



Biological Safety Manual

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INTRODUCTION

This manual is applicable to all laboratory, research, service and support activities that may involve exposure to biohazardous agents or materials and that may come under the oversight of the Institutional Biosafety Committee ([IBC](#)). This manual does not address issues of chemical or radiation safety. Those issues are covered in separate manuals available on the EVMS Environmental Health and Safety ([EH&S](#)) website: www.evms.edu/ehs.

If you are working with potentially biohazardous materials, it is *your* responsibility to

- Know the hazards that may be associated with your experiments
- Follow the best guidelines for safe practice in your work
- Satisfy the requirements for reporting and registration review that the Federal Government has established.

If you are working with bacteria, fungi, parasites or live viruses, it is your responsibility to understand the appropriate risk groups by consulting the National Institute of Health ([NIH](#)) and Centers for Disease Control and Prevention ([CDC](#)) guidelines. The Biological Safety Officer (*BSO*) and IBC can assist you in obtaining this information.

Regulatory Requirements and Guidelines

Guidelines developed by the NIH and CDC form the basis for the biological safety practices in this manual. These guidelines **must** be followed to ensure the continuation of grant support from Federal agencies.

National Institutes of Health (NIH)

The NIH, through the Office of Biotechnology Activities ([OBA](#)), issued [Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules \(NIH Guidelines\)](#) to:

- Mandate the establishment of an IBC for the review and oversight of biological research
- Outline roles and responsibility within an institution for biological safety
- Establish the practices, procedures and conditions under which recombinant DNA (rDNA) and synthetic nucleic acid work **must** be conducted.

Centers for Disease Control and Prevention (CDC)

The companion to the NIH Guidelines is the CDC's [Biosafety in Microbiological and Biomedical Laboratories \(BMBL\)](#). This guide addresses the appropriate measures and facilities for work with **all** microbial agents.

Occupational Safety and Health Administration (OSHA)

The requirements of OSHA's [Occupational Exposure to Bloodborne Pathogen](#) standard apply to anyone who works with blood or other potentially infectious (human) materials. Special training, medical surveillance, procedures and equipment that should be implemented are described in the EVMS [Bloodborne Pathogen Exposure Control Plan](#).

Virginia Department of Environmental Quality (DEQ)

The DEQ regulates and monitors the handling and disposal of biohazardous materials under its [Regulated Medical Waste Management Regulations](#) found in Virginia's Environmental Code Section 9 VAC 20-120. The procedures for biohazardous waste handling are outlined in the "Biohazardous Waste" section of this manual and comply with the requirements of these regulations.

Department of Transportation (DOT)

The requirements for packaging and shipment of biological materials are provided in the regulations issued in the Department of Transportation (DOT) final rule "Hazardous Materials: Infectious substances; Harmonization with the United Nations Recommendations" ([71 FR 32244; June 2, 2006](#)) and parts of the Hazardous Materials regulations in [49 CFR, Parts 171-180](#). Information on shipping procedures that comply with these regulations is found in the "Packaging and Shipping Biological Materials" section of this manual.

Training at EVMS also satisfies requirements for the International Air Transport Association ([IATA](#)) for shipping to other countries.

Biological Safety Program at EVMS

The EVMS biological safety program developed from the institution's commitment to address and comply with the *NIH Guidelines* regarding safe research with rDNA and associated infectious materials. The oversight of biosafety at EVMS is charged to the IBC. The fundamental components of the program are:

- The Institutional Biosafety Committee
- The Environmental Health & Safety Department
- The Biological Safety Officer
- The Principal Investigator
- Occupational Health Department
- The EVMS Veterinarian / Department of Comparative Medicine
- Other associated committees (IRB, IACUC, etc.)

The roles of each are described below.

Institutional Biosafety Committee (IBC)

IBC members include faculty and administrators, the Biological Safety Officer, Occupational Health representative, representative from Comparative Medicine and representatives from the community. The IBC:

- Ensures that research involving human and animal pathogens, tissues and toxins are reviewed and found to comply with all federal, state and local requirements
- Ensures that all rDNA and synthetic nucleic acid registrations and research are in compliance with NIH guidelines
- Establishes policies and procedures ensuring biological materials are handled and disposed of safely and in the proper manner.

The IBC conducts business through BioRAFT, a modular software platform for research management and compliance oversight. IBC members, Principal Investigators (*PI*), and laboratory personnel can

access BioRAFT at <https://evms.bioraft.com/>. Lab submissions, agent forms, biosafety-related training and each laboratory's status are contained in BioRAFT.

Environmental Health and Safety (EH&S)

The EH&S Department is responsible for

- Addressing biosafety issues related to research and experiments
- Assisting personnel with compliance to regulatory requirements and best management practices pertaining to biological and laboratory safety
- Training PIs, staff and other personnel on safety issues and regulations.

Biological Safety Officer (BSO)

The BSO is responsible for

- Developing protocols and procedures to address issues of biological safety
- Providing training in safe use and procedures for those working with potentially biohazardous materials and research activities
- Advises PIs on proper waste disposal methods based on Federal, State and local regulations
- Advises personnel on correct shipping requirements for biological substances
- Advises the IBC and other committees on issues related to biosafety
- Inspection of laboratories to ensure compliance with accepted biosafety practices and with the IBC approved registrations
- Assists PIs with laboratory registrations and forms required by the IBC.

Principal Investigator (PI)

The PI is responsible for

- Completing and submitting an IBC Registration for laboratories conducting work with biological materials and agents
- Accepting responsibility for the health and safety of their laboratory personnel
- Completing registrations in a timely and proper manner
- Ensuring proper training and instruction in safe practices and procedures for laboratory personnel involved with handling potentially biohazardous materials
- Ensuring compliance by laboratory personnel with all relevant regulations, guidelines and policies
- Reporting accidents, spills, or contamination to the EH&S and the IBC
- Ensuring all laboratory personnel are aware of the lab-specific hazards involved in their work as well as ensuring personnel are conducting work as specified in an IBC approved registration.

Occupational Health Department

The Occupational Health Department:

- Provides medical review and medical surveillance, as appropriate, for infectious agent workers, those exposed to laboratory animals and those in the Bloodborne Pathogens (*BBP*) program
- Advises investigators, animal care personnel, clinical staff and institutional committees on potential exposures and risks of personal injury associated with laboratory or clinical procedures

- Advises PIs and staff on practices and procedures for reducing or eliminating exposure and injury

Veterinarian

The Veterinarian provides training to all animal users in safe animal-handling procedures. Additionally, the Veterinarian advises the IBC and IACUC on issues of animal safety and procedures when necessary.

IBC Administrator (IBCA)

The IBCA is a non-voting, support staff from the Office of Research. The IBCA sends all correspondence from the IBC to the Investigators. The IBCA is charged with the responsibility of IBC records (meeting minutes, registration and PI files, etc.) and is also responsible for meeting agendas, committee minutes, delegating reviewers for registrations, distributing supplemental information to IBC members and initial reviews of adverse event reports.

All form submissions, attachments and other materials requested by the IBC are to be sent to the IBCA. The PI is to send all training documentation to the IBCA at the time of their laboratory submission. If a PI has a change in laboratory personnel, the PI is to send a letter to the IBCA requesting the change. The IBCA can then administratively approve changes in personnel on the BioRAFT registration

Associated Committees

The Institutional Review Board ([IRB](#)) reviews and oversees research involving human subjects. The Institutional Animal Care and Use Committee ([IACUC](#)) reviews and oversees research involving laboratory animals. Both of these committees consult and coordinate with the IBC on any proposals under their purview that involves the use of potentially biohazardous materials or activities.

RESEARCH REGISTRATION AND APPROVAL

This document provides a reference for PIs to consult when submitting a registration. For a complete account of IBC operations and procedures, consult the [IBC Standard Operating Procedures](#).

