ONLY ONE):</p> <p><input type="checkbox"/> Has never contained or been contaminated with hazardous materials (chemical, biological or radiological).</p> <p><input type="checkbox"/> Has been decontaminated in accordance with procedures approved by EH&S and/or Prospective Health. (Identify Hazard Category: <input type="checkbox"/> Chemical <input type="checkbox"/> Biological <input type="checkbox"/> Radiological)</p>		
Description:		ECU Tag #:
Department & Location:		
Equipment Owner (print):		
Signature:		
Authorized by: <input type="checkbox"/> EH&S <input type="checkbox"/> Prospective Health Initial _____		
COMPLETED TAG MUST BE ATTACHED TO EQUIPMENT		

Appendix J

Pipetting

Aerosols are created during pipetting procedures by liquid dropping from a pipette to a work surface, by mixing cultures by alternate suction and blowing, by forceful ejection of an inoculum onto a culture dish, or by blowing out the last drop. It has been demonstrated by high-speed photography that an aerosol of approximately 15,000 droplets, most under ten micrometers, is produced when the last drop of fluid in the tip of the pipette is blown out with moderate force. While the aerosol hazard associated with pipetting procedures can only be reduced by use of safe techniques and of biological safety cabinets, the potential hazard associated with oral ingestion can be eliminated by use of mechanical pipetting aids.

There are many commercially available safety pipetting aids that can be used. A particular type of pipetting aid that may be satisfactory to one individual or kind of operation may not meet the requirements of others; therefore, different types should be tried in each situation. Ease of manipulation and accuracy of delivery by the aids are important factors in their selection. Care should be taken in selecting a device so that its design or use does not contribute to exposure to hazardous contaminants such as by leakage, that the suction end of the pipette and other exposed parts (other than deliver tips) are kept free of hazardous substances, and that the unit can be easily sterilized and cleaned after use. Pipetting techniques that reduce aerosols include:

1. Never use mouth pipetting. Always use some type of pipetting aid.
2. If working with biohazardous materials, pipetting operations should be confined to a safety cabinet or hood.
3. Pipettes used for the pipetting of biohazardous or toxic materials always should be plugged with cotton (even when safety pipetting aids are used).
4. No biohazardous material should be prepared by bubbling expiratory air through a liquid with a pipette.
5. Biohazardous material should not be mixed by suction and expulsion through a pipette.
6. No biohazardous material should be forcibly expelled out of a pipette.
7. When pipettes are used, avoid accidentally dropping infectious cultures from the pipette. Place a disinfectant-soaked towel on the working surface and autoclave the towel after use.
8. Mark-to-mark pipettes are preferable to other types, since they do not require expulsion of the last drop.
9. Discharge from pipettes should be as close as possible to the fluid or agar level, or the contents should be allowed to run down the wall of the tube or bottle whenever possible, not dropped from a height.
10. Contaminated pipettes should be placed horizontally in a pan containing enough suitable disinfectant to allow complete immersion. They should not be placed vertically in a cylinder.
11. Discard pans for used pipettes are to be housed within the biological safety cabinet.
12. The pan and pipettes should be autoclaved as a unit. The replacement unit should be a clean pan with fresh disinfectant.¹

¹ Taken from "Laboratory techniques for biohazard control" from the *Biosafety Manual*, University of North Carolina at Chapel Hill. 2000.

Appendix K

Opening Culture Plates, Tubes, Bottles, and Ampoules

In the absence of definite accidents or obvious spillage, it is not certain that opening of plates, tubes and bottles of other microorganisms has caused laboratory infection. However, it is probable that among the highly infective agents some infections have occurred by this means. Particular care is required when opening plates, tubes, or bottles containing fungi, for this operation may release a large number of spores. Such cultures should be manipulated in a biological safety cabinet.

To assure a homogenous suspension that will provide a representative sample, liquid cultures are agitated before a sample is taken. Vigorous shaking will create a heavy aerosol. A swirling action will generally create a homogenous suspension with a minimum of aerosol. When a liquid culture is resuspended, a few minutes should elapse prior to opening the container to reduce the aerosol.

The insertion of a sterile, hot wire loop or needle into a liquid or slant culture can cause spattering and release of an aerosol. To minimize the aerosol production, the loop should be allowed to cool in the air or be cooled by touching it to the inside of the container or to the agar surface where no growth is evident prior to contact with the culture or colony. Following use of the inoculating loop or needle, it is preferable to sterilize the instrument in an electric or gas incinerator specifically designed for this purpose rather than heating in an open flame. These small incinerators have a shield to contain any material that may spatter from the loop. Disposable inoculating loops are available commercially. Rather than decontaminating them immediately after use with heat, they are discarded first in a disinfectant solution.

The practice of streaking an inoculum on rough agar results in aerosol production, created by the vibrating loop or needle. This generally does not occur if the operation is performed on smooth agar. It is good safety practice to discard all rough agar poured plates that are intended for streaking purposes with a wire loop.

Water of syneresis in Petri dish cultures usually contains viable microorganisms and forms a film between the rim and lip of the inverted plate. Aerosols are dispersed when this film is broken by opening the plate. Vented plastic Petri dishes where the lid touches the rim at only three points are less likely to offer this hazard. The risk may also be minimized by using properly dried plates, but even these (when incubated anaerobically) are likely to be wet after removal from an anaerobic jar. Filter papers fitted into the lids reduce, but do not prevent, dispersal. If plates are obviously wet, they should be opened in the biological safety cabinet. Less obvious is the release of aerosols when screw-capped bottles or plugged tubes are opened. This happens when a film of contaminated liquid, which may collect between the rim and the liner, is broken during removal of the closure. The practice of removing cotton plugs or other closures from flasks, bottles, and centrifuge tubes, etc., immediately following shaking or centrifugation can generate aerosols and cause environmental contamination. The technique of shaking tissue cultures with glass beads to release viruses can create a virus laden aerosol. Removal of wet closures, which can occur if the flask or centrifuge tube is not held in an upright position, is also hazardous. In addition, when using the centrifuge, there may be a small amount of foaming and the closures may become slightly moistened. Because of these possibilities, it is good safety practice to open all liquid cultures of infectious or hazardous material in a biological safety cabinet wearing gloves and a long-sleeved laboratory garment.

Dried, infectious culture material may also collect at or near the rim or neck of culture tubes and may be dispersed into the air when disturbed. Containers of dry powdered hazardous materials should be opened only in a biological safety cabinet.

When a sealed ampoule containing a lyophilized or liquid culture is opened, an aerosol may be created. Aerosol creation should be prevented or minimized, and opening of ampoules should be done in safety cabinets.

When recovering the contents of an ampoule, care should be taken not to cut the gloves or hands or disperse broken glass into the eyes, face, or laboratory environment. In addition, the biological product itself should not be contaminated with foreign organisms or with disinfectants. To accomplish this, work in a safety cabinet and wear gloves. Nick the ampoule with a file near the neck. Wrap the ampoule in disinfectant-wetted cotton. Snap the ampoule open at the nick, being sure to hold the ampoule upright. Alternatively, at the file mark on the neck of the ampoule, apply a hot wire or rod to develop a crack. Then, wrap the ampoule in disinfectant-wetted cotton, and snap it open. Discard cotton and ampoule tip into disinfectant. The contents of the ampoule are reconstituted by slowly adding fluid to avoid aerosolizing the dried material. Mix contents without bubbling, and withdraw it into a fresh container. Some researchers may desire to use commercially available ampoules prescored for easy opening. However, there is the possibility to consider that this may weaken the ampoule and cause it to break during handling and storage. Ampoules of liquid cultures are opened in a similar way.²

² Taken from "Laboratory techniques for biohazard control" from the *Biosafety Manual*, University of North Carolina at Chapel Hill. 2000.

Appendix L

Blenders, Mixers, Sonicators, and Cell Disruption Equipment

Hazardous aerosols are created by most laboratory operations concerned with blending, mixing, stirring, grinding or disrupting materials such as cells, tissues, blood samples, freeze dried sera, and environmental samples that may contain infectious, toxic or otherwise hazardous materials. Even use of the mortar and pestle can be a hazardous operation. Ball mills, colloid mills, jet mills, tissue grinders, magnetic mixers, stirrers, sonic cleaning devices, ultrasonic cell disintegrators, and shakers are other devices that can produce hazardous aerosols.

Aerosols of infectious or hazardous materials can escape from sonicators because of loosely fitting covers, loose gaskets at the bottom of the cup, or when the contents are removed from the cup. It is also recommended that sonicators be used in biological safety cabinets. Adequate decontamination of equipment potentially contaminated with infectious material prior to sonic cleaning is essential because of the hazard of creating aerosols during the sonic treatment.

Safe laboratory practices that are required generally when using blenders, mixers, ultrasonic disintegrators, colloid mills, jet mills, grinders, and mortars and pestles with hazardous biological or chemical materials are as follows:

- Operate blending and cell disruption and grinding equipment in a biological safety cabinet.
- Use safety blenders designed to prevent leakage from the rotor bearing at the bottom of the bowl. In the absence of a leak proof rotor, inspect the rotor bearing at the bottom of the blender bowl for leakage prior to operation. Test it in a preliminary run with sterile water, saline or methylene blue solution prior to use
- If the blender is used with infectious material, use a towel moistened with disinfectant over the top of the blender. Sterilize the device and residual contents promptly after use.
- Glass blender bowls are undesirable for use with infectious material because of potential breakage. If used, they should be covered with a propylene jar to prevent dispersal of glass in the event the bowl breaks.
- Heat-sealed flexible disposable plastic film enclosure can be used for a grinder or blender. The safest practice is to use these within a biological safety cabinet.
- Before opening the safety blender bowl, permit the blender to rest for at least one minute to allow settling of the aerosol cloud.³

³ Taken from "Laboratory techniques for biohazard control" from the *Biosafety Manual*, University of North Carolina at Chapel Hill, 2000.

Appendix M

Centrifuge Safety

Centrifugation presents two serious hazards: mechanical failure and dispersion of aerosols. A mechanical failure, such as a broken drive shaft, a faulty bearing, or a disintegrated rotor, can produce not only aerosols but also hazardous fragments moving at great velocity. These fragments, if they escape the protective bowl of the centrifuge, could produce traumatic injury to personnel. A well- functioning centrifuge, however, is still capable of producing hazardous aerosols of biological material or chemicals if improperly used or in the absence of good laboratory practices. Mechanical failure can be minimized by meticulous observance of the manufacturers' instructions, and aerosols can be avoided by observing sound laboratory practices and use of appropriate centrifuge safety equipment or biological safety cabinets.

Although accidents from improper use of centrifuges and equipment associated therewith are far less frequent than with pipettes or syringes and needles, when they do occur, aerosols usually are created, and the possibility of causing multiple exposures is considerably greater.

Activities, such as filling centrifuge tubes, removing cotton plugs and rubber caps from tubes after centrifugation, removing the supernatant and resuspending the cells, are capable of releasing aerosols into the environment. The greatest hazard associated with centrifuging biohazardous materials is created when a centrifuge tube breaks. When tubes break or crack and a fluid containing microorganisms remains in the cup under centrifugal force, relatively few organisms are released into the air compared to breakage that releases the fluid into the centrifuge chamber.

Safety Procedures Applicable to All Centrifuging

A safety centrifuge cabinet or safety centrifuge trunnion cup should be used when centrifuging hazardous or infectious substances. When bench-type centrifuges are used in a biological safety cabinet, the centrifuge operation creates air currents that may cause the escape of agent from an open cabinet. Class III, biological safety cabinets provide the best containment of the high velocity aerosols which may be generated during centrifugation.

Centrifuge tubes and trunnion cups should be filled and opened in a biological safety cabinet. If centrifugation is to be performed outside the cabinet, the safety trunnion cup should be used. After it is filled and sealed, it should be considered potentially contaminated and should be wiped with a cloth soaked in disinfectant or passed through a disinfectant dunk bath. Since some disinfectants are corrosive to centrifuge cups and heads, a rinse of the cup with clean water is desirable after an appropriate contact time has elapsed.

In some situations, in the absence of "O" ring sealed trunnion cup caps, specimens can be enclosed in sealed plastic bags before centrifugation. In the event of breakage, however, the plastic bag is likely to be ruptured. Thus, this technique normally only prevents the escape of organisms that contaminate the outside of the cup.

Before centrifuging, eliminate tubes with cracks and chipped rims, inspect the inside of the trunnion cup and correct rough walls caused by erosion or adhering matter, and carefully remove bits of glass and other debris from the rubber cushion.

A disinfectant should be added between the tube and trunnion cup to disinfect the materials in case of accidental breakage. This practice also provides an excellent cushion against shocks that might otherwise break the tube. Care must be taken, however, not to contaminate the culture material with the disinfectant. It must be recognized also that the disinfectant may not completely inactivate the infectious material when the tube breaks because of the dilution of the disinfectant and the high concentration and packing of cells.

Avoid pouring the supernatant material from centrifuge tubes. If you must do so, wipe off the outer rim with a disinfectant afterwards; otherwise, in a subsequent step, biohazardous fluid may be spun off as droplets that form an aerosol. Use of a vacuum system with appropriate in-line safety reservoirs and filters is preferable to pouring from centrifuge tubes or bottles.

If the sediment is packed, infectious microorganisms or other hazardous material must be carefully resuspended in order to minimize the amount of aerosol created, it is better to use a swirling, rotary motion rather than shaking. If vigorous shaking is essential to suspend the material or achieve homogeneity, a few minutes should elapse before opening the container to allow the aerosol to settle. Shaking always contaminates the closure; thus, there is the added hazard of liquids dropping from the closure or running down the outside of the container. A biological safety cabinet and the use of gloves may be required to assure safety to the laboratory worker when performing some of these operations.

Avoid filling the centrifuge tube to the point that the rim, cap, or cotton plug becomes wet with culture. Screw caps or caps that fit over the rim outside the centrifuge tube are safer than plug-in closures. Some fluids usually collect between a plug-in closure and the rim of the tube. Even screw-capped bottles are not without risk, however, if the rim is soiled and sealed imperfectly, some fluid will escape down the outside of the tube. Aluminum foil should not be used to cap centrifuge tubes containing toxic or infectious materials because these lightweight caps often become detached or ruptured during handling and centrifuging.

The balancing of buckets and trunnion cups is often improperly performed. Care must be taken to ensure that matched sets of trunnions, buckets and plastic inserts do not become mixed. If the components are not inscribed with their weights by the manufacturer, colored stains can be applied for identification to avoid confusion. When the tubes are balanced, the buckets, trunnions and inserts, including any disinfectant solution or water added for balancing, should be included in the procedure. The basic concern is that the centers of gravity of the tubes are equidistant from the axis of rotation. To illustrate the importance of this, two identical tubes containing 20 g. of mercury and 20 g. of water, respectively, will balance perfectly on the scales; however, their performance in motion is totally different, leading to violent vibration with all its attendant hazards.

Older Type and Small Portable Centrifuges

Older type centrifuges that do not have aerosol-tight chambers have been shown to allow the escape of aerosol created from various sources:

- Biohazardous fluid remaining on the lip of the tube after decanting the supernatant fluid
- Leakage from a tube in an angle-head centrifuge resulting from over-filling a tube and placing aslant in the centrifuge
- Leakage from nongrid tubes that distort under centrifugal forces, or
- Fluid trapped in the threads of screw caps.

Safety trunnion cups should be used to prevent escape of aerosol in the event the primary culture container held in the cup should break or in any other manner allow the release of agent into the cup. The handling of the culture, the filling of centrifuge tubes and placing them in the safety trunnion cups should be done in a biological safety cabinet. The outside of the trunnion cup should be decontaminated before the cup is removed for centrifuging. Subsequently, the cup should be returned to and opened in a biological safety cabinet. Where applicable, the centrifuge itself should be placed in the cabinet, and, if need be, a cabinet should be specifically constructed for the centrifuge.

Small portable, "clinical" centrifuges have been shown to be hazardous. The microhematocrit centrifuge, in particular, has been shown to produce aerosols. A frequent practice is to centrifuge blood samples in tubes without closures or to use cotton plugs secured in the tubes by means of tape or pins. It should be recognized that some tissue specimens contain viable infectious microorganisms, particularly hepatitis virus, and that open tubes, contaminated closures, and

release of aerosols from blood samples and tissues suspensions can be hazardous to laboratory personnel.

Sharples Centrifuges

Using the Sharples centrifuge with infectious or hazardous materials poses both engineering design and safety problems. The Sharples centrifuge is driven by a steam or air turbine, requires refrigeration around the bowl, and is equipped with feed and effluent lines. It has a continuous feed that could involve large volumes of liquid material, depending on the amount of solids in the material to be handled and the type of bowl. The centrifuge generates a massive aerosol that is almost impossible to contain within the instrument even with a hermetically sealed bowl. For these reasons, a ventilated safety cabinet is necessary to enclose the centrifuge. It may be desirable to accommodate the material to be centrifuged and the effluent in the cabinet or handle it by means of connectors through the walls of the cabinet. If the rotor must be transferred to another cabinet after use, it should be passed through a dunk bath, wrapped in a disinfectant-soaked towel, or placed in another container, the outside of which is decontaminated. Decontamination of the centrifuge bowl, lines, and surrounding cabinet can be accomplished by liquid disinfectants, formaldehyde vapor or ethylene oxide, followed by additional cleaning and rinsing. The rotor can be steam sterilized.

High-Speed Centrifuges

Centrifugation at high speeds presents additional hazards because of the higher stresses and forces applied to components of the system. In addition to the recommended practices listed above, precautions should be taken to filter the air exhausted from the vacuum lines, to avoid metal fatigue resulting in disintegration of rotors, and to apply proper techniques in cleaning, handling, and using centrifuge components. Some of these precautions are discussed briefly below.

In high-speed centrifuges, the chamber is connected to a vacuum pump. If there is breakage or accidental dispersion of infected particles, the pump and the oil in it will become contaminated. A HEPA filter should be placed between the centrifuge and the pump.

High-speed rotor heads are prone to metal fatigue, and, where there is a chance that rotors may be used on more than one machine, each rotor should be accompanied by its own log book indicating the number of hours run at top or de-rated speeds. Failure to observe this precaution can result in dangerous and expensive disintegration. Frequent inspection, cleaning, and drying are important to ensure absence of corrosion or other damage that may lead to the development of cracks. If the rotor is treated with a disinfectant, it should be rinsed with clean water and dried as soon as the disinfectant has adequately decontaminated the rotor. Rubber "O" rings and tube closures must be examined for deterioration and be kept lubricated with the material recommended by the makers. Where tubes of different materials are provided (e.g., celluloid, polypropylene, stainless steel), care must be taken that the tube closures designed specifically for the type of tube in use are employed. These caps are often similar in appearance, but are prone to leakage if applied to tubes of the wrong material. When properly designed tubes and rotors are well maintained and handled, leaking should never occur.

Cleaning and disinfection of tubes, rotors and other components require considerable care. It is unfortunate that no single process is suitable for all items, and the various manufacturers' recommendations must be followed meticulously if fatigue, distortion and corrosion are to be avoided. This is not the place to catalogue recommended methods, but one less well appreciated fact is worthy of mention. Celluloid (cellulose nitrate) centrifuge tubes are not only highly flammable and prone to shrinkage with age and distortion on boiling, but also can be highly explosive in an autoclave.

Large-Scale Zonal Centrifuges

Zonal centrifuges have been developed to process relatively large volumes, 5 to 150 liters, of material. The pumps, valves, seals, feed lines, connectors, and vacuum and cooling systems, associated with these centrifuges, as well as the large volumes processed at high speeds create the potential for leakage and generation of hazardous aerosols leading to the contamination both of the environment and of the operating personnel. The following areas have been identified as the principal sources of potential leakage: the centrifuge lip and face seals, the coolant system, the turbine exhaust air, various lines and connectors, the feed system, fraction collection, and during decontamination. In addition, the possibility of spills occurring during loading, unloading, sample collection, decontamination, and other procedures must be recognized.

The several seals in the equipment pose the greatest potential for escape of hazardous material because inherent to the system is the necessity for pressurizing the process fluid to obtain flow through the rotor; in addition, leaks may occur because of the large centrifugal forces exerted at all points in the rotating component. Procedural hazards identified include: (a) the danger of snagging or rupturing one of the numerous lines (influent, effluent, rotor by-pass, etc.), particularly when hemostats are used as clamps; (b) undetected over-pressurization of lines resulting in a rupture of a line or failure of a connection because flow was obstructed by bubbles caught in the system; (c) manual making and breaking of connections during the centrifuge operation; (d) inadequate precautions in handling gradient fractions containing very high concentrations of the purified material; and (e) incomplete or ineffective decontamination procedures of the rotor, feed lines and other components of the equipment before disassembly and cleanup.⁴

⁴ Taken from "Laboratory techniques for biohazard control" from the *Biosafety Manual*, University of North Carolina at Chapel Hill, 2000.

Appendix N

Policy: **Cleaning, Disinfection, and Sterilization**

Purpose:

The need for appropriate cleaning, disinfection, and sterilization of patient-care items has been emphasized by published reports documenting infection after improper decontamination practices. Because it is neither necessary nor possible to sterilize all patient-care items, clinical policies must identify whether cleaning, disinfection, or sterilization is indicated, based primarily on an item's use. This policy provides a practical approach to the prudent selection and proper disinfection and sterilization processes.

Cleaning

Cleaning is the physical removal of foreign material, e.g., dust, soil, organic material such as blood, secretions, excretions and microorganisms. Cleaning physically removes rather than kills microorganisms. It is accomplished with water, detergents and mechanical action. Cleaning is always essential prior to disinfection or sterilization. An item that has not been cleaned cannot be assuredly disinfected or sterilized. Organic material left on a medical device can shield microorganisms and protect them from the action of disinfectants or sterilants or interact with the disinfectant or sterilant to neutralize the activity of the process.

Disinfection

Disinfection is the inactivation of disease producing microorganisms. Disinfection does not destroy bacterial spores. Disinfectants are used on inanimate objects; antiseptics are used on living tissue. Disinfection usually involves chemicals, heat, or ultraviolet light. Levels of disinfection vary with the type of product used.

Levels of Disinfection

Low Level Disinfection: required when processing noncritical items or some environmental surfaces. Low level disinfectants kill most vegetative bacteria and some fungi as well as enveloped (lipid) viruses (e.g., hepatitis B, C, Hantavirus, and HIV). Low level disinfectants do not kill mycobacteria or bacterial spores. Low levels disinfectants-detergents are used to clean environmental surfaces

Intermediate Level Disinfection: required for some semi critical items. Intermediate level disinfectants kill vegetative bacteria, most viruses and most fungi but not resistant bacterial spores

High Level Disinfection: required when processing semicritical items. High level disinfection processes destroy vegetative bacteria, mycobacteria, fungi and enveloped (lipid) and non enveloped (non lipid) viruses, but not necessarily bacterial spores. High level disinfectant chemicals must be capable of sterilization when contact time is extended. Items must be thoroughly cleaned prior to high level disinfection.

Sterilization

Sterilization is the destruction of all forms of microbial life including bacteria, viruses, spores and fungi. Items must be cleaned thoroughly before effective sterilization can take place.

E.H. Spaulding devised a classic approach to cleaning, disinfecting, and sterilizing patient-care equipment. He believed that the nature of disinfection would be understood more readily if items for patient care were divided into 3 categories based on the potential risk of infection involved in the use of the items. The 3 categories of risk of patient-care items suggested by Spaulding were critical, semicritical, and non critical. (Table 1)

Critical Items: instruments and devices that enter sterile tissues, including the vascular system. Critical items present a high risk of infection. Reprocessing critical items requires meticulous cleaning followed by sterilization.

Semicritical Items: devices that come in contact with nonintact skin or mucous membranes but ordinarily do not penetrate them. Reprocessing semicritical items involves meticulous cleaning followed by high level disinfection/

Non Critical Items: those that either touch only intact skin but not mucous membrane or do not directly touch the patient. Reprocessing of non critical items involves cleaning or low level disinfection.

Table 1.

Object Classification	Use of Item	Example	Decontamination required after cleaning
Critical	Enters vascular system or sterile body tissues	Scalpels and other surgical instruments, intravascular instruments, biopsy forceps	Sterilization and holding in sterilized state. High level disinfection is not sufficient
Semi-Critical	Comes in contact with mucous membranes	Thermometer, vaginal speculum, endoscopes, vaginal ultrasound probes, nasal specula	High level disinfection (by heat or chemicals)
Non-Critical	Comes in contact with intact skin	Examination table top, blood pressure cuff, stethoscope, baby weigh scales	Intermediate or low level disinfection

Table 2. Summary of Approved Sterilization Methods

Method	Processing Times	Application examples
Steam	~ 40 min.	Heat tolerant critical and semi-critical patient care items
Dry Heat	1-6 hours depending on temperature	Heat tolerant critical and semi-critical patient care items
Ethylene Oxide Gas	~ 15 hours	Heat sensitive critical and semi-critical patient care items
Hydrogen peroxide gas plasma	~ 50 min.	Heat sensitive critical and semi-critical patient care items
Chemical Sterilants 2.65% glutaraldehyde	10 hrs @ 22°C	Heat sensitive critical and semi-critical patient care items that can be immersed
1.12% glut. And 1.93% phenol	12 hrs	
7.35% hydrogen peroxide and 0.23% paracetic acid	3 hrs @ 20°C	
7.5% hydrogen peroxide	6 hrs @ 20°C	
1.0% hydrogen peroxide and 0.08% paracetic acid	8 hrs	
> 0.2% paracetic acid	~50 min	

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Table 3. Summary of Approved Agents for Disinfection