IBC Reviewable Registrations

Each PI is responsible for the preparation of an IBC Registration for their laboratory. The IBC is charged with review of all registrations utilizing

- Recombinant DNA and synthetic nucleic acid, including all methodologies that involve the isolation, amplification, hybridization and other uses of DNA or RNA from any organism
- Culture of human or animal pathogen(s)
- Laboratory-induced infection of a human or animal with any human or animal pathogen(s)
- All human tissues and cells
- All other tissues and cells
- Studies referred to the IBC from the Institutional Animal Care and Use Committee ([IACUC](#)) or the Institutional Review Board ([IRB](#)).

Submission Process

Laboratory submissions are entered and maintained in the BioRAFT system. A hard copy of the submission should be in each lab and be readily accessible to all personnel. The following procedures should be followed for submitting registrations for review to the EVMS Institutional Biosafety Committee.

Initial Registration Review

Schedule

Registrations are certified in BioRAFT by 5:00pm on the Monday **two weeks** prior to the next scheduled IBC meeting. The registration is then prescreened by the IBCA and the BSO. Any requested modifications to the registration must be completed and the registration re-certified by 12:00pm the following Friday.

Example: Submission due in July 2008. BioRAFT submission date is 5:00pm Monday June 30, 2008. The requested modifications are to be completed and the submission re-certified by 12:00pm Friday July 3, 2008. The IBC meeting is held Monday July 14, 2008

Registration

All laboratory registrations are to be completed on the BioRAFT website, <https://evms.bioraft.com>.

Once in the BioRAFT system, the PI must complete several components in order to certify the registration.

1. Enter background laboratory information including the laboratory focus, project titles and personnel.

2. Complete the project information section detailing funding sources, materials and methods used, specific aims of the research, and lab techniques employed. All information should be addressed with respect to biological safety and risk assessment of the laboratory.
3. Complete each survey as required by the BioRAFT system.
4. Complete Viral Vector and/or Human Pathogen Registration Forms (as necessary).

The IBC has a help page dedicated to using the BioRAFT system on its MyPortal page at https://myportal.evms.edu/research/research_compliance/committees/institutional_biosafety_committee/.

All registrations must be certified in the system *two weeks* before the scheduled IBC meeting. Unacceptable submissions will be returned to the Principal Investigator for correction and resubmission.

Exemptions

Some registrations do not fall under the *NIH Guidelines Section III A-E* or under the EVMS “Reviewable Registrations” criteria. These registrations can be granted Exempt Status. It is the responsibility of the IBC, not the PI, to determine if the research qualifies for exempt status. **Designation of Exempted status is determined on an individual basis and is at the discretion of the IBC.**

The application deadline to have a registration evaluated for exempt status is the same as that for a convened committee review. The PI must still submit a laboratory registration through BioRAFT. The Exempt Subcommittee, comprised of an IBC Chair and two additional IBC members, will then determine whether a registration qualifies for exempt status.

If the Exempt subcommittee determines the research is exempt, the Investigator will be informed of the IBC’s decision via written letter. The Investigator will still have the responsibility of notifying the IBCA (1) if there are any changes to the research that might deem it non-exempt, and (2) an annual communication to verify the exempt status of the research. Exempt registrations will be tracked by the IBC until completion, but formal annual reviews are not required from the PI.

If the Exempt subcommittee determines the research is not exempt, the registration will be included in the upcoming fully convened IBC meeting.

Amendment Procedure

An immediate notification to the IBCA is required when the Biosafety Level of a registration is changed.

The Principal Investigator is responsible for submitting all proposed methodology changes to the registration, by modifying and recertifying the registration. The IBCA will send the recertified registration to the IBC Chairs and the Chairs will determine if the changes go before the committee. If presented with the amendment request, the committee will review and vote on the proposal. The PI will then be informed of the IBC’s decision.

The IBCA may administratively approve changes in personnel on a registration in between annual reviews. The PI must also ensure proper training for any additional personnel; this training includes Biosafety Training, Autoclave Training, Shipping Training (if applicable) and Bloodborne Pathogen Training.

The Committee *shall not* accept a letter changing or transferring a registration to a new PI. The transfer of a registration to a new PI will require the submittal of letters from both the original and new PI for review and approval. The new PI must certify a new registration if they are new faculty, or amend their existing registration to include the new research. The original PI must submit a letter indicating the study is terminated and the disposition of materials (see [Completed/Terminated Registrations](#)).

Minor modifications may be processed with the IBC Chair's approval.

Requested Revisions

The following are changes requested by the IBC for approval of an IBC registration. The Committee will decide on which method of resubmittal is needed on a case-by-case basis. Until the final approval is given for a registration, the registration will not be considered approved by the IBC or designated as "Approved" in the BioRAFT system.

Minor Revisions

Minor revisions can be made by submitting the clarified and re-certified registration in BioRAFT. These revisions will then be reviewed by the BSO. If acceptable, the BSO will send the registration to the Chair or a designated IBC member for final approval. Final approval is sent to the investigator in the form of an email through BioRAFT stating their status has been marked as approved.

Resubmission

Revision(s) requiring the full Committee's re-evaluation must be certified in BioRAFT and the Committee will review the registration at the following IBC Meeting. The PI is notified in writing of the Committee's decision and requirement(s) for resubmission, if any.

Annual Review

An IBC registration must be re-evaluated and approved on an annual basis. The PI will receive an email from the IBCA, at approximately 60 days before the expiration of a registration's approval. This email will include the date of the next IBC meeting as well as a submission date.

The month prior to the expiration date of the existing approval period, the PI is to send a letter to the IBCA declaring any changes to the registration. Any changes in the registration must be in emphasized font, with the review year preceding the stated changes (see examples below).

- If the registration has no changes, the PI is to include this as a statement at the bottom of the project description, for each project, by the deadline. The IBC Chair will then review the registration and impart a decision.

- Example: 2013 ANNUAL REVIEW – THERE ARE NO CHANGES TO BIOLOGICAL AGENTS, OR TECHNIQUES BEING USED FOR THIS PROJECT.
- If the registration has changes, the PI is to list these changes in a statement at the bottom of the project description for each project by the deadline. The IBC Chair will then review the proposed registration and determine whether the changes warrant the registration going to full committee for review.
 - Example: 2013 ANNUAL REVIEW – E.COLI STRAIN XYZ WAS ADDED AND WE NOW DO SELDI ANALYSIS FOR THIS PROJECT.

Non-Compliance

Occasionally, a deadline for an initial registration, requested revision or renewal lapses without a submission. The following administrative steps will be taken in such cases:

- **Expiration date** - a reminder is sent from the IBC to the investigator.
- **One (1) month post-expiration date** - a reminder is sent from the IBC to the investigator and the investigator's department chair-person.
- **Two (2) months after the expiration date** - a letter will be sent to the investigator's department chair-person stating registration termination within 30 days.
- **After three (3) months** - the Office of Research will administratively terminate the registration. At such time, re-activation of the registration will require submission of a new registration.

Important: The submission of a late registration *does not* change the registration review date. The original review date will remain for subsequent submissions.

Completed/Terminated Registrations

The following applies to terminated *registrations*, not completed or terminated projects. Terminated projects are to be included in annual review submissions.

For all completed or terminated registrations, the PI will be required to submit a letter to the IBC Chair(s) *two weeks* prior to a scheduled IBC meeting. In the closure letter, the PI will convey the disposition of the biohazardous materials related to the registration in the form of one of three options:

- the PI may transfer the materials to another PI or responsible party
- the PI can make arrangements for proper disposal of the materials
- the PI can state that the materials will remain stored under their supervision

If the biohazardous materials have been *transferred to another PI*, both the original PI and the new responsible party must include a signature stating where and to whom the material is assigned and their acceptance of said material. The new responsible party must also modify or create their IBC registration to include these materials.

If materials are to be *disposed of*, it is the responsibility of the PI to ensure that all biohazardous and regulated medical waste is disposed of in the proper manner.

If these materials will be *stored*, the PI will be required to document in detail where the material is located (i.e. building, room number, freezer number, labeling, etc.).

If a Principal Investigator fails to inform the committee regarding the closing of their registration, a **90-day** limit for the disposal or transfer of materials will be given once the Committee is aware of the termination of the research. After 90 days, Environmental Health & Safety will have the authority to dispose of the biological materials as they see fit and the PI's Registration will be administratively closed.