Agent	Advantages	Disadvantages	Efficacy	Indications
Alcohol Ethyl and isopropyl 60-90%	Fast acting, Nonstaining	Flammable, drying, evaporates quickly	Excellent activity against gram + and gram-organisms; rapidly bactericidal against vegetative bacterial; tuberculocidal, virucidal, and fungicidal but not sporicidal	Intermediate level disinfectant. Used primarily for disinfecting external surfaces (e.g., stethoscopes) and as an antiseptic for disinfection of the skin
Hydrogen peroxide	Sporicidal at higher concentrations; by-products environmentally friendly	Oxidizing properties may be damaging to endoscopes; some reports of pseudo-membranous colitis associated with 3% hydrogen peroxide	Bactericidal, fungicidal, virucidal, sporicidal; MEC is 6% for 7.5% hydrogen peroxide and 0.85% phosphoric acid combination; no difference in germicidal effectiveness between 2% and 7.5% hydrogen peroxide; 30 min at 20° HLD claim	Used for disinfection of soft contact lens, tonometers, and ventilators; 7.5% hydrogen peroxide/0.85% phosphoric acid solution acceptable for endoscope reprocessing unless incompatible with endoscopic material
Chlorine Household bleach (5% sodium hypochlorite solution with 50,000 ppm available chlorine)	Fast acting, low level of toxicity; low cost; broad spectrum activity	Corrosive; inactivated by organic material; unstable; efficacy decreases with increase in Ph; CDC guidelines recommend that chlorine solutions be made fresh daily; Should not be used on instruments and medical devices	Effective against bacteria, mycobacteria, fungi, and viruses, high concentrations of chlorine required to kill mycobacterium tuberculosis ; not sporicidal	Depending on concentration, is HLD, ILD, LLD Use 1 part bleach with 9 parts water (1:10 or 500 ppm) for disinfection of blood spills Use 1 part bleach to 99 parts water (1:100 or 5,000 ppm)) for disinfection of countertops and floors
Iodophors (combination of iodine and carrier; best	Rapid action; low toxicity and irritation; effective carrier	Corrosive; inactivated by organic material; staining;; may	Bactericidal, virucidal, mycobacteriocidal, fungicidal; not	Primarily used as an antiseptic; has also been used to disinfect blood

known is povidone-iodine) Manufacturer instructions must be followed for dilution		burn tissue	sporicidal	culture tubes, thermometers and hydrotherapy tanks; concentrations differ significantly between iodophor antiseptics and disinfectants; therefore antiseptic formulations are not indicated for disinfectant use
Phenolics (used in 0.4%-5% aqueous solutions;	Broad spectrum	Leaves film on surfaces; inactivated by organic matter; may cause tissue irritation; corrosive to certain materials; has been associated with hyperbilirubinemia when used in nursery	Bactericidal, virucidal, fungicidal, and tuberculocidal; not sporicidal	Considered ILD to LLD; Used to decontaminate hospital environment (walls, floors, furnishings); should not be used on instruments and medical devices
QACs Quaternary Ammonium Compounds (used in 0.4%-1.6 aqueous solution)	Good cleaning agent; less irritating to hands than some detergents	Not sporicidal, tuberculocidal, or virucidal against hydrophilic viruses; less microbiocidal in presence of organic matter; less microbiocidal in presence of materials such as gauze and cotton; incompatible with soap	Bactericidal, fungicidal; effective against lipophilic viruses	Considered LLD; Used for decontamination of hospital environment (walls, floors, furnishings); should not be used for disinfecting instruments and medical devices
Glutaraldehyde Chemical sterilant	Effective in presence of organic matter; noncorrosive; noncoagulation of protein material	Unstable; may cause contact dermatitis following skin exposure and may be irritating to eyes, nose and throat; effective concentrations dependent on use conditions	Bactericidal, fungicidal, virucidal, mycobactericidal, and sporicidal; MEC is 1%-1.5%	HLD for medical equipment and instruments, endoscopes, RT equipment, anesthesia equipment, transducers; not for use on noncritical items due to toxicity and cost

		and organic stress		
Formaldehyde (liquid and gaseous forms in limited use; used primarily as water based solution formalin (37% formaldehyde by weight); used as HLD or ILD; 4% is tuberculocidal agent)	Broad spectrum; not affected by organic matter	Potential carcinogen; irritating fumes and odor; OSHA limits exposure in workplace (8 hr TWA 0.75 ppm)	Bactericidal, tuberculocidal, fungicidal, virucidal, and sporicidal	Used primarily for preparing viral vaccines, preserving specimens, and as embalming agent; sometimes used to reprocess hemodialyzers; CDC recommends 4% concentration with 24hr exposure time for hemodialyzers reused for same pt.
Paracetic Acid (chemical sterilant formed by reaction between hydrogen peroxide, acetic acid and water)	By products not harmful; no residue; effective in presence of organic matter; sporicidal at low temperatures; rapid action	Can be corrosive to copper, brass, bronze, plain steel, and galvanized iron; unstable when diluted; severe burns may result from direct contact to skin; irreversible damage or blindness can occur following direct contact of eyes; inhalation of vapor or mist may irritate nose, throat, and lungs	Bactericidal, tuberculocidal, fungicidal, virucidal, and sporicidal	Chemically sterilize medical, surgical, and dental instruments; also used with automated endoscopic reprocessing systems (for use with immersible endoscopes)
Paracetic acid/hydrogen peroxide (sterilant primarily used as HLD; 25min at 20°C HLD claim; product containing 0.08% paracetic acid plus 1.0% hydrogen peroxide has been cleared by FDA as liquid chemical sterilant)	No activation required; mild odor	Materials compatibility issues with lead, brass, copper, and zinc	Effective against all microorganisms except bacterial spores	Has been used for disinfecting hemodialyzers; consult with manufacturers for product compatibility

Orthophthalaldehyde (e.g., Cidex OPA) HLD; contains 0.55.% orthophthalaldehyde	Stable over wide Ph range of 3-9; nonirritating to eyes and nose; requires no activation; materials compatibility with wide range of devices	Can be staining to skin and clothing	Excellent microbiocidal activity	Has FDA clearance for use as liquid HLD on flexible endoscopes
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FDA-Cleared Sterilants and High Level Disinfectants with General Claims for Processing Reusable Medical and Dental Devices available in the ECU Medical Store Room

Manufacturer	Active Ingredients	Sterilant Contact Conditions	High level Disinfectant contact conditions
Cidex™ Activated Dialdehyde Solution			
Johnson & Johnson Medical Products	2.4% glutaraldehyde	Indication for device sterilization. 10 hrs at 25°C 14 days Maximum Reuse Contact conditions based on AOAC Sporocidal Activity Test only.	45 min at 25°C 14 days Maximum Reuse Contact conditions based on literature references.
K875280?? STERIS 20™ Sterilant <i>Note: Cleared for use with the STERIS System 1™ Processor only.</i>			
STERIS Corporation	0.2% peracetic acid	Indication for device sterilization. 12 min at 50-56°C Single use only. Contact conditions established by simulated use testing with endoscopes and passing a modified AOAC Sporocidal Activity Test.	No indication for high level disinfection.

See **Gluteraldehyde Usage Guidelines policy** for complete instructions on high level disinfection and sterilization using Cidex.

For a complete listing of FDA cleared sterilants and high level disinfectants with general claims for processing reusable medical equipment and dental devices, refer to <http://www.fda.gov/cdrh/ode/germlab.html>.

EPA Registered Intermediate and Low Level Disinfectants Available in the ECU Medical Store room

Product	EPA Reg. #	Active Ingredient	Contact Time
Clorox Bleach (not a cleaner,	5813-1	Sodium	1:10 dilution* at 2min. for blood

must first remove blood or other organic matter prior to disinfection)		hypochlorite 5.25%	and body fluids If stored in closed opaque bottle, at room temp., this dilution may be used up to 30 days. 1:500 dilution** at 1 ½ min. or air dry for general cleaning (e.g., walls, floors, counter tops etc.). This dilution must be mixed fresh daily
PDI Sani Cloth Germicidal Wipes	9480	Isopropanol 55%	2 min.
Dispatch Hospital Cleaner with Bleach (can be used as a cleaner due to added surfactants and deodorizer)	56392-7	Sodium hypochlorite 0.55%	2 min.
Lysol Disinfectant Spray	777-72-875	Ethanol 79%	2-3 sec. spray until surface is covered in mist. Allow to stand for 10 min. or air dry.

*1:10 dilution = 1 part bleach in 10 parts water = 5,000 ppm

**1:500 dilution = 1 part bleach to 500 parts water = 100 ppm

For a complete listing of all EPA registered disinfectants refer to:

<http://www.epa.gov/oppad001/chemregindex.htm>.

Appendix O

Biosafety Considerations for Research with Lentiviral Vectors

Recombinant DNA Advisory Committee (RAC) Guidance Document

Background: The use of lentiviral vectors has been increasing because the vector system has attractive features; however, such research also raises Biosafety issues. The NIH Office of Biotechnology Activities has received frequent questions regarding the appropriate containment for lentiviral vectors, particularly those derived from HIV-1. Because the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* do not explicitly address containment for research with lentiviral vectors, the RAC was asked to provide additional guidance for institutional Biosafety committees (IBCs) and investigators on how to conduct a risk assessment for lentiviral vector research. At the March RAC 2006 meeting (webcast), the RAC offered the following findings and recommendations.

Risks of lentivirus vectors: The major risks to be considered for research with HIV-1 based lentivirus vectors are

- potential for generation of replication-competent lentivirus (RCL), and
- potential for oncogenesis.

These risks can be mitigated by the nature of the vector system (and its safety features) or exacerbated by the nature of the transgene insert encoded by the vector.

General criteria for risk assessment of lentivirus vectors: Decisions about containment should take into account a range of parameters/considerations including:

- the nature of the vector system and the potential for regeneration of replication competent virus from the vector components,
- the nature of the transgene insert (e.g., known oncogenes or genes with high oncogenic potential may merit special care)
- the vector titer and the total amount of vector,
- the inherent biological containment of the animal host, if relevant,
- negative RCL testing (*see section below*)

General containment considerations: Either BL2 containment or enhanced BL2 containment is often appropriate in the laboratory setting for research involving the use of advanced lentivirus vector systems that have multiple safety features and that segregate vector and packaging functions onto four or more plasmids. Enhanced BL2 containment may include in addition to attention to sharps (and use of safety needles where feasible), the use of personal protective equipment intended to reduce the potential for mucosal exposure to the vector. In most such research, these levels of containment are also expected to be appropriate even when producing large volumes of HIV-1 vectors (>10 L).

The appropriate containment level for specific lentivirus vector research is, of course, determined following a complete risk assessment and local IBC review. The following sections discuss some considerations which should form an important part of the Biosafety assessment for research involving lentivirus vectors.

Potential for generation of replication competent lentivirus (RCL) from HIV-1 based lentivirus vectors: The potential for generation of RCL from HIV-1 based lentivirus vectors depends upon several parameters, the most important of which are

- the number of recombination events necessary to reassemble a replication competent virus genome and
- the number of essential genes that have been deleted from the vector/packaging system.

On this basis, later generation lentivirus vector systems are likely to provide for a greater margin of personal and public safety than earlier vectors, because

- they use a heterologous coat protein (e.g., VSV-G) in place of the native HIV-1 envelope protein (However, the use of the certain coat proteins, such as VSV-G, may broaden the host cell and tissue tropism of lentivirus vectors, which should also be considered in the overall safety assessment by the IBC),
- they separate vector and packaging functions onto four or more plasmids and
- they include additional safety features (e.g., they do not encode Tat, which is essential for replication of wild-type HIV-1).

In contrast, earlier vector systems (such as two-plasmid vector systems) may have a higher potential for generation of RCL.

RCL testing: The National Gene Vector Laboratory (NGVL) has produced over 60 liters of HIV-1 vector and has screened supernatant and cells from different vector systems, using different assays, without detecting RCL (K. Cornetta, personal communication of unpublished data). This suggests that the frequency of RCL generation using lentivirus vectors is very low. It may not, however, be zero. There is a need for continued investigation of RCL generation using lentivirus vectors, in order to inform and advance the field of lentivirus vector technology.

The FDA requires that lentiviral vector stocks used in human clinical trials be tested for RCL. Individual research laboratories conducting preclinical research often use only small volumes (e.g., a few milliliters) of lentivirus vectors expressing lower risk transgenes such as GFP. While these laboratories are not mandated to characterize vector stocks, such testing should be encouraged. However, RCL testing requires expertise with the appropriate assays and such expertise may not be available in laboratories that do not work regularly with infectious lentiviruses. In such laboratories, the use of a positive control may increase risk to the investigator as compared to use of the test material. IBCs may make containment assignments without requiring such testing by undertaking a risk assessment that considers the nature of the specific vector system being used and overall past experience with the system.

Animal studies: Some animals, such as wild-type mice, cannot support replication of infectious HIV-1. As a result, the potential for shedding of RCL from such animals is very low (even if RCL were present in the original vector inoculum). IBCs may consider the biosafety issues associated with animal husbandry and housing *after* the initial injection separately from the initial inoculation itself. In general, the initial delivery of vector should be performed under BL2 or BL2-N, according to the animal model, or under enhanced BL-2/BL2-N containment (see "*General containment considerations*"), so as to minimize the risk of autoinoculation by the investigator. However, it may be permissible to reduce the containment level at some point following vector delivery. For example, if there is no expectation of infection (see below), the site of inoculation has been thoroughly cleansed, and the bedding changed, it may be acceptable to consider reducing containment from BL2/BL2-N to BL1/BL1-N within a few days (the specific time

period can be specified by the local IBC, and may vary anywhere from 1-7 days depending on local and experimental considerations). Animals engrafted with human cells or animal hosts that are permissive for HIV-1 replication constitute a special case, in light of their potential to support replication of infectious HIV-1. Use of lentivirus vectors in these animals requires a higher level of containment.

Other lentivirus vectors: Some non-human lentivirus vectors (e.g., FIV, SIV, EIAV, etc.) are also in use. Of these, the most frequently encountered are feline immunodeficiency virus (FIV) vectors. In the Appendix B-V of the *NIH Guidelines*, a containment level appropriate for Risk Group 1 agents is recommended for use of certain animal viral etiologic agents not associated with disease in healthy human adults. However, replication-defective vectors in which a heterologous envelope (such as VSV-G) is used for vector packaging may require BL2 containment in the laboratory setting since these vectors have the potential to infect human cells, and thus have the potential to cause insertional mutagenesis. Under circumstances in which mice are not permissive hosts for FIV replication, BL1 containment may be acceptable for mouse housing and husbandry when dealing with mice that have received FIV vectors (subject to the considerations noted above).

Summary: A comprehensive risk assessment and determination of containment for research with lentiviral vectors should consider the nature of the vector system, transgene insert, and type of manipulations involved. For many experiments, either BL-2 or enhanced BL-2 will be appropriate. Examples of Biosafety considerations and risk assessments for three different scenarios are included below.