WORKING WITH BIOLOGICAL MATERIALS

Exposure Control

Biosafety issues are addressed in terms of physical and biological containment. The necessary containment can be achieved by using the appropriate combination of:

1. Proper practices and techniques
2. Safety equipment
3. Laboratory design

Laboratory Practices

The most crucial component of containment is stringent adherence to standard microbiological practices and techniques. Personnel working with infectious agents or biohazardous materials must be aware of the potential danger, and must be trained and proficient in the practices and techniques required for handling such material safely.

Each PI should identify specific hazards that will occur in the course of the research project, and consider practices and procedures needed to minimize or eliminate risks. *Personnel should be advised of special hazards and are expected to follow the required practices and procedures.*

When [standard laboratory practices](#) are not sufficient to control the hazards associated with a particular agent or procedure, additional measures may be needed. The PIs, with assistance from the Biological Safety Officer, are responsible for selecting additional safety practices, which must be in keeping with the hazards associated with the agent or procedure. Laboratory personnel, safety practices and techniques must be supplemented by appropriate facility design and engineering features, safety equipment and management practices.

Safety Equipment (Primary Barriers)

Safety equipment includes engineering controls and personal protective equipment (PPE), where both serve as primary barriers against exposure to biohazardous materials.

Biological Safety Cabinet

Biological safety cabinets (BSC), enclosed containers, safety centrifuge cups and other engineering controls are designed to eliminate or minimize the exposure to hazardous biological materials. The BSC is the principle device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. There are three types of BSCs (Class I, II, III) used in microbiological laboratories. Open-fronted Class I and Class II BSCs are primary barriers that offer significant levels of protection to both laboratory personnel and the environment when used with good microbiological techniques. Class II biological safety cabinets also provide protection from external contamination of the materials being manipulated inside the cabinet. Class III BSCs are enclosed, gas-tight and provide the highest attainable level of protection to personnel and the environment.

An inspection/field test and certification of the BSC must take place at the time of installation and at least **annually**. Cabinet inspections must also be conducted when HEPA filters are changed,

when maintenance repairs have been made to internal parts, when the BSC has been relocated and when other manufacturer's guidelines apply. (*ANSI Standard 49, Annex F.1*)

The inspection and certification of biological safety cabinets are the responsibility of the PI and must be completed by an accredited and certified vendor.

Semi-annual cleaning of biological safety cabinets is *strongly recommended*. General guidelines on how to clean a BSC using 10% household bleach are:

1. Raise the front window to its highest level.
2. Remove and clean the removable grille inside the cabinet.
3. Remove and clean the one piece work surface or individual work surfaces. Clean both sides.
4. Open the drain valve in the cabinet pan and place a leak-proof container under the opening.
5. Clean all cabinet surfaces, including the pan, back wall, and side panels. Let disinfectant sit 10 minutes before wiping. Gently wipe pooled disinfectant towards the cabinet drain and into the leak-proof container.
6. Spray and wipe entire cabinet with water to prevent pitting of the metal.
7. Reassemble the cabinet interior. Remember to close drain valve!
8. Clean the front window.
9. Dispose of collected liquid down the drain.

Centrifuge

A centrifuge can be an important tool in a clinical or research laboratory. Most hazards with centrifuges stem from two sources: mechanical conditions and processing hazardous materials. Stress, fatigue and corrosion of mechanical parts on a centrifuge are all serious problems.

- Large, ultra-centrifuges **must be inspected annually** by an accredited and certified vendor to ensure these issues do not become serious problems.
- EH&S **recommends** high-speed and micro centrifuges be inspected annually.

Centrifugation of hazardous samples can result in exposures to chemical, biological or even radiological agents. Careful consideration must be given to work practices to avoid hazards. Use the following guidelines when hazardous materials are centrifuged:

- Load and unload hazardous samples in ventilated enclosures (biosafety cabinets)
- When hazardous samples are centrifuged, contain samples in safety cups, sealed tubes, or safety rotors
- **Wait at least 10 minutes after centrifuge has stopped before opening.** This allows any aerosols generated in the chamber to settle.
- Clean and decontaminate all parts after each use, according to manufacturer's instructions.

Personal Protective Equipment

Safety equipment also includes PPE. This equipment consists of gloves, lab coats, gowns, shoe covers, boots, respirators, face shields, safety glasses or goggles. Appropriate PPE must be worn whenever work is being conducted. *At a minimum, appropriate PPE includes lab coats, gloves and eye protection (if there is the possibility of splashes)*. PPE is to be made available to employees by the PI or the department.

PPE is often used in combination with BSCs and other containment devices. In some situations in which it is impractical to work in BSCs, personal protective equipment may form the primary barrier between personnel and the infectious material.

While PPE should be worn while in the lab, it is to be disposed of *before* exiting.

- *Do not wear laboratory-used PPE in common areas, such as hallways and offices.*

Facility Design (Secondary Barriers)

The design of a facility is important in providing a barrier to protect those working inside and outside the laboratory and to protect people or animals in the community from infectious agents. Facilities must be commensurate with the laboratory's function and the recommended biosafety level for the agents being manipulated.

The secondary barrier(s) will depend on the risk of transmission of specific agents. Secondary barriers in these laboratories include separation of the laboratory work area from public access, availability of a decontamination facility (e.g. autoclave) and hand washing facilities.

Vaccinations

Vaccinations can be made available for many etiologic agents used in the laboratory *if* the need is great enough. The Occupational Health Department, in conjunction with the IBC, will make the recommendation for the use of vaccinations on a case-by-case basis.

Training

Responsibility

Those individuals working in an active biological laboratory, or a laboratory with biological hazards present, are required to complete biological safety training. Ensuring the completion of proper training by laboratory staff is the responsibility of the individual Principal Investigator. All biological laboratory personnel, including the Principal Investigator, must be trained for the hazards contained in the laboratory, and the training must be up-to-date.

Types of Training

Training on BioRAFT

Training on BioRAFT is located in the Course Directory under the "Training" tab. For each course/training, select "Launch course," view the presentation, complete the quiz and submit.

Biosafety Training

It is mandatory that all faculty, staff, and students working in a laboratory setting containing biohazardous materials must satisfactorily complete Biological Safety training. Completion of the biosafety training is required **every five (5) years**.

Autoclave Training

It is mandatory that all faculty, staff, and students working in a laboratory setting containing biohazardous materials must satisfactorily complete Autoclave Safety training. Completion of the autoclave training is required **every five (5) years**.

Shipping Training

It is mandatory that all faculty, staff, and students who are involved in shipping or receiving biological materials must satisfactorily complete Shipping Biological Materials training. Completion of this course satisfies requirements of both the International Air Transport Association (*IATA*) and the Department of Transportation (*DOT*) for infectious substances shipping training. Completion of the autoclave training is required **every two (2) years**, as long as the laboratory/person is involved with shipping.

NIH Guidelines Training

NIH Guidelines Training is recommended by NIH for all IBC Members and at a minimum, faculty involved in recombinant DNA research. The PI must review the training presentation **every five (5) years**, at the time of new submission (or 5-year resubmission) if not taken within the previous year. Principal Investigators are responsible for the training of their laboratory members in the NIH Guidelines.

Bloodborne Pathogen Training

Investigators performing studies utilizing human blood or other potentially infectious materials covered by the OSHA Bloodborne Pathogen Standards are required to complete annual Bloodborne Pathogen Training. Other potentially infectious material (OPIM) is defined as:

Body Fluids

Amniotic Fluid	Pleural Fluid
Breast Milk	Saliva from dental procedures
Cerebrospinal Fluid	Semen
Joint Fluid	Vaginal Secretions
Pericardial Fluid	Any body fluid visibly contaminated with blood
Peritoneal Fluid	Unidentifiable body fluids

Other Materials

Any unfixed tissue or organ of human origin

Investigators are required to use “Standard Precautions” when handling specimens of blood, blood products, or “other potentially infectious material” as stipulated in the EVMS Biosafety Procedure Manual. Standard Precautions involves treating materials as though infectious no matter the circumstance; this involves always using standard PPE and treating the materials with universal precautions.