Examples of Biosafety Considerations

Vector Considerations

- Potential for generation of RCL
 - Vector and packaging functions separated onto multiple plasmids
 - Deletion of viral genes
- Viral Env used in packaging system
 - Non-native Env (decrease potential for generation of RCL)
 - Coat protein that increases species or cell type tropism of parent virus (e.g., VSV-G)
- Safety modifications (e.g., no expression of Tat)

Transgene Considerations

- Oncogene
- Non-oncogene

Vector Generation Considerations


- Laboratory scale
- Large scale

Animal Research Considerations

- Permissive host
- Non-permissive host
- Animal engrafted with permissive cells
- Vector Administration (e.g., injection)
- Housing and husbandry

Practices, Containment Equipment and Training Considerations

- Training in use of PPE
- Availability of safety equipment (e.g., sealed centrifuge rotor cups)
- Laboratory-specific safety and spill cleanup protocols
- Availability of on-site occupational health support in the event of accident

Biosafety Considerations and Risk Levels		
Biosafety Considerations	Higher Risk	Lower Risk
		
Vector Design	<ul style="list-style-type: none"> • Vector packaging functions on two plasmids • Expression of viral genes 	<ul style="list-style-type: none"> • Vector and packaging functions separated onto multiple plasmids • Deletion of viral genes
Transgene	<ul style="list-style-type: none"> • Oncogene 	<ul style="list-style-type: none"> • Non-oncogene
Vector Generation	<ul style="list-style-type: none"> • Large scale 	<ul style="list-style-type: none"> • Laboratory scale
Animal Hosts	<ul style="list-style-type: none"> • Permissive host • Animals engrafted with human cells 	<ul style="list-style-type: none"> • Non-permissive host
Animal Manipulation	<ul style="list-style-type: none"> • Vector administration (e.g., use of sharps during injection) 	<ul style="list-style-type: none"> • Housing and husbandry (no use of sharps)

EXAMPLE SCENARIOS

EXAMPLE ONE: *In vitro* study A:

Use of a 4-plasmid derived lentivirus vector encoding siRNA against Lck in primary human T cells.

Considerations

1. *What is the amount of vector to be produced?* A = LOW (100 ml)
2. *What is the nature of the vector?* A = 4-Plasmid System
3. *What is the nature of the insert?* A = Non-Oncogenic

Tentative Safety Assessment = BL2

(Note that the use of primary human cells would require BL2 containment, independent of the vector, as well as use of Universal Precautions and compliance with the OSHA standard for Bloodborne Pathogens – 29 CFR 1910.1030)

EXAMPLE TWO: *In vitro* study B:

Use of a 2-plasmid derived lentivirus vector encoding luciferase in a human cell line (A549 cells).

Considerations

1. *What is the amount of vector to be produced?* A = LOW (100 ml)
2. *What is the nature of the vector?* A = 2-Plasmid System (non-commercial)
3. *What is the nature of the insert?* A = Non-Oncogenic

Tentative Safety Assessment = BL2 enhanced

BL2 “enhanced” stipulations might include:

- Avoidance of needles and sharps, where possible
- Use of a containment hood for all work with the vector (including the loading and unloading of centrifuge rotors, which should have an aerosol-tight seal)
- Use of personal protective equipment [PPE] designed to prevent a mucosal exposure/splash to the face and exposure of hands (especially in persons with broken skin or open cuts)
- A requirement for an in-person consultation between Biosafety staff and lab personnel prior to initiation of experiments

EXAMPLE THREE: *In vivo* study A

Use of a 4-plasmid derived lentivirus vector encoding brain-derived neurotrophic factor (BDNF) in mouse brain

Considerations

1. *What is the amount of vector to be produced?* A = LOW (100 ml)
2. *What is the nature of the vector?* A = 4-Plasmid System
3. *What is the nature of the insert?* A = Non-Oncogenic (*: see below)
4. *What is the nature of the animal host?* A = Non-permissive for HIV-1

Tentative Safety Assessment = BL2 for lab work and initial injection of mice (which would probably be done using a stereotactic frame); after 1-7 days, animals could be moved to BL1 containment.

Added explanation:

- Even though BDNF is a growth factor for neurons, it has no known oncogenic activity for skin or blood cells that might be the target of a potential needle stick. Hence, this insert would not automatically trigger a requirement for increased biocontainment.
- Stereotactic injection frames cannot easily be placed into a laminar flow hood, and may use a syringe or pulled glass pipette for inoculation; they may also use a pump to ensure a slow rate of delivery of the agent. BL2 containment does NOT require

the use of a Biosafety cabinet, and is therefore compatible with the use of a stereotactic frame, even if that frame is not contained within a laminar flow cabinet.

Additional points to consider:

- An in-person consultation between Biosafety staff and lab personnel prior to initiation of experiments may be a useful stipulation
- One might also impose additional Biosafety enhancements during the injection process, perhaps by requiring use of additional PPE above and beyond the stipulated requirements associated with BL2/BL2-N. See Example 2 for examples of such stipulations.

APPENDIX

Sections from the *NIH Guidelines*

General Considerations

Section II-A. Risk Assessment

Section II-A-3. Comprehensive Risk Assessment. BL2 containment is recommended for activities involving all blood-contaminated clinical specimens, body fluids, and tissues from all humans, or from HIV – or HBV-infected or inoculated laboratory animals. Activities such as the production of research-laboratory scale quantities of HIV or other Biosafety pathogens, manipulating concentrated virus preparations, or conducting procedures that may produce droplets or aerosols, are performed in a BL2 facility using the additional practices and containment equipment recommended for BL3. Activities involving industrial scale volumes or preparations of concentrated HIV are conducted in a BL3 facility, or BL3 Large Scale if appropriate, using BL3 practices and containment equipment.

Appendix

Section III-D. Experiments That Require Institutional Biosafety Committee Approval Before Initiation

Section III-D-3. Experiments Involving the Use of Infectious DNA or RNA Viruses or Defective DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems. Recombinant DNA or RNA molecules derived therefrom, which contain less than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family (see Section V-J, *Footnotes and References of Sections I-IV*) being considered identical (see Section V-K, *Footnotes and References of Sections I-IV*), are considered defective and may be used in the absence of helper under the conditions specified in Section III-E-1, *Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus*.

Section III-E. Experiments That Require Institutional Biosafety Committee Notice Simultaneous with Initiation

Section III-E-1. Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus. Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family being considered identical [see Section V-J, *Footnotes and References of Sections I-IV*]) may be propagated and maintained in cells in tissue culture using BL1 containment. For such experiments, it must be demonstrated that the cells lack helper virus for the specific Families of defective viruses being used. If helper virus is present, procedures specified under Section III-D-3, *Experiments Involving the Use of Infectious Animal or Plant DNA or RNA Viruses or Defective Animal or Plant DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems*, should be used. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than two-thirds of a genome.

Animal Studies

Section III-D-4-a. Recombinant DNA, or DNA or RNA molecules derived therefrom, from any source except for greater than two-thirds of eukaryotic viral genome may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of physical containment

comparable to BL1 or BL1-N and appropriate to the organism under study (see Section V-B, *Footnotes and References of Sections I-IV*). Animals that contain sequences from viral vectors, which do not lead to transmissible infection either directly or indirectly as a result of complementation or recombination in animals, may be propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study. Experiments involving the introduction of other sequences from eukaryotic viral genomes into animals are covered under Section III-D-4-b, *Experiments Involving Whole Animals*. For experiments involving recombinant DNA-modified Risk Groups 2, 3, 4, or restricted organisms, see Sections V-A, V-G, and V-L, *Footnotes and References of Sections I-IV*. It is important that the investigator demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see Section V-G, *Footnotes and References of Sections I-IV*).

APPENDIX B. CLASSIFICATION OF HUMAN ETIOLOGIC AGENTS ON THE BASIS OF HAZARD

Appendix B-III-D. Risk Group 3 (RG3) – Viruses and Prions

Retroviruses

--Human immunodeficiency virus (HIV) types 1 and 2

BL2 Facilities

Appendix G-II-B-3. Containment Equipment (BL2)

Appendix G-II-B-3-a. Biological safety cabinets (Class I or II) (see Appendix G-III-L, *Footnotes and References of Appendix G*) or other appropriate personal protective or physical containment devices are used whenever:

Appendix G-II-B-3-a-(1). Procedures with a high potential for creating aerosols are conducted (see Appendix G-III-O, *Footnotes and References of Appendix G*). These may include 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.

Appendix G-II-B-3-a-(2). High concentrations or large volumes of organisms containing recombinant DNA molecules are used. Such materials may be centrifuged in the open laboratory if sealed beads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

Appendix G-II-B-4. Laboratory Facilities (BL2)

Appendix G-II-B-4-a. The laboratory is designed so that it can be easily cleaned.

Appendix G-II-B-4-b. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G-II-B-4-c. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G-II-B-4-d. Each laboratory contains a sink for hand washing.

Appendix G-II-B-4-e. If the laboratory has windows that open, they are fitted with fly screens.

Appendix G-II-B-4-f. An autoclave for decontaminating laboratory wastes is available.

BSLs for HIV-1 Lentivirus Vectors

The University of Pittsburgh has historically required all HIV-1 lentivirus work to be done at the BSL-2+ level. We are presently amending our approval process to allow certain types of this work to be done at BSL-2. It follows the recent NIH-RAC guidance for 4-plasmid systems plus allowing 3-plasmid systems to be used at BSL-2 if certain criteria are met (no oncogenes; <100 ml; RCV negative – see Table below).

No. of plasmids	Oncogenic transgene or production > 100 ml	RCV testing	Vector production	Use of viral vector in vitro	Use of viral vector in animals	Use of virus-transfected cells in animals
4 or more	Yes	Not required	BSL-2+	BSL-2+	ABSL-2+	ABSL-2+
4 or more	No	Not required	BSL-2	BSL-2	ABSL-2	ABSL-2
3	Yes	With or without RCV testing	BSL-2+	BSL-2+	ABSL-2+	ABSL-2+
3	No	Elect to test for RCV	BSL-2 Work may not proceed beyond this point until RCV data approved by IBC	BSL-2 Only after final approval by IBC following acceptance of RCV data	ABSL-2 Only after final approval by IBC following acceptance of RCV data	ABSL-2 Only after final approval by IBC following acceptance of RCV data
3	No	No RCV test	BSL-2+	BSL-2+	ABSL-2+	ABSL-2+

EXAMPLE FROM UNIVERSITY OF PITTSBURGH

Appendix P

Template for Reporting Adverse Events In Human Gene Transfer Trials

This template is intended to facilitate the reporting of adverse events in human gene transfer trials. You may download this as a Word document and the fields will expand according to the amount of text entered. Use of this template is not required and other formats (e.g. AdEERS reports, MedWatch forms) may be acceptable provided that they include all the information specified in M-I-C-4-a of the *NIH Guidelines for Research Involving Recombinant DNA Molecules*. (<http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html>)

Submitting this completed template to the NIH Office of Biotechnology Activities alone does NOT fulfill the reporting requirements of other agencies. However, some agencies may accept submission of a duplicate copy of this completed template. You should verify with the other parties to whom you report whether the use of this template is acceptable.

Completed reports may be sent via U.S. mail, courier service, e-mail, or facsimile to:

NIH Office of Biotechnology Activities
6705 Rockledge Drive, Suite 750
Bethesda, Maryland 20892-7985
 (For all non-USPS deliveries use Zip Code 20817)
 Telephone 301-496-9838
 Fax 301-496-9839

E-mail address for Reporting Adverse Events: GeMCRIS@od.nih.gov
 General E-mail: oba@od.nih.gov
 Website: www4.od.nih.gov/oba/

PROTOCOL AND EVENT TYPE

NIH/OBA (RAC) Protocol Number	
FDA IND number	
Date this report completed:	
Seriousness of the AE (choose one)	Death Life-threatening Initial or prolonged hospitalization Disability Congenital anomaly Required intervention to prevent permanent impairment/damage Other medically important condition Non-serious
Severity of Event	Minimal Moderate Severe Life- Threatening Fatal
Was this event expected in terms of its severity?	Yes No
Was this event expected in terms of its specificity?	Yes No
Relationship of Event to gene transfer product	Unrelated Unlikely Possible Probable Definite

Attribution of AE Attribution of AE, continued	Concomitant medication Product Intervention Underlying disease Route of administration Other suspected cause (describe)
Type of report	Initial Follow-up
<u>DEMOGRAPHICS</u>	
PI Name	
Name of Clinical Trial Site/Organization	
PI Telephone Number	
PI E-mail Address	
Reporter name	
Reporter Telephone number	
Reporter E-mail address	
Research Participant's study identification number	
Research Participant's gender	
Research Participant's date of birth	
Research Participant's date of death	
Research Participant's weight in kgs	
Research Participant's height in cms	
Which Arm/Cohort/treatment group was the subject assigned to?	
Was subject dosed?	Yes No Information Not Available
What study agent was received:	IND agent Placebo Blinded Study Agent
Were there any Protocol Deviations/Violations/Exceptions for this participant?	Yes: _____ _____ No
<u>DETAILED ADVERSE EVENT INFORMATION</u>	
Adverse Event Date	
Description of Event	
Relevant tests (e.g. x-rays) and results	

Treatment (s) of Adverse Event (Include medications used to treat this event.)	
Name of Concomitant Medications (Do not include medications used to treat this event.)	
Pre-existing conditions/ relevant clinical history (if this is an oncology trial, please designate primary disease, e.g. ovarian cancer)	
Date(s) of treatment(s) of the adverse event	
Was autopsy performed?	Yes No
Date of autopsy	_____ or Not Applicable _____
Outcome of the event	Recovered/resolved Recovering/resolving Not recovered/not resolved Recovered/resolved with sequelae Fatal Unknown
Documentation accompanying the report (e.g., H& P, Progress Notes, Discharge Summary, Lab or Autopsy Reports, Other, etc.)	
Description of any "other" documentation	

PRODUCT AND DOSING INFORMATION

Name of gene transfer product	
Vector type (e.g. adenovirus)	
Vector sub-type (e.g. type 5, also include relevant deletions)	
Lot number	
Was the agent manufactured at an NGVL?	
Route of administration	
Site of administration	
Did subject receive the dose specified in the protocol?	
If not, what dose was given?	
Date of first exposure to study agent?	
Date of most recent exposure to study agent?	
Total dose received prior to this event?	
Total dose quantity administered to subject to date	
Unit of measure for a single dose	

Dose quantity in a single administration	
If courses used, how many were given prior to this event?	
How many doses on the last course were given?	
Was the administration of this product stopped because of this adverse event?	
Name of other treatment (s) (medications, radiation, surgery) received by research participant as required by the protocol	

Template for Reporting Incidents Involving Recombinant DNA to the NIH Office of Biotechnology Activities (OBA)

The *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* states that "...any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses" must be reported to NIH OBA within 30 days. Certain types of incidents must be reported on a more expedited basis. Spills or accidents in BL2 laboratories resulting in an overt exposure must be immediately reported to NIH OBA. Spills or accidents occurring in high containment (BL3 or BL4) laboratories resulting in an overt or potential exposure must be immediately reported to NIH OBA.