Personnel who qualify to be exempted from the annual Bloodborne Pathogen Training requirement must contact the Occupational Health department to complete a waiver form. Questions and concerns about Bloodborne Pathogen Training can be submitted to the Occupational Health Director at 446-5870.

EVMS Bloodborne Pathogen Training is provided for EVMS personnel only. For guest/visiting researchers and research staff who will be working with human blood or body fluids, Bloodborne Pathogen Training should be completed annually with their employer or school.

These researchers should provide the IBCA with documentation stating the last training completion date.

Live Training

Contact Environmental Health and Safety Department for scheduling live training.

Chemical Hygiene Plan (CHP)

All permanent faculty and staff working in a laboratory must complete Chemical Hygiene Plan Training. CHP training credit is obtained by attendance in Environmental Health & Safety's CHP course. An online CHP Refresher course must be completed **every five (5)** years.

Radiation Safety Training

Radiation safety training is required when personnel will be working with radioactive materials or products containing radioactive material. If working with radioactive materials, completion of the "Radiation Safety in the Laboratory" coursework is mandatory. After successful completion, personnel will receive their user documentation and may need to apply to the EVMS Radiation Safety Committee for approval to work with radioactive materials. Radiation Safety refresher training must be completed **annually** to be compliant.

If personnel will only be working in the vicinity of radioactive materials (such as animals containing radioactive products), they may complete an abridged training course. While being able to work around radioactive materials, these personnel will not be users and are therefore not permitted to handle radioactive materials.

Other Training

Animal Users

Training requirements for laboratories utilizing animal models can be found on the [IACUC MyPortal](#) page. Additionally, there is a facility orientation as well as a live training session for Rodent Users. Please contact the Comparative Medicine Program Director for details and scheduling.

Risk Assessment

Risk assessments are an important responsibility for principal investigators. The PI, Biological Safety Officer and others share in the risk assessment process. A risk assessment is a method used to:

- Identify the hazardous characteristics of the agent or material involved in an investigator's work, such as
 - Risk Group of the agent or material
 - Route of transmission
 - Agent stability
 - Infectious dose
 - Origin of material
- The activities and research methods that can result in a person's exposure to an agent, for example
 - Work requiring high concentration doses of virus
 - Centrifugation

- Amplification
- The likelihood that such exposure will cause an infection
- The probable consequences of such an infection

The information identified by a risk assessment provides a guide for

- The selection of appropriate Biological Safety Level (*BSL*) for conducting the research
- Appropriate microbiological practices
- Sufficient safety equipment
- Proper facility safeguards that can prevent laboratory acquired infections

Risk assessments are accomplished through BioRAFT registration reviews. Principal investigators will use the risk assessments to alert their laboratory personnel to the hazards working with infectious agents and to the need for developing proficiency in the use of selected safe practices and containment equipment.

Risk assessments are to be conducted at several points. They should be done

- Prior to working with an agent
- At regular intervals during registration approval period or work
- At least annually
- Whenever changes occur in the laboratory, such as
 - A move or renovation
 - A new employee begins working in the lab
 - New agent introduced
 - New or different piece of equipment utilized in the work
 - New techniques or procedures are employed in the work

Successful control of hazards in the laboratory also protects persons not directly associated with the laboratory, such as other occupants of the same building and the public.

Laboratory Inspections

According to the *NIH Guidelines for Research Involving Recombinant DNA Molecules*, periodic laboratory inspections are required in order to ensure biological safety standards are followed (**Section IV-B-3-c-(1)**) and to ensure that research is in compliance with the *Guidelines* (**Section IV-B-2-b-(5)**).

All Principal Investigators with approved IBC Registrations (including those granted with IBC Exemptions) will have **annual** inspections. Inspections will be conducted by the Biological Safety Officer. The general procedure for inspections will be as follows:

1. A communication will be sent to the laboratory/Principal Investigator that will be inspected in order to select a date and time.
2. A reminder will be sent to the laboratory **one week** before the inspection.
3. Within **2 days** after the inspection, a memo will be sent to the PI listing any deficiencies.
4. A response to the memo will be due within **1 week**. The response should include the signed checklist, any corrected deficiencies, and any plans for corrections not made.

These inspections are to ensure compliance not only with safe laboratory practices, but also with each laboratory's submitted and approved registration. All laboratories listed under a Principal Investigator will be inspected at the same time. If a laboratory comes up for their Annual Review and has not **scheduled** their inspection with the BSO, the BSO reserves the right to withhold their registration from going through to the IBC until the inspection is scheduled.

Laboratory Procedures

The following are general laboratory guidelines for EVMS.

Standard Practices

- Limit access to the laboratory.
- Lab coats are to be worn at all times in the laboratory. Lab coats should be changed when they become contaminated.
- Do not eat, drink, store food, smoke, handle contact lenses or apply cosmetics in the laboratory. *There is no such thing as a “clean area” inside a laboratory in which to eat and drink.*
- Persons wearing contact lenses should wear non-vented goggles or a face shield. However, it is best to avoid wearing contact lenses if possible.
- Pants or long skirts should be worn in the laboratory. Only closed-toe shoes are to be worn while in the laboratory.
- Remove all protective equipment before leaving the laboratory (lab coat, gloves, etc.).
- Always wear gloves when using biological materials or handling animals. Change gloves often even if you don't think they are contaminated.
- Remove gloves before handling non-contaminated materials.
- Wash hands after removing gloves and before leaving the work area.
- Minimize the production of splashes and aerosols.
- Use sharps only when necessary and dispose of them in appropriate sharps containers.
- Never manipulate sharps or needles in a manner that involves direct contact or directing a movement toward the body.
- Report spills and accidents involving infectious materials to the PI and EH&S immediately.
- Decontaminate work surfaces after every spill of infectious material. Also, decontaminate work surfaces before and after working on them.
- Do not mouth pipette.
- Decontaminate all waste before disposal.

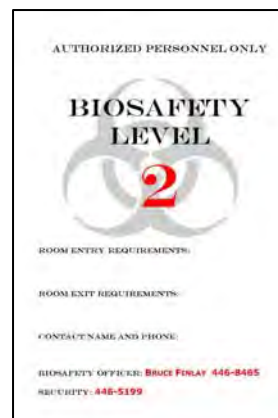
Basic Facilities

Microorganisms, which present minimal hazards, are used in a basic laboratory facility. Each laboratory at EVMS has the same basic design. The basic laboratory design must include

- A sink for hand washing with hot water, soap and towels located preferably near the lab entrance/exit
- Easy-to-clean areas
- Bench tops which are waterproof and resistant to acids, alkalis, organic solvents, and moderate heat
- Sturdy lab furniture
- Fly screens on windows if they open
- Doors should open inward, be self-closing, lockable, and kept closed when work is being performed
- Laboratory rooms should have directional airflow into the laboratory (not into common areas like hallways)
- Do not recirculate exhaust air in animal facilities

Signs

A Biohazard Warning Sign, having the biohazard symbol, must be posted at each entrance to all laboratories at Biosafety Level 2 and higher. Signs must identify the Biological Safety Level, list the name and phone number of laboratory contacts, and indicate any special requirements for entering and exiting the laboratory (such as immunizations and training).



Laundry

For Biological Safety Level 2 facilities, including clinical areas, decontamination of laundry prior to transport to a commercial laundry facility is not necessary. Commercial laundry facilities use water temperatures of at least 160°F and 50-150 ppm of chlorine bleach to remove significant quantities of microorganisms from grossly contaminated laundry.

Each PI or their department is responsible for maintaining a laundry service if reusable PPE is used in the laboratory. It is the responsibility of the Principal Investigator to ensure that laundry services for all laboratory employees' lab coats are acceptable and performed at adequate intervals.

In the event laundry, whether in the laboratory or clinical setting, becomes soiled, the following procedures shall be followed:

1. DO NOT sort, rinse, or soak laundry in the location of use. Employees are not to take contaminated laundry home for any reason.
2. While wearing appropriate PPE, remove the contaminated piece of laundry.
3. Place laundry in an appropriately labeled bag or container at the location where it was used.
 - a. Bag or container must be leak-proof and labeled with the Biohazard symbol.
 - b. *Note:* Commercial laundry bags from a contracted vendor are appropriately labeled and are coated in a liquid-resistant material to prevent leaks.
4. If laundry is wet and presents a reasonable likelihood of leakage to the exterior of a single container, it must be placed in a second container (or "double-bagged").
5. After all soiled laundry has been placed in the appropriate container(s), dispose of used PPE in the medical waste container.
6. All laundry shall be sent to a commercial laundry facility for decontamination and cleaning at no expense to the employee.

Emergency Freezers

The Environmental Health and Safety Department is dedicated to assisting EVMS research personnel in the safety and efficiency of their work. As part of this endeavor, EH&S is responsible for the Emergency Freezers located in Lewis Hall.

The Emergency Freezers are utilized as *temporary* cold storage equipment in cases of an individual laboratory's emergency need. A complete explanation of the procedures for using the freezers is located in the Appendices.

Pest Management

Animals and plants are not permitted in the laboratory unless they are associated with the work being performed. Pest management is the responsibility of the Physical Facilities Department. To report pest issues, contact Physical Facilities at 446-5035.

Moving Equipment

In order to move or dispose of biological equipment, it must first be decontaminated. Biological Safety Cabinets must be decontaminated by an outside vendor due to the contained HEPA filters. All other equipment may be decontaminated by laboratory staff using an approved chemical decontaminate.

Biological Safety Cabinets must be inspected and certified once they have been installed in a new location regardless of when the previous certification expires. The BSC needs to be tested to ensure proper functioning with the new work space and airflow.

Biosecurity

The objective of security, or in this case biosecurity, is to prevent loss, theft or misuse of microorganisms, biological materials and research-related information. This is accomplished by limiting access to facilities, research materials and information.

Biosecurity is based upon risk assessment and management methodology; personnel expertise and responsibility; control and accountability for research materials including microorganisms and culture stocks; access control elements, material transfer documentation, training, emergency planning and program management. Each PI shares in the responsibility of securing the materials in their laboratories. Simple mechanisms such as locking a laboratory as you exit and having emergency procedures prepared are part of biosecurity.

Select Agents

The Centers for Disease Control and Prevention (CDC) regulates the possession, use and transfer of select agents and toxins that have the potential to pose a severe threat to public health and safety. The U.S. Departments of Health and Human Services (HHS) and Agriculture (USDA) published final rules for the possession, use, and transfer of select agents and toxins (42 C.F.R. Part 73, 7 C.F.R. Part 331, and 9 C.F.R. Part 121) in the Federal Register on March 18, 2005. A curr listing of Select Agents and Toxins can be found in Appendix A or at <http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20List.html>.

Principal Investigators are to identify all Select Agents and Toxins desired to be used and stored in the laboratory in a written communication to Environmental Health & Safety *before* ordering. The BSO will respond each request on an individual basis. The PI is *not* to order or receive any Select Agent or Toxin until given final approval from EH&S and the IBC.

Dual Use Research

“Dual Use” refers to research materials or technology that, based on current understanding, can be reasonably anticipated to provide knowledge, products, or technologies that could be directly misapplied by others to pose a threat to public health, agriculture, plants, animals, the environment, or materiel. It is

imperative that researchers and IBCs increase their awareness of biological security issues and learn to assess research in terms of modern security concerns to minimize the chance of malicious consequences of their research.

There are *seven (7)* specific experiments considered “Dual Use Experiments.”

1. Demonstrate how to render human or animal vaccines ineffective.
2. Confer resistance to therapeutically useful antibiotics or antiviral agents for humans, animals, or crops.
3. Enhance the virulence of human, animal, or plant pathogens, or make non-pathogens virulent.
4. Increase the transmissibility of pathogens.
5. Alter the host range of pathogens.
6. Enable the evasion of diagnostic or detection methods.
7. Enable the weaponization of biological agents or toxins.

The Office Biotechnology Activities' measure to address such “dual use” research includes convening and managing the **National Science Advisory Board for Biosecurity** ([NSABB](#)). The purposes of the NSABB are to:

- Advise the Federal government on strategies to minimize the risks and harm that could result from malevolent use of legitimate research
- Support development of Federal and institutional oversight guidelines
- Promote awareness in the research community about the dual-use issue
- Foster international collaboration on issues related to dual-use research

Extra scrutiny and precautions should be given to potential dual-use research by both the PI and the IBC.

BIOLOGICAL SAFETY LEVELS (BSL)

Biosafety levels are standards that specify the combinations of laboratory practices, safety equipment and facility design that are appropriate for the biohazards of an operation. These standards are found in the [CDC](#)'s *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* and the [NIH](#) *Guidelines for Research Involving Recombinant DNA Molecules*. This manual lists a summary of these standards.

Be aware that Risk Groups are *not* the same as Biological Safety Levels. Risk Groups deal only with an organism's characteristics, such as mode of transmission and infectious dose. Determination of a Biological Safety Level includes an organism's Risk Group, but also includes the facility design, required PPE, specific safety practices and hazard to the surroundings.

In addition, there is a distinction between a laboratory and an animal facility or animal room. This distinction exists because of the additional hazards an animal can introduce, such as biting and husbandry. For instance, a biosafety level for a laboratory may be referred to as Biosafety Level 1 (BSL1), while a biosafety level for an animal facility or room may be referred to as Animal Biosafety Level 1 (ABSL1). Because there are few differences, the requirements for both biosafety level types are presented together.

All laboratory work should be evaluated and approved for the biosafety level by the BSO. **EVMS does not currently have appropriate facilities for work at BSL3 and/or BSL4 levels. Therefore, organisms that require such facilities are not handled in EVMS laboratories.**

BSL	Agents	Practices	Safety Equipment (Primary Barrier)	Facilities
1	Not known to consistently cause disease in health 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 	<u>Primary Barriers</u> <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 <u>PPE</u> <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 lab clothing before laundering ➤ Baseline Serum 	<u>Primary Barriers</u> <ul style="list-style-type: none"> ➤ Class I or II BSCs or other physical containment devices used for all open manipulations of agents <u>PPE</u> <ul style="list-style-type: none"> ➤ Protective lab 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 ➤ Exhausted 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 lab infections; 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 	<u>Primary Barriers</u> All procedures conducted in: <ul style="list-style-type: none"> ➤ Class III BSCs or <ul style="list-style-type: none"> ➤ 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 decon systems ➤ Other requirements outlined in the text

Table 1: BSL Summary (*BMBL, 5th edition*). To view a complete description of the CDC’s BSLs, visit <http://www.cdc.gov/biosafety/publications/bmb15/index.htm>.

Biosafety Level 1 (BSL1) and Animal Biosafety Level 1 (ABSL1)

Biosafety Level 1 is appropriate for work involved with well-characterized strains of viable microorganisms, which have no known or minimal hazard to healthy adults. BSL-1 laboratories do not necessarily have to be separated from the general traffic patterns in the building. Working on bench tops is permitted while using standard practices and wearing protective equipment and clothing such as face shield, gloves and a lab coat when necessary.

Access to the animal facility is limited to persons who have been informed of the hazards. Persons having a higher than normal sensitivity to infection should not be allowed in the animal facility.

Biosafety Level 2 (BSL2) and Animal Biosafety Level 2 (ABSL2)

BSL2 and ABSL2 correspond to work conducted with organisms causing disease in humans where vaccines and therapies are usually available. Work can be conducted on the open bench unless there will be aerosolization of material or if sterility is needed. Examples of agents and materials that can be worked with in a BSL2 setting include:

- Human tissues and fluids
- Hepatitis B virus
- Hepatitis C virus
- *Adenovirus*
- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*

Access to these laboratories and animal facilities is limited to persons who have been informed of the hazards. Persons having higher than normal sensitivity to an infectious agent should not be permitted to enter.