This template is intended to facilitate the reporting of incidents that occur during the conduct of research subject to the *NIH Guidelines*. You may download this template as a Word document and the fields will expand according to the amount of text entered. Use of this template is not required and other formats may be acceptable.

A separate template for reporting Human Gene Transfer Adverse Events is available at: http://www4.od.nih.gov/oba/RAC/Adverse_Event_Template.doc

Please note that submitting this completed template to NIH OBA does NOT fulfill the reporting requirements of other agencies. You should verify with the other parties to whom you must report whether the use of this template is acceptable.

Completed reports may be sent via U.S. mail, courier service, e-mail, or facsimile to:

Attention: Incident Reports
NIH Office of Biotechnology Activities
6705 Rockledge Drive, Suite 750
Bethesda, Maryland 20892-7985
(For all non-USPS deliveries use Zip Code 20817)
Telephone 301-496-9838
Fax 301-496-9839
E-mail: oba@od.nih.gov

NIH OBA Incident Reporting Template

For reporting Human Gene Transfer Adverse Events a separate template is available at: http://www4.od.nih.gov/oba/RAC/Adverse_Event_Template.doc

Does this incident involve research subject to the <i>NIH Guidelines</i> ?	YES NO If no, this incident does not have to be reported to OBA
--	--

Institution name:	
Date of report:	
Reporter name and position:	
Reporter telephone:	
Reporter email:	
Date of incident:	
Name of principal investigator:	
Is this an NIH funded project?	YES NO
If yes, please provide:	NIH grant or contract number: NIH funding institute or center: NIH program officer contact information (name, email etc):
What was the nature of incident?	Personnel exposure Spill Loss of containment Loss of transgenic animal Failure to obtain IBC approval Failure to follow approved containment conditions Other – please describe:

Did the Institutional Biosafety Committee (IBC) approve this research	YES NO
If yes, please provide:	If yes, on what date? Approval date: Approved Biosafety level for the research: Additional approval requirements:
What section(s) of the <i>NIH Guidelines</i> is the research subject to?	
Has a report of this incident been made to other federal or local agencies? If so please indicate by checking the appropriate box.	CDC USDA FDA EPA OSHA Research Funding Agency/Sponsor: (name) _____ State/Local Public Health Federal/State/Local Law Enforcement Other – please describe:

Please provide a narrative of the incident including a timeline of events. The incident should be described in sufficient detail to allow for an understanding of the nature and consequences of the incident. **Include the following information as applicable.**

A description of:

- The recombinant agent or material involved.
- The incident/violation location (e.g. laboratory Biosafety level, vivarium, non-laboratory space).
- Who was involved in the incident/violation, including others present at the incident location? **Note – please do not identify individuals by name. Provide only position titles (e.g., graduate student, post doc, animal care worker, facility maintenance worker).**
- Actions taken immediately following the incident/violation, and by whom, to limit any health or environmental consequences of the event.
- The training received by the individual(s) involved and the date(s) the training was conducted.
- The institutional or laboratory standard operating procedures (SOPs) for the research and whether there was any deviation from these SOPs at the time of the incident/violation.
- Any deviation from the IBC approved containment level or other IBC approval conditions at the time of the incident/violation.
- The personal protective equipment in use at the time of the incident/violation.
- The occupational health requirements for laboratory personnel involved in the research.
- Any medical advice/treatment/surveillance provided or recommended after the incident.
- Any injury or illness associated with the incident.
- Medical surveillance results (if not available at the time of initial report please indicate when results will be available).
- Equipment failures.

DESCRIPTION OF INCIDENT: (use additional space as necessary)

DESCRIPTION OF INCIDENT: (continued)

		YES	NO
		If yes, please provide a copy the minutes of the IBC meeting in which the incident was reviewed.	
Has the IBC reviewed this incident?		YES	NO
Has a root cause for this incident been identified?		YES	NO
Describe measures taken by the institution to mitigate any problems identified. For measures identified but not yet taken, please include a timeline for their implementation: (use additional space as necessary)		If yes please describe:	

- Please provide copies of any documents referenced in this report.
- Additional information may be requested by OBA after review of this report depending on the nature of the incident.

Appendix Q

Genetically Modified (Transgenic) Animals and the Use of Recombinant or Synthetic Nucleic Acid Molecules in Animals

FAQs for Research Subject to the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*

1. Under which section of the *NIH Guidelines* does the generation of transgenic rodents fall?

The creation of transgenic rodents falls under one of two sections of the *NIH Guidelines* depending on the containment level required to house the rodents. Experiments involving the creation of transgenic rodents that can be housed under Biosafety Level 1 conditions are covered under **Section III-E-3**. Experiments involving the generation of transgenic rodents requiring BL2, BL3 and BL4 containment are covered under **Section III-D-4**.

2. Under which section of the *NIH Guidelines* does the generation of transgenic animals other than rodents fall?

The creation of all transgenic animals (other than rodents that can be housed under BL1 containment conditions) is covered under **Section III-D-4** of the *NIH Guidelines*.

3. Would the breeding of two different lines of knock-out mice require IBC approval under the *NIH Guidelines*?

If the technique used to create the knock-out mice involves the stable introduction of recombinant or synthetic nucleic acid molecules into the animal's genome, the animals will be considered transgenic. As the breeding of two different lines of knock-out animals will potentially generate a novel line of transgenic animal, the work may be covered under the *NIH Guidelines* and require IBC review and approval. Sections in the *NIH Guidelines* that cover work with rodents include **III-E-3** for work that requires Biosafety Level (BL) 1 containment and **III-D-4** for work that requires BL2, BL3 and BL4 containment. Certain breeding experiments are exempt under Appendix C-VII the *NIH Guidelines*. This exemption covers the breeding of two different lines of transgenic rodents or the breeding of a transgenic rodent and a non-transgenic rodent with the intent of creating a new line of transgenic rodent that can be housed at BL1 if:

- (1) both parental rodents can be housed under BL1 containment; and
- (2) neither parental transgenic rodent contains the following genetic modifications: (i) incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or (ii) incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); and
- (3) the transgenic rodent that results from this breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses.

4. Is IBC registration and approval needed for the maintenance of a transgenic animal colony?

The maintenance of a transgenic rodent colony (i.e. breeding within a particular transgenic line) that can be housed at BL1 is an activity that is exempt from the *NIH Guidelines* and, as such, does not require IBC registration and approval. The maintenance of a transgenic rodent colony at BL2 or higher falls under **Section III-D-4-b** and requires IBC approval. The

breeding of all other transgenic animals is subject to the *NIH Guidelines* under Section **III-D-4-a** or **III-D-4-b** depending on the containment level required.

5. Is the purchase and transfer of transgenic rodents exempt from the *NIH Guidelines*?

Under **Appendix C-VI** of the *NIH Guidelines*, the purchase or transfer of transgenic rodents that may be housed under BL1 containment are activities that are exempt from the *NIH Guidelines*. The purchase or transfer of transgenic rodents that require BL2 or higher containment is not exempt from the *NIH Guidelines*. These animals are covered under **Section III-D-4**, and purchase and transfer of such animals requires IBC registration and approval.

It should be noted that the subsequent use of transgenic rodents may not be exempt from the *NIH Guidelines*. Experiments using transgenic rodents at BL1 are exempt from the *NIH Guidelines* if the research does not involve the use of recombinant or synthetic nucleic acid molecules. If the protocol does involve the use of recombinant or synthetic nucleic acid molecules then the work falls under **III-D-4** of the *NIH Guidelines* and as such requires IBC review and approval prior to initiation.

6. Is the purchase and transfer of transgenic animals other than rodents exempt from the *NIH Guidelines*?

No, only the purchase or transfer of transgenic rodents that may be maintained at BL1 containment is exempt from the *NIH Guidelines*. The purchase or transfer of any other animal for research purposes at any biosafety level (including BL1) is not exempt, nor is the purchase and transfer of transgenic rodents that require BL2 or higher containment.

7. Are gene ablation studies covered by the *NIH Guidelines*?

The answer to this question depends on the technique employed in the study. If recombinant techniques are used to knock out the gene, then the work would be covered under the *NIH Guidelines*.

8. Who has the responsibility to review the generation of transgenic animals if an institution is generating animals for investigators who are not affiliated with that institution?

The generation (creation) of transgenic animals is an activity covered under the *NIH Guidelines*. The IBC at the institution where that activity is occurring has the responsibility to review and approve that activity (if the institution is subject to the requirements of the *NIH Guidelines*). The subsequent use of the animals by an investigator not at that institution would need to be reviewed and approved by the IBC at that investigator's institution if that institution conducts or supports research with recombinant or synthetic nucleic acid molecules, receives NIH support for such research, and the activity covered under the *NIH Guidelines*.

9. When a core facility generates transgenic mice as a "fee for service" for a Principal Investigator (PI), is it the responsibility of the PI or the core facility to register the generation of the mice with the IBC?

Section IV-B-7-a-(1) of the *NIH Guidelines* articulates one of the responsibilities of the PI as 'initiating no recombinant or synthetic nucleic acid molecules research which requires IBC approval prior to initiation until that research has been approved by the IBC and has met all other requirements of the *NIH Guidelines*.' It would be acceptable for either the PI or the core facility or the PI purchasing the transgenic animals to fulfill the responsibility to register the generation of the animals. In many cases, the animals being generated will be subsequently used in experiments that are subject to the *NIH Guidelines*, and the registration of the research

with the IBC may encompass both the generation and subsequent experimentation with the animals.

10. When existing transgenic animals at an institution are purchased or transferred to an investigator outside the institution, who should review and approve the use of these animals?

An institution's IBC does not need to review and approve the use of transgenic animals at another institution. If the receiving institution is subject to the *NIH Guidelines* (i.e. receives NIH support for research with recombinant or synthetic nucleic acid molecules), then the purchase and transfer of animals (other than rodents that can be housed under BL1 containment), along with any experiments subject to the *NIH Guideline*, would require review and approval by the IBC at that institution.

11. What are the *NIH Guidelines* requirements for research with large transgenic animals (sheep, pigs, etc.), or research with recombinant or synthetic nucleic acid-modified microorganisms in such animals?

When conducting research with recombinant or synthetic nucleic acid molecules in large animals, the work is covered under Appendix Q of the *NIH Guidelines*. **Appendix Q** specifies containment and confinement practices when animals are of a size or have growth requirements that preclude the use of laboratory containment of animals. The *NIH Guidelines* include provisions for tracking and inventorying these animals (**Appendix Q-1-B-2** states that a permanent record must be maintained of the experimental use and disposal of each animal).

Animal carcasses must be disposed of as to avoid their use as food for human beings or animals unless food use is specifically authorized by an appropriate federal agency (**Appendix Q-1-B-1**). An acceptable method, for example, would be incineration.

12. Are recombinant or synthetic nucleic acid modifications to the somatic cells of non-transgenic animals subject to the *NIH Guidelines*?

Yes, these experiments are subject to the *NIH Guidelines*.

- **Sections III-D-1-a** through **III-D-1-d** cover experiments using Risk Group 2, 3, 4, or restricted agents in whole animals. See the *NIH Guidelines* for the appropriate containment for such experiments
- **Section III-D-4-a** covers experiments involving viable recombinant or synthetic nucleic acid-modified microorganisms tested on whole animals. Recombinant or synthetic nucleic acid from any source except for greater than two-thirds of eukaryotic viral genome may be transferred to any animal and propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study.
- **Section III-D-4-b** covers recombinant or synthetic nucleic acid, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals that are not covered by **Sections III-D-1** or **III-D-4-a**. The appropriate containment for these experiments is determined by the IBC.
- Experiments not included in **Sections III-A, III-B, III-C, III-D, III-F**, fall into **Section III-E**. Experiments covered by **Section III-E** may be conducted at BL1 containment.

The *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)* are available at

http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

For further information about the requirements of the *NIH Guidelines*, please visit the NIH Office of Biotechnology Activities web page at <http://oba.od.nih.gov> or write to us at oba@od.nih.gov

Appendix R

Experiments that are Exempt from the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)* Frequently Asked Questions

1. Are there experiments that are exempt from the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*?

Section III-F of the *NIH Guidelines* details experiments that are exempt from the requirements of the *NIH Guidelines*. The following molecules are exempt:

- For synthetic nucleic acids, those that: (1) can neither replicate nor generate nucleic acids that can replicate in any living cell (e.g., oligonucleotides or other synthetic nucleic acids that do not contain an origin of replication or contain elements known to interact with either DNA or RNA polymerase), and (2) are not designed to integrate into DNA and (3) do not produce a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight. If a synthetic nucleic acid is deliberately transferred into one or more human research participants and meets the criteria of Section III-C, it is not exempt under this Section. (See Section III-F-1)
- Those that are not in organisms, cells, or viruses and that have not been modified or manipulated (e.g., encapsulated into synthetic or natural vehicles) to render them capable of penetrating cellular membranes. (See Section III-F-2)
- Those that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature. (See Section III-F-3)
- Those that consist entirely of nucleic acids 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. (See Section III-F-4)
- Those that consist entirely of nucleic acids from a 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). (See Section III-F-5)
- Those that 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. (See Section III-F-6)
- Those genomic DNA molecules that have acquired a transposable element, provided the transposable element does not contain any recombinant and/or synthetic DNA. (See Section III-F-7)

- Those that do not present a significant risk to health or the environment as determined by the NIH Director, with advice from the RAC and public comment. (See Section III-F-8) Appendix C of the *NIH Guidelines* details the specific classes of experiments that may be exempt under Section III-F-8.

2. Are the experiments described in Section III-F and Appendix C of the *NIH Guidelines* always exempt, or are there circumstances in which the research might require IBC review and approval under *NIH Guidelines*?