In addition to BSL1/ABSL1 requirements:

- Laboratory personnel must have specific training in handling pathogenic agents and be supervised by PIs competent in handling infectious agents and associated procedures
- Access to laboratory is restricted when work is being conducted
- Plants or animals not related to the research should not be allowed in the labs or animal facilities
- Use biohazard warning signs
- Implement a medical surveillance program (*when applicable*)
- Use extra caution with sharps. Sharps protective devices (“safety” devices) should be used when working with human material.
- Use Class II biological safety cabinets when there is a likelihood of generating aerosols
- Use the appropriate containment equipment for animals
- If splashing is anticipated, use face protection such as goggles, safety glasses and a NIOSH N95 HEPA-filtered respirator, or a face shield
- Wear a NIOSH N95 HEPA-filtered respirator when unable to contain aerosols
- Use protective laboratory clothing such as laboratory coats or gowns
- An eyewash and an autoclave must be available
- Decontaminate animal cages before washing

Although ***BSL3 and BSL4 facilities are not currently available*** at EVMS, it is important to know and understand the agents and requirements involved in these Biosafety Levels.

Biosafety Level 3 (BSL3) and Animal Biosafety Level 3 (ABSL3)

BSL3 and ABSL3 are for work done with indigenous or exotic agents which can be associated with respiratory transmission and which may cause serious and potentially lethal disease in humans. Vaccines and therapies are not usually available for these microorganisms. Other safety measures such as controlled access and special ventilation systems must be implemented. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents and must be supervised by scientists competent in handling infectious agents and associated procedures. Examples of agents that can be worked with in a BSL3 setting are:

- *M. tuberculosis*
- *St. Louis encephalitis*
- *Coxiella burnetii*

In addition to BSL3 and ABSL3 requirements:

- All manipulation of infectious materials must be conducted within BSCs, other physical containment devices, or by personnel wearing appropriate PPE.
- Restrict traffic flow
- Anteroom (changing room) is required
- Use disposable and impermeable laboratory clothing
- Decontaminate all waste
- The entrance must have two sets of self-closing doors and have locks in accordance with institutional policy
- The sink must be hands-free or automatically operated and near the exit
- The interior surfaces of the ceilings, walls and floors must be water resistant and sealed
- The windows must be closed and sealed
- The exhaust system must be ducted and create directional air flow in the laboratory from clean to contaminated areas and discharged outside
- If possible, decontamination methods should be available within the laboratory
- Air from Class II biosafety cabinets may be recirculated, provided the cabinet is certified at least every twelve (12) months
- Aerosol producing equipment must exhaust air through HEPA filters
- Protect vacuum lines with liquid disinfectant traps and HEPA filters

Biosafety Level 4 (BSL4) and Animal Biosafety Level 4 (ABSL4)

Work done at BSL4/ABSL4 corresponds to dangerous and exotic agents that pose a high individual risk of life-threatening disease, aerosol transmission, or related agent with unknown risk of transmission. No vaccines or therapies are available for these agents. Class III BSCs or air-supplied positive pressure suits (full-body) must be used. This facility must be separate or completely isolated from any other facility. Examples of agents that can be worked with in a BSL4 setting include:

- *Ebola Zaire*
- *Sin Nombre virus*
- *Rift Valley Fever*

This biosafety level includes BSL3/ABSL3 requirements, plus:

- The laboratory or animal facility must be in separate buildings or an isolated area within a building
- Access to the facility is tightly controlled, with a logbook or other means of documenting date and time of all persons entering and leaving the laboratory must be maintained
- A room for changing into laboratory clothing must be provided

- Shower before exiting the facility
- Decontaminate all materials and waste before removing them from the facility
- Use Class III biological safety cabinets or partial containment equipment in combination with a full-body, air-supplied respirator
- Use special sterilization procedures
- Sterilize everything before removing from the facility, except for biological materials intended for transfer
- Sterilize all laboratory effluents
- The ventilation system must be dedicated, non-recirculating and have HEPA filters
- Use utility services with backflow protected systems
- Entrance doors must be self-closing and self-locking

DECONTAMINATION: DISINFECTION AND STERILIZATION

To prevent laboratory-induced disease and infection, the number of microorganisms in the open laboratory must be reduced. This reduction is achieved by using a number of chemical and physical mechanisms.

Three mechanisms used to reduce the number of microorganisms are heat, chemical and radiation. Heat treatment is used for **sterilization**, or destroying all microorganisms including spores. Chemical and radiation treatments are used for **disinfection**, or they destroy all microorganisms *except* spores. **Decontamination** is a general term, which can mean either sterilization or disinfection.

Wet Heat (Steam)

Autoclaving, or steam sterilization, involves exposing infectious materials to steam at a specified temperature and pressure for a defined period. In general, gravity displacement autoclaves should be operated at 121°C (250°F) at 15 PSI for 90 minutes. The appropriate autoclaving settings vary according to the type and amount of biological materials as well as the physical characteristics (types of containers) of the load.

The large autoclaves were originally installed as core equipment for use by all research laboratory personnel. As such, these autoclaves are considered facility equipment. The locations of the “core” autoclaves are Lewis Hall 2110, Lewis Hall 3003, Lewis Hall 3163, Williams Hall (Pediatrics) A14 and Lester Hall 447.

Responsibilities

Physical Facilities is responsible for the installation and repair of core autoclaves.

Environmental Health & Safety (EH&S) is responsible for the certification and maintenance of these autoclaves. EH&S has established a preventative maintenance contract with an outside vendor to help ensure the safe functioning of the units. EH&S is also responsible for the monthly spore testing of the autoclaves and for the upkeep of the recording paper.

Laboratory Personnel are to complete Autoclave Training, available on the EVMS BioRAFT system (<https://evms.bioraft.com/raft/training/courses>), before utilizing the core autoclaves. Personnel are also responsible for the general preservation of the machines by using them in a clean and timely manner. By emptying the chamber after a cycle or by not leaving material in the autoclave overnight, the autoclaves can be kept efficiently operational and serviceable to all.

Also, laboratory personnel should enter a record into the autoclave’s log book for each use. There is a separate log book for each autoclave, whether it is utilized for sterilization of equipment or for general use. EH&S collects and stores these Log records in Lewis Hall 2142.

For additional safety and operational suggestions, personnel can consult the Autoclave Safety Guidelines, posted on the EH&S intranet site as well as in the core autoclave rooms.

Accessibility

The core autoclaves and the equipment rooms they are housed in should be accessible to all laboratory staff. Autoclave rooms should not be locked to approved, trained laboratory personnel. As each technician or investigator follows approved autoclave guidelines, including proper containment of materials and correct documentation of usage, each are to have complete access to the autoclaves.

Please contact EH&S at 446-5798 if a problem or concern arises with the autoclaves, so that the autoclaves can return to service as quickly as possible.

Spore Testing Procedure

If you wish to conduct a spore test on the core autoclaves, or on your lab's personal autoclave, follow the procedure below.

1. Plug in incubator at least **30 minutes** before beginning testing process
2. Place biological indicator vial horizontal or cap up in the test tray or appropriate package
3. Place tray or package in the area of the drain on the bottom shelf
4. Process load
5. Remove load from autoclave
 - » Always wear appropriate PPE
6. Allow indicator to stand an additional **10 minutes**
 - » Crushing indicator before cooling may cause ampoule to burst
7. Follow manufacturer's instructions on processing the vial
8. Place lid onto incubator and incubate vials for **48 hours**
9. Record results
10. Place used vials in the medical waste container for disposal off-site

Dry Heat

This method may be used for sterilizing hard surfaces such as glassware. Generally sterilization takes place at 160° - 170°C (320° - 338°F) after 2 – 4 hours. Because each load may contain different types and quantities of objects and infectious material, the time for sterilization may vary. Operational checks and sterilization checks of each load or of like loads should be performed regularly. Sterility checks can be done using an appropriate sterility indicator such as *Bacillus stearothermophilus* spore strips.

Radiation

Ultraviolet (UV) radiation can be used to inactivate microorganisms in the air and on surfaces, such as in biosafety cabinets. The wavelength range used for decontamination is known as the germicidal range and is 210 – 310 nm. UV radiation is not a recommended method of decontamination at EVMS.

The NIH does **not** recommend or support the use of UV radiation in laboratories. Although UV is effective against most microbes, it requires an understanding of its abilities and limitations. The 253.7-nm wavelength emitted by the germicidal lamp has limited penetrating power and is primarily effective against unprotected microbes on exposed surfaces or in the air. It does not penetrate soil or dust. The intensity or destructive power decreases by the square of the distance from the lamp. Thus, exposure time is always related to the distance. The intensity of the lamp diminishes over time. This requires periodic monitoring with a UV meter. The intensity of the lamp is drastically affected by the accumulation of dust and dirt on it. The bulbs require frequent maintenance. In addition, there are safety hazards associated

with the use of UV that require personal protective equipment or other safety devices to protect users. UV lights in biosafety cabinets require the cabinet be decontaminated prior to performing maintenance on the system.

Chemical

Chemical decontamination can be done with liquids, vapors and gases. Liquid disinfectants are used to decontaminate work surfaces and liquid waste. Vapors and gases are used to disinfect items that are not easily disinfected or cannot be disinfected without damage by other methods. See [Table 2](#) for a summary of liquid chemical disinfectants.

A 10% sodium hypochlorite (bleach) solution can be used for most disinfection procedures. A 10% bleach solution equates to 1 part bleach in 9 parts water (e.g. 100 mL bleach + 900 mL water = 1L of 10% bleach solution). To allow for maximum effectiveness, allow a contact time of at least **10 minutes**. Bleach solutions should be prepared *weekly* since they quickly lose their effectiveness.

Contact EH&S if using other chemical disinfectants.

Table 2: Chemical Disinfectants Comparison

Disinfectant	Requirements			Inactivates				Application					Other					
	Dilution	Virus with Envelope ^b	Broad Spectrum ^b	Bacteria	Virus with Envelope	Virus without Envelope	Bact. Spores	Work Surfaces	Glass-ware	Large Area Decon	Liquid for Discard	Penetrating Decon	Eff. Shelf Life ^e	Corro-sive	Flamm-able	Skin Irritant	Eye Irritant	Toxic
<i>Phenolic Compounds</i>	1-5%	10	NE	+	+	^c		+	+				+	+		+	+	+
<i>Chlorine Compounds</i>	5000 ppm^a	10	30	+	+	+	+	+	+		+			+		+	+	+
<i>Ethyl Alcohol</i>	70 - 85%	10	NE	+	+	^c		+	+				+		+		+	+
<i>Isopropyl Alcohol</i>	70 - 85%	10	NE	+	+	^c		+	+				+		+		+	+
<i>Iodophor</i>	25 - 1600 ppm	10	30	+	+	+	+	+	+				+	+	+	+	+	+
<i>Gluteraldehyde</i>	2%	10	30	+	+	+	+					^d +	+			+	+	+

NE - not effective

^a Available halogen (Bleach = 1:10 dilution)

^b Contact time (minutes)

^c Variable results depending on the virus

^d Gas may be flammable or explosive if used improperly

^e approx. 1 week, protected from light and air

* Commercial formulations of these disinfectants are generally available. Principal Investigators should ensure that commercial products are registered with the Environmental Protection Agency (EPA) and that their use is in accordance with the manufacturer's recommendations.

EXPOSURE TO INFECTIOUS AGENTS

EVMS recommends the following guidelines in the event of an exposure to an infectious agent or material:

Intact Skin

1. Remove contaminated clothing.
2. Vigorously wash contaminated skin for at least 1 minute with soap and warm water.
3. Report the event to the Occupational Health Department as soon as possible.

Damaged Skin or Puncture Wound

1. Remove contaminated clothing.
2. Vigorously wash contaminated skin for at least 5 minutes with soap and warm water.
3. Report the event to the Occupational Health Department as soon as possible.

Eye Exposure

1. Immediately flush eyes with water for 15 minutes, preferably using an eyewash.
2. Seek medical attention from the Occupational Health Department. For after hours, call 911 and seek assistance from Sentara Norfolk General Hospital's Emergency Department.

Ingestion or Inhalation

1. Seek medical attention immediately from the Occupational Health Department. For after hours, call 911 and seek assistance from Sentara Norfolk General Hospital's Emergency Department.
2. Do not induce vomiting unless advised to do so by a health care provider.

Laboratory Acquired Infections

In the event of a possible laboratory-related illness, consultation between Occupational Health, Environmental Health & Safety, the employee and the employee's supervisor is required for proper medical management and recordkeeping. The following procedures should be followed if an EVMS employee suspects an illness is related to infectious agents in their work area.

1. Treat any exposure site(s) immediately.
2. Contact supervisor and EVMS Occupational Health Department.
 - » During normal business hours: 757-446-5870
 - » Evenings, nights, weekends, holidays: 757-669-1157 (*pager*)
3. Report to Occupational Health to receive counseling, baseline lab testing and prophylaxis if indicated.

Reporting rDNA Exposures and Adverse Events

The following applies to research involving recombinant DNA.

Definitions

Recombinant DNA (rDNA) and synthetic nucleic acids are defined as:

1. Molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e., recombinant nucleic acid
2. Nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e. synthetic nucleic acids, or
3. Molecules that result from the replication of those described in 1 or 2 above.

An “exposure” is any event involving exposure of any person to infectious materials, biological toxins or human blood/OPIM.

A “serious adverse event” is any event occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening event
- In-patient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization also may be considered a serious adverse event when, upon the basis of appropriate medical judgment, they may jeopardize the human gene transfer research subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

An adverse event is “associated with the use of a gene transfer product” when there is a reasonable possibility that the event may have been caused by the use of that product.

An “unexpected serious adverse event” is any serious adverse event for which the specificity or severity is not consistent with the risk information available in the current investigator’s registration.

Procedure

All exposures (accidents, injuries, illnesses, etc.) must be reported to the Occupational Health Department (446-5870) as soon as possible. After regular business hours (e.g. evenings, nights, weekends, holidays), exposures must be reported by calling the EVMS Exposure Pager at 669-1157.

Any serious adverse event or adverse event must be reported, in writing, to the Chair of the IBC within **three business days** of the event. Even if an adverse effect was anticipated by the registration (and disclosed to a subject), if the effect has changed in nature, severity or frequency in the study, this must be reported to the IBC.

Required reporting also includes, but is not limited to, any procedural errors during the research, a breach in confidentiality or privacy, emotional disturbances, noncompliance with the regulations of

IBC policies, or any other problems occurring during the research. Principal Investigators should err on the side of caution when determining whether an event is reportable to the IBC.

Adverse event reports, when involving rDNA, must be reported under the [NIH Guidelines](#). Any serious adverse event that is fatal or life threatening, which is unexpected and associated with the use of a gene transfer product, must be reported to the NIH OBA no later than **seven (7) calendar days** after the event.

Serious adverse events that are unexpected and associated with the use of a gene transfer product, but are not fatal or life-threatening, must be reported to the NIH OBA no later than **15 calendar days** after the event.

Any significant problems with or violations of the NIH Guidelines and any significant research-related accidents or illnesses must be reported to the appropriate institutional official and NIH/OBA within **30 days**, unless the IBC determines that a report has already been filed by the PI. Reports to the NIH/OBA shall be sent to:

Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive, Suite 750, MSC 7985
Bethesda MD 20892-7985
Phone: 301-496-9838
Fax: 301-496-9839
Email: oba@od.nih.gov

In the case of any adverse event involving hazardous biological materials, an IBC meeting will be called at the earliest date possible. The IBC's review of adverse event reports will focus on reasons for exposures and any risks that may have changed. The IBC will determine if there is a need to revise the registration and whether approval should continue. In addition, increased monitoring, training and safety measures may be required.

BIOHAZARDOUS WASTE

The EVMS Environmental Health and Safety Department has prepared these guidelines to assist laboratory personnel in safely and legally managing biohazardous waste. Federal, State and local laws and regulations govern how biohazardous waste must be managed. These guidelines apply to *anyone* who generates biohazardous waste.

What is biohazardous waste?