If an experiment falls under Sections III-A, III-B, or III-C and also III-F, then the research is not exempt (see the note Under Section III of the *NIH Guidelines*). The three types of experiments that would not be exempt under this provision are:

- The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine or agriculture [Section III-A];
- Deliberate formation of recombinant or synthetic nucleic acid molecules containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin). [Section III-B]; or
- The deliberate transfer into human research participants of either:
 - Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acid molecules, or
 - Synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acid molecules, that meet any one of the following criteria:
 - Contain more than 100 nucleotides; or
 - Possess biological properties that enable integration into the genome (e.g., *cis* elements involved in integration); or
 - Have the potential to replicate in a cell; or
 - Can be translated or transcribed. [Section III-C].

In addition, there are certain exceptions to the exemptions described in Appendix C of the *NIH Guidelines*. [See Appendix C-1-A, through C-V1-A]. In addition to the three types of experiments listed above, these exceptions include experiments involving:

- DNA from Risk Group 3, 4, or restricted organisms or cells known to be infected with these agents ☐ Whole plants regenerated from plant cells and tissues cultures that do not remain axenic
- cultures ☐ Large scale experiments (more than 10 liters of volume in a single culture vessel)
 - ☐ Deliberate introduction of genes coding for the biosynthesis of molecules that are toxic for vertebrates with an LD50 greater than 100 nanograms/kg but less than or equal to 100 micrograms/kg (see Appendix F)

3. The *NIH Guidelines* exempt certain experiments that do not pose a threat to health or the environment. Can an Institutional Biosafety Committee (IBC) or Principal Investigator (PI) determine if an experiment does not pose such a threat and is therefore exempt?

Section III-F-8 of the *NIH Guidelines* refers to categories of experiments that the NIH Director has determined do not present a significant risk to health or the environment and are therefore exempt. This determination was made with the advice of the RAC, following appropriate notice and opportunity for public comment. **PIs and IBCs cannot make the determination that a class of experiments other than the ones listed below poses no significant risk.**

The following classes of experiments are exempt under Section III-F-8:

- Certain recombinant or synthetic nucleic acid molecules that contain less than one-half of any eukaryotic viral genome when propagated and maintained in cells in tissue culture [Appendix C-I – see question 8 below for more information on the limits of this exemption]
- *Escherichia coli* K-12 host-vector systems [Appendix C-II]
- *Saccharomyces cerevisiae* or *Saccharomyces uvarum* host-vector systems [Appendix C-III]
- *Kluyveromyces lactis* host-vector Systems [Appendix C-IV]
- *Bacillus subtilis* or *Bacillus licheniformis* host-vector systems [Appendix C-V]
- Extrachromosomal elements of gram positive organisms [see specific list of organisms in Appendix C-VI]
- The purchase or transfer of transgenic rodents [Appendix C-VII]
- Generation of certain BL1 Transgenic Rodents via Breeding [Appendix C-VIII]

A full description of the exemptions with exceptions can be found in Appendix C of the *NIH Guidelines*.

4. How do I know if I am working with host-vector system that is exempt from the *NIH Guidelines*?

Only certain experiments that use *E. coli* K-12, *Saccharomyces cerevisiae* or *Saccharomyces uvarum*, *Kluyveromyces lactis*, *Bacillus subtilis* or *Bacillus licheniformis* host-vector systems are exempt from the *NIH Guidelines* (see Appendix C). If you are obtaining an *E. coli* host-vector system from a commercial supplier, genotype information may be available to permit determination of the strain from which the host is derived.

1. Nucleic acid molecules resulting from the replication of recombinant or synthetic nucleic acids are subject to the *NIH Guidelines*. Are any other materials derived from or produced by genetically engineered organisms subject to the requirements of the *NIH Guidelines*?

No. For example, proteins produced by genetically engineered organisms are not subject to the *NIH Guidelines*.

1. Are any types of human gene transfer trials exempt from the requirements of the *NIH Guidelines*?

If the investigational product meets the criteria described in Section III-C of the *NIH Guidelines* and involves the deliberate transfer of recombinant or synthetic nucleic acid molecules, or DNA or RNA derived from recombinant or synthetic nucleic acid molecules, into one or more human research participants, then the research will be subject to the *NIH Guidelines*.

Synthetic nucleic acid molecules that contain less than 100 nucleotides, or do not possess biological properties that enable integration into the genome (e.g., *cis* elements involved in integration); or do not have the potential to replicate in a cell; or do not have the potential to be translated or transcribed are not covered under Section III-C.

In addition, Appendix M-VI-A of the *NIH Guidelines* exempts certain types of vaccine trials from the requirements for submission of the protocol to NIH OBA, RAC review, and subsequent reporting (Appendix M-I). Specifically, this exemption applies to "human studies in which induction or enhancement of an immune response to a vector-encoded microbial immunogen is the major goal, such an immune response has been demonstrated in model systems, and the persistence of the vector-encoded immunogen is not expected."

Trials with these characteristics do not have to be registered with NIH OBA or undergo RAC review, but can be submitted on a voluntary basis, particularly if the investigator believes that a trial presents scientific, safety, or ethical concerns that would benefit from RAC review and public discussion. Investigators that submit trials voluntarily will be expected to comply with all aspects of the protocol review and reporting requirements. OBA encourages investigators and institutional review bodies to contact us (oba@od.nih.gov) for assistance in determining whether this exemption applies to a particular trial.

It is important to note that Appendix M-VI-A does not exempt these vaccine trials from other requirements specified in the *NIH Guidelines*, including biosafety review. Thus, vaccine trials, like other human gene transfer trials subject to the *NIH Guidelines*, must be reviewed and approved by an IBC before research participants can be enrolled. For further information see the FAQ on experiments that are exempt from the *NIH Guidelines* at: http://oba.od.nih.gov/rdna/rdna_faq_list.html

7. There is a note at the beginning of Section III of the *NIH Guidelines* that states "If an experiment falls into Section III-F and into either Sections III-D or III-E as well, the experiment is considered exempt from the *NIH Guidelines*." What is meant by this note?

If an experiment falls into Section III-D or III-E of the *NIH Guidelines* and also falls into section III-F, it is exempt. An example of such an experiment is the following:

Staphylococcus aureus (a Risk Group 2 bacterium) contains a recombinant plasmid. The plasmid is indigenous to *S. aureus*, was created in vitro, and contains only DNA from *S. aureus* (i.e., the DNA inserted into the plasmid was *S. aureus* DNA).

Rationale: The introduction of recombinant DNA into Risk Group 2 agents is usually covered

under Section III-D-1-a. However, because the experiments are only performed in *S. aureus*, this work would fall under III-F-4 (experiments that consists entirely of DNA from a prokaryotic host including its indigenous plasmids when propagated only in that host or a closely related strain of the same species). Thus this experiment falls into both Sections III-D and III-F and is exempt, due to the above note, from the requirements of the *NIH Guidelines* for IBC review and approval.

It should be noted that only experiments covered by both III-D or III-E and III-F are considered to be exempt. If an experiment falls into Section III-A, III-B or III-C and any one of the other sections, then the rules pertaining to Sections III-A, III-B or III-C **must** be followed.

8. Appendix C-1 of the *NIH Guidelines* exempts experiments involving recombinant or synthetic nucleic acid molecules in tissue culture. I have a cell line that was created by the introduction of recombinant DNA. Are all experiments that I conduct with this cell line exempt from the requirements of the *NIH Guidelines*?

No. Although Appendix C-1 does exempt the use of recombinant or synthetic nucleic acid molecules in tissue culture, there are exceptions to this exemption. Existing tissue culture cell lines created by the introduction of recombinant or synthetic nucleic acid molecules are exempt from the *NIH Guidelines* unless, the cell line:

- was modified using DNA from Risk Group 3 or 4 agents, or from restricted agents [Section III-D];
- contains a toxin with an LD50 of less than 100 nanograms per kilogram/kg body weight [Section III-B-1];
- contains viral DNA in a quantity exceeding 50% of any viral genome [Appendix C-I];
- is used in conjunction with defective viruses in the presence of helper virus [Section III-D 3];
- is used in an experiment involving the deliberate transfer of the cell line into humans [Section III-C-1]; or
- is grown in a volume exceeding 10 liters of culture [Section III-D-6].

Appendix S

Animal experiments covered under the *NIH Guidelines for Research Involving Recombinant DNA Molecules*

ACTIVITY	MINIMUM BSL	SECTION
CREATION OF TRANSGENIC ANIMALS		
Creation of transgenic rodents	BL1	III-E-3
Creation of transgenic rodents	BL2 or higher	III-D-4-b
Creation of transgenic animals other than rodents	BL1/BL1-N	III-D-4-a
Creation of transgenic animals other than rodents	BL2/BL2-N or higher	III-D-4-b
Creation of recombinant DNA modified arthropods	BL1	III-D-4-a
Creation of recombinant DNA modified arthropods	BL2 or higher	III-D-4-b
Creation of knock-out rodents	BL1	III-E-3
Creation of knock-out rodents	BL2 or higher	III-D-4-b
BREEDING OF TRANSGENIC ANIMALS**		
Breeding rodents from one strain (propagation/colony maintenance)	BL1	Exempt (III-F-4)
Breeding rodents from one strain (propagation/colony maintenance)	BL2 or higher	III-D-4-b
Breeding rodents from two strains (generating new strain)	BL1	III-E-3
Breeding rodents from two strains (generating new strain)	BL2 or higher	III-D-4-b
Breeding of transgenic animals other than rodents	BL1	III-D-4
Breeding of transgenic animals other than rodents	BL2 or higher	III-D-4
Breeding of recombinant DNA modified arthropods	BL1	Exempt (III-F-4)
Breeding of recombinant DNA modified arthropods	BL2 or higher	III-D-4-b
Breeding of knockouts (propagation)	BL1	Exempt (III-F-4)
Breeding of knockouts (propagation)	BL2 or higher	III-D-4-b
Breeding of knockouts from two strains (generating new strain)	BL1	III-E-3 or Exempt
Breeding of knockouts from two strains (generating new strain)	BL2 or higher	III-D-4-b
<p>**The breeding of two different transgenic rodents or the breeding of a transgenic rodent and a non-transgenic rodent with the intent of creating a new strain of transgenic rodent that can be housed at BL1 containment will be exempt from the NIH Guidelines if</p> <ol style="list-style-type: none"> 1. Both parental rodents can be housed under BL1 containment, and 2. Neither parental transgenic rodent contains the following genetic modifications <ol style="list-style-type: none"> a. Incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses, or b. Incorporation of a transgenic that is under the control of a gammaretroviral long terminal repeat (LTR), and 3. The transgenic rodent that results from this breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses. 		
EXPERIMENTS WITH TRANSGENIC ANIMALS		
Experiment with transgenic rodents	BL1	III-D-4-a* (see note)
Experiment with transgenic rodents	≥ BL2 set by IBC	III-D-4-b
Experiments with transgenic animals other than rodents	BL1	III-D-4-a
Experiments with transgenic animals other than rodents	≥ BL2 set by IBC	III-D-4-b
Experiments with recombinant DNA modified arthropods associated with plants	BL1	III-E-2-b(5)
Experiments with recombinant DNA modified arthropods associated with plants	BL2 or higher	III-E-2
Experiments with recombinant DNA modified arthropods not associated with plants	BL1	III-D-4-a
Experiments with recombinant DNA modified arthropods not	BL2 or higher	III-D-4-b

associated with plants		
*The purchase or transfer of transgenic rodents requiring BL1 container is exempt under Appendix C-6. Subsequent use of these animals is also exempt providing the experimental protocol does not involve the use of recombinant DNA. If the protocol does involve the use of recombinant DNA then the research is covered under III-D-4-a. All experiments involving the use of other transgenic animals at any Biosafety Level and the use of transgenic rodents requiring BL2 or higher containment are subject to the <i>NIH Guidelines</i> . See above for applicable sections.		
EXPERIMENTS WITH R-DNA IN AN ANIMAL (TRANSGENIC OR OTHERWISE)		
Experiments with r-DNA modified microbes in any animal (transgenic or otherwise)	BL1/BL1-N	Not permitted at BL1 *
Experiments with RG2 r-DNA modified microbes in any animal (transgenic or otherwise)	BL2/BL2-N	III-D-1-a
Experiments with RG3 r-DNA modified microbes in any animal (transgenic or otherwise)	BL3/BL3-N	III-D-1-b
Experiments with RG4 r-DNA modified microbes in any animal (transgenic or otherwise)	BL4/BL4-N	III-D-1-c
Experiments with r-DNA modified restricted agent in any animal (transgenic or otherwise)	BL4/BL4-N	III-D-1-d
Experiments with r-DNA modified animal pathogens in any animal (transgenic or otherwise)	BL4/BL4-N	III-D-1-d
Introduction of less than 2/3 of eukaryotic viral genome into a non-human vertebrate or invertebrate	BL1/BL1-N	III-D-4-a
Propagation of animals containing viral vector sequences not leading to transmissible infection	BL1/BL1-N	III-D-4-a
Experiments with r-DNA involving whole animals not covered by Sections III-D-1 or III-D-4-a	Set by IBC	III-D-4-b
*Other than viruses which are only transmitted vertically, the experiments may not be conducted at BL1. A minimum of BL2 or BL2-N is required		
CLONING ANIMALS		
Cloning animals	BL1 or higher	Not covered
PURCHASE OR TRANSFER OF TRANSGENIC ANIMALS		
Purchase or transfer of transgenic rodents	BL1	Exempt (Appendix C-6)
Purchase or transfer of transgenic rodents	BL2 or higher	III-D-4
Purchase or transfer of transgenic animals other than rodents	BL1	III-D-4
Purchase or transfer of transgenic animals other than rodents	BL2 or higher	III-D-4
Purchase or transfer of recombinant DNA modified arthropods	BL1	III-D-4
Purchase or transfer of recombinant DNA modified arthropods	BL2 or higher	III-D-4
PLANT EXPERIMENTS WITH ANIMALS OR ARTHROPODS		
Experiments with microorganisms or insects containing recombinant DNA with the potential for detrimental impact to ecosystems.	BL3-P or BL2-P plus biological containment	III-D-5-a or III-D-5-b
Experiments with exotic infectious agents in the presence of arthropod vectors	BL4-P	III-D-5-c
Experiments with microbial pathogens of insects or small animals associated with plants with the potential for detrimental impact to ecosystem.	BL3-P or BL2-P plus biological containment	III-D-5-e
Small animals associated with recombinant DNA-modified plants.	BL1	III-E-2
Experiments with Rdna-modified arthropods or small animals associated with plants	BL1	III-E-2-b-(5)
OTHER		
Transfer of a drug resistance to microorganisms compromising the use in veterinary medicine	Set by NIH (case by case)	III-A-1-a

The NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) are available at

<http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html>

For further information about the requirements of the *NIH Guidelines*, please visit the NIH office of Biotechnology Activities web page at <http://www4.od.nih.gov/oba/> or write to us at oba@od.nih.gov

Appendix T

FAQS about Incident Reporting

Reporting of Incidents Involving Recombinant DNA to the NIH Office of Biotechnology Activities (OBA)

- **What kinds of incidents involving recombinant DNA must be reported to the NIH OBA?**

The *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* states that "...any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses" must be reported to NIH OBA within 30 days. Certain types of accidents must be reported on a more expedited basis. Spills or accidents in BL2 laboratories resulting in an overt exposure must be immediately reported to NIH OBA. Spills or accidents occurring in high containment (BL3 or BL4) laboratories resulting in an overt or potential exposure must be immediately reported to NIH OBA.

- **How serious must a problem be to warrant reporting to OBA?**

Any spill or accident involving recombinant DNA research of the nature described above or that otherwise leads to personal injury or illness or to a breach of containment must be reported to OBA. These kinds of events might include skin punctures with needles containing recombinant DNA, the escape or improper disposition of a transgenic animal, or spills of high-risk recombinant materials occurring outside of a Biosafety cabinet. Failure to adhere to the containment and Biosafety practices articulated in the *NIH Guidelines* must also be reported to OBA.

Minor spills of low-risk agents not involving a breach of containment that were properly cleaned and decontaminated generally do not need to be reported. OBA should be consulted if the Institutional Biosafety Committee (IBC), investigator, or other institutional staff are uncertain whether the nature or severity of the incident warrants reporting; OBA can assist in making this determination.

- **Who is responsible for reporting incidents involving recombinant DNA to NIH OBA?**

Under the *NIH Guidelines* incident reporting is articulated as a responsibility of the Institution, IBC, Biological Safety Officer, and Principal Investigator. Institutions have the discretion to determine which party should make these reports, and one report for each incident or set of information is generally sufficient.

- **What information should incident reports include?**

Incident reports should include sufficient information to allow for an understanding of the nature and consequences of the incident, as well as its cause. A detailed report should also include the measures that the institution took in response to mitigate the problem and to preclude its reoccurrence.

- **What other information needs to be provided?**

Depending on the severity of the incident, OBA staff may request the IBC meeting minutes documenting approval conditions for the research, minutes of IBC meetings where the incident was reviewed, policies in place at the time the incident occurred, or any revised policies prepared in response to the incident. Training records for the personnel involved in the incident may also be requested.

- **What does OBA do with this information?**

OBA staff review incident reports to assess whether the institutional response was sufficient. Depending on the adequacy of the institutional response, OBA may ask the institution to take additional measures as appropriate to promote safety and compliance with the *NIH Guidelines*.

- **Do adverse events experienced by participants in human gene transfer trials fall under this incident reporting requirement?**

No, adverse events in human gene transfer trials are subject to a separate set of reporting requirements. These are found in Appendices M-1-C-3 and M-1-C-4 of the *NIH Guidelines*. Serious adverse events that are unexpected and possibly associated with the gene transfer product should be reported to OBA within 15 calendar days of sponsor notification, unless they are fatal or life threatening, in which case they should be reported within 7 calendar days. Other serious adverse events should be reported to OBA as part of the Principal Investigator's annual report to OBA.

- **To report an incident involving an exposure, loss of containment, a violation of the *NIH Guidelines* or other compliance issue to OBA contact:**

Kathryn Harris, Ph.D., RBP
Senior Outreach and Education Specialist
6705 Rockledge Drive, Suite 750
Bethesda, MD 20892
Phone: 301-496-9838
Fax: 301-496-9839
Email: harriskath@od.nih.gov

BIOSAFETY LEVEL 2

**When Door is
CLOSED,
Work is in Progress**



STOP! DO NOT ENTER!

**Please Knock and
Wait to be Admitted**

Appendix V

Guidelines for the Research Use of Adjuvants

The use of adjuvants in animal research studies of basic immunological phenomena, and in applied immunology, requires careful consideration. The apparent requirement for non-specific inflammation to elicit robust immunity obliges the investigator to evaluate the cost of potential, local and/or systemic pain and/or distress of the research animal due to the inflammation with the presumed scientific benefit to be gained from the experiment. The validity and applicability of the scientific knowledge gained must be tempered with acknowledgement that the use of potent inflammatory agents, particularly Complete Freund's Adjuvant (CFA), should be considered early during the development of the experimental design. Whenever possible alternatives to CFA should be used.

Adjuvants known to produce less intense inflammatory responses should be strongly considered as alternatives to CFA. These include TiterMax, Ribi Adjuvant System (RAS), Montanides, Syntex Adjuvant Formulation (SAF), aluminum compounds (e.g., alum), capable of eliciting sufficient cellular and humoral antibody responses with fewer side effects than those commonly seen with CFA. Information on alternative adjuvants is available on-line (see references).

Personnel Safety

Handling of adjuvants that contain mycobacterial products can be an occupational hazard to laboratory personnel. Reports of accidental needle punctures in humans have been associated with clinical pain, inflammatory lesions, and abscess formation in tuberculin-positive individuals. Tuberculin-negative individuals have tested positive in subsequent tuberculin tests after accidental CFA exposure. At a minimum, goggles with or without a face shield are worn or mixing occurs under in a biosafety cabinet to avoid accidental splashing of CFA in the eyes. Nitrile gloves and appropriate laboratory attire including a lab coat are also worn when working with CFA. It is the investigator's responsibility to ensure that all personnel understand the hazards of working with CFA, that CFA lab SOP's are developed and implemented for safe use in mixing and injecting and that inexperienced personnel have direct investigator oversight until personnel have mastered proper and safe mixing and injecting technique.

Appendix W

Transgenic Plant Containment for Laboratories, Growth Chambers and Greenhouses

For more
information on
working with
transgenic plants:

A Practical Guide to Containment

http://www.kb.utd.edu/greenhouses/green_manual.htm

NIH Guidelines for Plant Research

http://www1.od.nih.gov/ohrt/bioethics/ethics_02/appendix_p.htm

USDA/APHIS

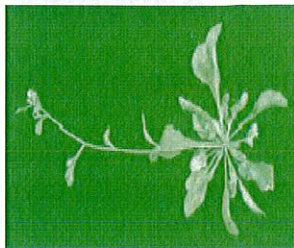
<http://www.aphis.usda.gov/electronic/biology/>

Arabidopsis Information

<http://www.arabidopsis.com/>

Transgenic Plant Containment
for Laboratories, Growth
Chambers, and Greenhouses

University of Kentucky
Department of Biological Safety



UK

UNIVERSITY OF KENTUCKY

Department of
Biological Safety

Barbara Flanagan
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505 Oldham Court
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Phone: 359-257-4049
Fax: 359-257-3636

Elements of Containment



The goal of biosafety with regard to transgenic plants is to prevent dissemination of the modified species outside the growing area. Containment is crucial in transgenic plant research in the labs, greenhouses, and growth chambers. Field trials with transgenic plants require compliance with USDA regulations.

Please consult the USDA guidelines for containment requirements. This pamphlet provides information for transgenic plant and seed containment. Individual research projects will require different containment procedures based upon the experiments performed.

The goals of transgenic plant containment are:

- Prevent interbreeding with native species
- Transgenic plant waste must be decontaminated or inactivated prior to disposal
- Contain species that could detrimentally impact local and agriculturally important species
- Control insect vectors
- Contain seeds and pollen.

All researchers working with transgenic plants must register with the IBC, determine the appropriate biosafety level for their work, and have standard operating procedures in place for:

- Storage, transport, and handling of transgenic seeds and plant materials
- Labeling and segregation of transgenic and non-transgenic plant materials
- Preventing release of transgenic seed to the environment
- Preventing dissemination of genetic material to the environment

Storage, transport, and handling of transgenic seeds and plant materials

Transgenic seed should be stored in a locked cabinet located preferably in or near the greenhouse or growth chamber. When stored or handled outside of a confined space, such as on a lab bench or potting bench, seed should be in a spill-proof container. White paper can be utilized on lab benches in conjunction with a tray to allow for easy identification and containment of stray seeds.



White paper and trays can assist in containing transgenic seed on the lab bench.

Labeling and segregation of transgenic and non-transgenic plant materials

All transgenic seeds and plants should be clearly identified and labeled to distinguish them from other stored seeds, plants, or materials. If transgenic and nontransgenic plants must be grown in the same location, such as an open lab or mixed use greenhouse, all work must be completed at the biosafety level approved for the transgenic plant work.



Transgenic plants should be clearly labeled to distinguish them from non-transgenic plants.

Preventing release of transgenic seed

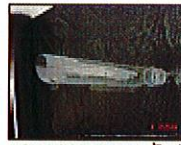
Seed is easily tracked out of facilities on shoes and this inadvertent dissemination can be easily prevented through the use of shoe covers and/or sticky mats. Seed is also easily carried out of facilities on clothing and this can be prevented with the use of disposable lab gowns that are dedicated for use in the plant growth chamber or greenhouse. Good housekeeping practices can help prevent

release of transgenic seed by keeping loose seed off the floor. Daily use of a Sulfur can be an easy way to remove loose seed from floors.



Shoe covers, sticky mats, isolation gowns, and sulfur help prevent release of transgenic seed.

Preventing dissemination of genetic material
Growing plants need to be contained to prevent the dissemination of genetic material. This can be achieved by covering or removing flower and seed heads to prevent seed dispersal, harvesting plant material prior to sexual maturity, or utilizing male sterile lines. Various commercial containment systems are available or inexpensive systems can be constructed with disposable plastic sheeting. These systems contain seeds, soil, plant parts resulting in less housekeeping and less contamination between shelves. These systems also provide better humidity control resulting in less watering of plants.



There are many containment systems available for transgenic plant research.

Department of
Biological Safety

Marla F. Kase
Biological Safety Officer
305 Oldham Court
Lexington, KY 40502
Phone: 859-257-1049
Fax: 859-257-3008
E-mail: mfk2@uky.edu
Web: http://ky.edu/bio/bio

Appendix X

Biohazard Waste Disposal SOP



The Brody School of Medicine Office of Prospective Health

East Carolina University
188 Warren Life Sciences Building • Greenville, NC 27834
252-744-2070 office • 252-744-2417 fax

Occupational Medicine
Employee Health

Radiation Safety DATE: March 6th, 2019

Infection Control

Biological Safety TO: ECU Faculty, Staff and Students

FROM: Edward Johnson/John Baumgartner-ECU Biological Safety
Office of Prospective Health

SUBJECT: Biohazardous Waste Disposal Standard Operating Procedures

Please review the following items regarding disposal and storage of biohazardous waste in this Facility (note: nothing else shall be stored in this area, i.e., chemicals, glassware, etc.):

The OSHA Blood Borne Pathogen Standard, 29 CFR 1910.1030 states that sharps must be discarded immediately or as soon as feasible in appropriate sharps containers that are closeable, puncture resistant, leak proof on sides/bottoms, and are properly labeled/color coded with the universal biohazard symbol. Please **DO NOT** under any circumstances dispose of needles or sharps unless they are properly secured in an appropriate sharps container. This will prevent needle/sharps sticks to our Technicians that pick up and handle the waste.

Please place all non-sharp biohazardous waste in red or orange biohazard bags with the universal biohazard symbol imprint. NOTE: orange biowaste bags are specified only for waste that is to be autoclaved. Known infectious materials MUST always be autoclaved and all animal (example mice) and animal parts MUST be stored in designated freezers or refrigeration units. Our Environmental Health and Safety Hazardous Material Technicians, which pick up the biowaste, occasionally find orange bags that have not been autoclaved. Individuals must make arrangements to have this waste properly autoclaved prior to disposal. After the autoclave process, orange bags may be placed into red bags. The Medical Stockroom has several types of small biohazard bag in stock if needed by departments. **Please refer to the Institutional Biological Safety Manual (pp. 49-52) for autoclave information.**

Please do not place chemicals or chemical waste in this area. Chemicals should be stored and labeled appropriately in the laboratory. Any chemical waste should be stored in the labs satellite accumulation area. Also, please do not store any glassware in this area.

By adhering to the proper disposal procedures, you are ensuring the continued health and safety of those that collect the biohazardous waste generated by this facility. Thank you for your cooperation and support.

If you have any questions or have an urgent matter, please call 744-2070.



Appendix Y

Biosafety Cabinet Decon SOP for Changes/Electrical Service

EAST CAROLINA UNIVERSITY

Office of Prospective Health

Purpose: To ensure all personnel completing maintenance on Biological Safety Cabinets are protected against harmful infectious agents and radiological hazards.

Responsibilities and Procedures

Laboratory Personnel

1. Contact the Office of Prospective Health/Biological Safety for guidance. 744-3437 / 744-2237
2. Decontaminate equipment located in the Biological Safety Cabinet with an EPA approved disinfectant and remove it.
3. Decontaminate work surfaces within the Biological Safety Cabinet with an EPA approved disinfectant.
4. Leave blower on for several minutes with no activity so that any airborne contaminants will be purged from the work area.
5. After the Biological Safety Cabinet is decontaminated, no work shall be performed in the Cabinet until the lab is notified by Facility Services the maintenance/repair is complete.
6. Remove personal protective equipment and wash hands.

The Office of Prospective Health

1. Ensure the Biological Safety Cabinet is properly disinfected with an EPA approved disinfectant and/or surveyed for radiological contamination.
2. Oversee maintenance operations to ensure proper operation of the Biological Safety Cabinet is not altered or compromised.
3. Advise Facility Services of hazards associated with the laboratory and the Biological Safety Cabinet.
4. Notify Facility Services that the Biological Safety Cabinet has been decontaminated and they may begin work.

Facility Services

1. Wear appropriate personal protective equipment as advised by Prospective Health.
2. Conduct Maintenance or repair equipment as needed.
3. Remove personal protective equipment and wash hands.
4. Notify lab personnel maintenance/repair has been completed.

This equipment has been properly decontaminated and is safe for routine maintenance work to be completed.

Disinfectant Used: _____ Approved By/Date: _____
Prospective Health Biological Safety

Laboratory Personnel/Date: _____ Approved By/Date: _____
Radiation Safety

Work Order # _____ Completion Date: _____

Facility Contact: _____ Completion Date: _____

APPENDIX Z

SAFE WORK PRACTICES INFORMATION PAGE

Working with Tat and other Potentially Hazardous Proteins

Revised 11/11/09

I. PURPOSE: The potential immunosuppressive, cytotoxic, and carcinogenic properties which have been associated with Tat protein and related viral proteins and peptides may present a potential health threat to laboratory staff, animal handlers and other personnel who may accidentally come into contact with these agents. This page is intended to provide the research community with sufficient information regarding toxicology of Tat and other potentially hazardous proteins to assist in the development of research protocols that include measures for effectively reducing risk of occupational exposure.

II. BACKGROUND

A. Tat Protein: Trans-activating transduction (Tat) proteins are currently receiving much interest due to associated qualities which make them promising for use in a number of therapeutic applications. Tat is a regulatory protein of HIV-1 produced very early after infection and essential for HIV-1 gene expression, replication, and infectivity (Gavioli et al., 2004). Tat proteins are small (14 kDa) 86 – 1001 amino acid nuclear proteins transcribed from complex spliced mRNAs which function as transacting transcriptional activators and are involved in array of viral functions (Moreau et al. 2004). Tat protein, functions intracellularly as a *trans*-activating factor of the human immunodeficiency virus type 1 (HIV-1), and also acts as an extracellular molecule modulating gene expression, cell survival, growth, transformation, and angiogenesis (Ruznati et al., 2000). Recent research has focused on the potential for the use of Tat protein derived “Tat toxoids” as possible candidates for the development of an HIV-1 vaccine (Gallo, 1999). The ability of the Tat protein to readily permeate cell membranes and accept tags has also led to research of the feasibility of use of the Tat protein as a delivery vehicle for a wide range of therapeutic agents. (Moreau E, et. al, 2004) The key role of Tat protein in HIV-1-induced immune pathogenesis has been firmly established through a number of in vitro and in vivo studies (Gallo RC 1999, Moreau E 2004). Recent studies have indicated that exposure to Tat protein, even in the absence of HIV infection, can lead to serious health consequences (Kim et al., 2003).

B Other Potentially Hazardous Regulatory and Accessory Proteins: In addition to Tat Protein research involving other proteins related to HIV-1 and other retroviruses has also been widely undertaken. Deletion of the Nef protein (Negative Regulatory Factor) which serves as a viral infection promoter has shown mixed results in several HIV-1 vaccine studies (Verity et al. 2007). Immunosuppressive proteins Vif (Viral Infectivity Factor) and Vpr (Viral Infectivity factor), regulatory proteins such as Vpu (Progeny Virion Production) and Env (Envelope Glycoprotein), and other accessory proteins are now none to also be key components to HIV pathogenesis (Seelamgari et. al, 2004, Bour 2003, Ndolo et. al 2003). Cytotoxic and/carcinogenic properties have been identified in several of these regulatory and accessory proteins.

III. POLICY:

A. Tat Protein: The Institutional Biosafety Committee (IBC) has classified Tat protein as a reportable biological hazard which must be handled under Biosafety Level 2 (BSL-2) precautions. All use of Tat protein must be registered with the IBC via completion of a Memorandum of Understanding and Agreement (MUA). *in vivo* use of Tat protein must be reported via completion of Appendix C of Institutional Animal Care and Use Committee IACUC protocol.

B Other Potentially Hazardous Proteins and Peptides: Though not reportable at this time, Principal Investigators should conduct thorough hazard assessments, develop standard operating procedures, and train staff fully prior to undertaking research involving other potentially hazardous proteins (Nef, Vpu, Vpr, Vif, Vpx, Env, etc.). The IBC strongly recommends that all HIV and other retroviral proteins/peptides be handled under BSL-2 conditions.

IV. OCCUPATIONAL EXPOSURE HAZARDS: Extensive scientific literature is available regarding potential health threats associated with Tat protein exposure. Information regarding potential health threat posed by other regulatory and accessory proteins HIV and other retroviruses is somewhat limited. The section below focuses on the toxicologic effects of Tat protein with limited reference to other regulatory/accessory proteins:

A. Cytotoxic Effects:

1. Tat protein: Tat protein is secreted actively by infected cells through a leaderless secretion pathway and is free in the plasma, where it can interact and be taken up by infected and noninfected cells. Numerous deleterious effects have been described in scientific literature:

a. Tat protein-related depression of the immune system is a result of several complex interactions (Ibelgauft, 2007). Tat protein induces production of the highly immunosuppressive cytokine IL10 by peripheral blood monocytes (Badou et al, 2000). Inhibition and/or destruction of natural killer cells, T cell lymphocytes, monocytes, and other immune cells have been widely reported. (Travis, 1999, Kim, 2003, Gallo 1999).

b. In addition to inducing destruction of T cells/other immune cells, Tat protein plays a major role in damaging and/or causing apoptosis of other bystander cells and in the killing of cells which are actively infected with HIV. (Campbell et al., 2004, Gallo et al., 1999).

c. Neuropathological effects including breakdown of the cerebellum and cortex, brain edema, astrocytosis, neuronal dendrite degeneration, neuronal apoptosis have been observed in Tat protein studies. The related symptomology may include

tremor, ataxia, reduced cognitive and motor ability, seizures, and premature death. (Kim, 2003).

c. Exposure to Tat protein may lead to activation of quiescent proviruses resulting in latent infection (Gallo et al., 1999).

d. Other cytotoxic effects: The full effects rendered by extracellular Tat protein all still not completely understood. Concern has been raised that concentrations used in research applications may be several times more concentrated than would be experienced in natural infection (Travis, 1999).

2. Nef Protein:

a. In vivo studies (mice) involving exposure to solely the Nef protein indicated that the animals developed an AIDs-like disease exhibiting depletion of CD4 T-cells, alteration of T-cell activation and differentiation, and lymphoid organ disorders. Other studies have indicated that Nef exposure may lead to apoptosis of uninfected T-cells, disruption of cell signaling pathways, increase viral loads and disease progression, dendritic cell maturation, and interruption of B cells function (Ndolo et al., 2004, Mangino et al., 2007).

b. Research has also indicated that exposure to Nef protein may lead to a compromise of neuronal health due to cell apoptosis and other potential deleterious effects (Trill-Pazos et al., 1998).

3. Vpu Protein: *in vitro* experimentation has indicated that Vpu induces apoptosis of bystander cells through disruption of antiapoptotic genes (Akari et al., 2001).

B. Carcinogenic/Mutagenic Effects:

1. Indications of carcinogenic properties in association with Tat Protein have been widely demonstrated:

a. Studies have indicated that Tat expression in the liver predisposes to both initiation (promotion) of hepatocarcinogenesis and malignant progression of liver tumors (Altavilla et al., 2000, 2004).

b. In vitro studies have also implicated Tat protein as a major factor in the development of Kaposi Sarcoma in human Acquired Immune Deficiency Virus (AIDs) (Ensoli et al., 1990, 1994).

c. Angiogenic properties and enhancement of the effect of endogenous and exogenous carcinogens in human immunodeficiency virus-1-infected patients have been described (Analini, 1995).

d. *in vitro* and *in vivo* studies have indicated that Tat protein exposure may result in reduction of oncosuppressive properties and the induction of genes needed to proceed through the cell cycle including p107, cyclin A, and cyclin B (Lazzi et al., 2002).

2. Vdu Protein: Research has indicated a possible association between occurrence of AIDS-related B-cell non-Hodgkin's Lymphoma and exposure to Vpu (Henderson et al., 2004).

3. Nef Protein: The Nef protein is suspected of disabling the tumor suppressing protein p53 through active binding (Greenway et al., 2002). This finding may indicate potential oncogenic properties.

IV. SAFE WORK PRACTICES: The list of potential Tat protein-related health hazards identified above make it imperative that PIs conduct thorough risk assessments and prepare protocols which include standard operating procedures (SOPs) identifying appropriate administrative controls, personal protective equipment (PPE), work methods, engineering controls, and waste disposal procedures for eliminating or sufficiently reducing exposure threat to all staff involved in the affected research.

A. Administrative Controls

1. All use of Tat Protein must be registered with the IBC via submission of an MUA.

2. IACUC protocols involving *in vivo* use of Tat protein must include a completed "Research Involving Biohazards" form in Appendix C.

3. Principal investigators will develop and implement standard operating procedures (SOPs) by which laboratory staff and animal care workers will prepare/administer Tat protein and conduct animal husbandry with minimal potential for exposure. These SOPs will be incorporated into MUAs and IACUC protocols.

4. All tasks having potential for occupational exposure to Tat and other potentially hazardous proteins/peptides (via mixing of doses, dose preparation, administering of injections, etc.) will only be conducted by competent staff whom have received appropriate training (OSHA: "Worker Right to Know") regarding the related health and safety risks, SOPs, and procedures to be followed in event of an exposure incident.

5. *All staff* working in university and hospital laboratories is required to complete applicable modules of the VCU Laboratory Safety Training Modules. Principal Investigators are required to maintain documentation that staff has completed applicable training modules in their central files.

6. Special consideration should be given to protection of staff who may be HIV positive as exposure to Tat protein and other HIV-1 regulatory/accessory proteins may increase risk of developing AIDs.

B. Personal Protective Equipment: Staff involved with any tasks where potential for urethane exposure exists must don the following PPE:

1. Examination gloves.
2. Safety glasses or safety goggles (ANSI Z-87 approved).
3. Lab coat.
4. Appropriate laboratory attire.

5. If aerosol exposure threat exists suitable respiratory protection must be provided. Prior to instituting respiratory protection to personnel, the laboratory must participate in the university Respiratory Protection Program.

C. Work Methods: Biosafety Level 2 precautions must be employed whenever performing manipulations involving Tat protein. Biosafety Level 2 precautions are strongly advised whenever performing manipulations involving other HIV-1/retroviral proteins and peptides.

1. Whenever feasible, procedures with the potential for producing aerosols should be conducted with a certified biological safety cabinet or certified fume hood.

2. Needles used for Tat protein/other protein injection must be disposed of in approved sharps containers immediately following use.

3. Needles used for Tat protein/other protein injection should never be bent, sheared, or recapped. If recapping is absolutely necessary, a "Needle Recapping Waiver" must be submitted for IBC review/approval prior to proceeding.

4. Areas where Tat protein is prepared and/or administered should be cleaned/decontaminated immediately following completion of each task utilizing a detergent product containing protease enzyme (e. g. Terg-A-Zyme®) or a 10% bleach/water solution (prepare fresh stock as needed).

5. Avoid all contact with Tat and other proteins, immediately wash areas which come in contact with the agents with warm water and soap. Report any exposure incidents to Employee Health as soon as possible.

6. Wash hands thoroughly with soap and warm water immediately upon removing examination gloves.

D. Engineering controls

1. In cases where the recommended level of PPE does not provide sufficient protection (e.g.: splash potential, aerosolization potential) tasks must be conducted within a biological safety cabinet chemical fume hood utilizing sash for added protection.
2. It is recommended that syringes used for Tat and other potentially hazardous proteins injection be safety engineered (self-sheathing syringes, luer-lock syringes, etc.).

E. Waste Disposal

1. Surplus Tat protein stocks must be disposed of as a regulated medical waste (RMW) per the instructions provided on the University Bloodborne Pathogens - Infectious Waste Management website. Disposal via autoclaving and/or red bag procedures is deemed acceptable.
2. Waste materials known/suspected to be contaminated with Tat protein must be disposed of as a RMW per the instructions provided on the University Bloodborne Pathogens - Infectious Waste Management website. Disposal via autoclaving and/or red bag procedures is deemed acceptable.
3. Animal carcasses: University policy is to dispose of all animal carcasses used in research as RMW through the Department of Animal Resources.
4. All contaminated sharps waste materials must be placed in proper sharps container and disposed of as RMW.

F. Spills: Large spills of Tat protein and other potentially hazardous proteins/peptides should be reported to OEHS and should not be handled without OEHS assistance. Smaller spills (such as those occurring with a BSC or chemical fume hood) may be managed by the laboratory following the following procedures:

1. Don proper protective clothing: Examination gloves (2x layers), safety goggles (ANSI Z87 approved), laboratory coat, and proper laboratory attire.
2. Mist spill area with a detergent product containing protease enzyme (e. g. Terg-A-Zyme®), last stand for at least 20 minutes, then carefully wipe surface.
3. Mist surface with water and wipe, complete surface cleaning with final misting/wiping utilizing 70% ethyl alcohol solution.
4. Spills involving potential sharps hazards should be addressed utilizing the additional the enhanced safety procedures provided in Section V. of the VCU Bloodborne Pathogens - Infectious Waste Management webpage.