<i>Biohazardous Waste</i>	Solid waste (including animal carcasses) contaminated with infectious agents known to cause human illness and not contaminated with radioactive materials or hazardous chemicals.
<i>Biohazardous Sharps Waste</i>	Devices capable of cutting or piercing that are contaminated with biohazardous material. Includes: <ul style="list-style-type: none"> • Contaminated hypodermic needles • Scalpels • Razor blades • Contaminated pipette tips and micropipette tips • Broken microscope slides and Pasteur pipettes

What is NOT biohazardous waste?

<i>Radioactive Waste</i>	Or mixtures that contain radioactive components
<i>Chemical Waste</i>	Or mixtures of chemical and biohazardous waste
<i>BioWaste</i>	Not biohazardous, chemical or radioactive waste, but has the appearance of laboratory waste. Examples include plants, bacteria and viruses used in food production.
<i>Broken Glass</i>	Must not be contaminated with biohazardous, chemical, or radioactive wastes.

Segregating Waste

Segregating wastes at the point of generation is one of the most important steps in properly managing lab waste. Follow these rules to ensure compliance:

- Whenever possible, *do not* combine biohazardous waste with chemical or radioactive waste.
- Separate sharps from other waste by placing them in a puncture-resistant container.
- If different types of wastes are mixed, manage in the following manner (in descending order):
Radioactive → Chemical → Biohazardous → General Trash
- If safe to do so, you may decontaminate the biohazardous component and manage the other waste(s) as appropriate.

Containing Biohazardous Waste

As with all laboratory waste, biohazardous waste must be placed in a properly labeled container as soon as it is generated. Be mindful that the waste generated will be handled by other individuals, such as EH&S staff and medical waste contractors. Follow these steps to safely and legally contain biohazardous wastes.

Sharps Waste

- Place sharps waste in puncture-resistant containers labeled “*Sharps*” or in commercial sharps containers.
- Keep sharps containers upright and next to the work area (i.e. in the biosafety cabinet while working).
- Do not overfill sharps containers. *Tape sharps containers closed when they are 2/3 full.*

Biohazardous Waste (“Red Bag Waste”)

Place solid biohazardous waste in bags that meet the following requirements:

- Red or clear (with imprinted biohazard warning)
- Imprinted with a biohazard symbol and the words “Biohazardous Waste”
- Mil thickness of at least 1.5 mil
- Imprinted with UN number on the bags, on the box, or in the invoice
- Contained at all times in a secondary container

Secondary containers must:

- Have a biohazard bag lining the container
- Be rigid, leak-proof, puncture resistant
- Have a tight fitting lid
- Be labeled with a biohazard symbol on at least 2 sides
- Keep secondary containers closed unless waste is being added
- Do not use cloth hampers or wire racks as secondary container

Laboratories generating sufficient amounts of biohazardous waste are supplied with red bags and secondary containers once an account has been established with the current medical waste disposal contractor. It is up to the PI to establish an account with the disposal company; contact Materials Management (446 – 5224) for contract and purchasing information. If the laboratory does not generate biohazardous waste on a regular basis, consult with EH&S to determine whether a laboratory account is prudent.

Biohazardous Waste Pick-up

Biohazardous waste is picked up in two ways.

1. If the laboratory has an account with a medical waste disposal contractor:
 - Contact disposal contractor to schedule a pick-up when the secondary container is 2/3 full
 - Tie the open end of the red bag in a knot, then tape the top of the secondary container closed

2. If the laboratory does not have an account with the disposal company:
 - If the laboratory generates sufficient amounts of biohazardous waste (as determined by EH&S), the laboratory will be instructed to obtain an account with the disposal contractor and set up a pick-up.
 - If the laboratory does not generate sufficient amounts of biohazardous waste (as determined by EH&S), contact EH&S for a biohazardous waste pick-up.

Decontaminating Biohazardous Waste Containers

The law requires all secondary containers be kept clean and in good repair. These include laboratory and waste contractor supplied containers at pick-up locations.

If the laboratory utilizes a temporary, re-usable secondary container, this re-usable container must be cleaned before a new bag can be placed inside. To clean the re-usable:

- Ensure container is free from encrusted material
- Expose to hot water of at least 82°C (180°F) for a minimum of 15 seconds
or
- Sanitize container by rinsing with or immersing in a one-percent (1%) solution of household bleach or a quaternary ammonium solution (400 ppm active agent) for 10 minutes.

EMERGENCY PROCEDURES

In the event of a spill of biological material, the individual(s) who caused the spill is responsible for its cleanup. Biohazardous and biohazardous waste spills must be cleaned immediately. Any spilled biohazardous waste and all associated contaminated cleanup debris must be handles as biohazardous waste.

To minimize the consequences of any biological materials spill, work should be conducted on plastic-backed liner to absorb spilled material. A standard biological spill kit should also be on hand in case of a spill.

Spill Kits

A standard biological spill kit should include:

- Chlorine bleach (10%) or some other disinfectant
- Package or roll of paper towels
- Red biohazard bags
- Rubber gloves
- Forceps or tongs for picking up broken glass

Spills Inside Biological Safety Cabinets

1. LEAVE BIOLOGICAL SAFETY CABINET TURNED ON
2. While wearing gloves and lab coat, lay down paper towels over entire spill area
3. Spray 10% bleach solution on top of paper towels, going from the outer edge of the spill area to the center
4. Let sit for at least 10 minutes
5. Spray or wipe cabinet walls, work surfaces and equipment with bleach solution. If necessary, flood drain pans and catch basins below work surface with bleach solution and let stand 10 minutes.
6. Remove paper towels and discard into biohazard container. Pick up any associated broken glass and place in sharps container. Drain catch basin into a container.
7. Re-wipe spill area (including exhaust grill and tray) with water, to prevent pitting. Discard, along with gloves, into biohazard container.
8. Wash hands!

Spills Outside of Biological Safety Cabinets

Small Spills (<50mL)

1. Wearing gloves and lab coat, cover the spill with paper towels and gently apply bleach solution on top of paper towels, going from the outer edge of the spill area to the center.
2. Let stand at least 10minutes.
3. Place paper towels in biohazard container. Place any broken glass in sharps container.
4. Re-wipe the spill area with bleach solution or disinfectant. If inside a centrifuge, use bleach solution to disinfect cups and walls. Discard paper towels, along with gloves, into biohazard container.
5. Wash hands!

Large Spills ($\geq 50\text{mL}$)

1. Leave room immediately and close room door
2. Warn others to stay out of spill area to prevent spread of contamination
3. Remove any contaminated clothing and put into biohazard bag for autoclaving later. Scrubs should be available to replace contaminated clothing.
4. Wash hands and exposed skin; inform supervisor about the spill
5. Put on PPE (gloves, lab coat, shoe covers, etc) and assemble clean-up materials
6. Wait *30 minutes* before re-entering the contaminated area to allow dissipation of aerosols
7. Cover spill area with paper towels and gently apply bleach solution on top, going from outer edge of the spill area to the center
8. Let stand 10 minutes
9. Collect all treated material and discard into biohazard container. Pick up any broken glass with forceps and place into sharps container.
10. Re-wipe the spill area with bleach solution or disinfectant
11. Wash hands!

PACKAGING & SHIPPING INFECTIOUS MATERIAL

Packaging and shipping biological and infectious materials are regulated by government agencies and business organizations. The two main agencies involved in infectious substances shipping are the International Air Transport Association ([IATA](#)) and the US Department of Transportation ([DOT](#)). Infectious materials and other dangerous goods must always be transported according to the appropriate regulations. *Before shipping any infectious materials, you must consult with EH&S and complete “Biological & Infectious Materials Shipping” online training on the EVMS BioRAFT website.*

The following definitions and guidelines comply with IATA’s *Dangerous Goods Regulations* and *49 CFR 171-180*.

Definitions

- **Infectious substances** are defined as viable microorganisms, or their toxin, which causes or may cause disease in humans or animals and includes those agents listed in 42 CFR 72.3 and any other agent that causes or may cause severe, disabling or fatal disease. Infectious substance and **Etiologic Agent** are synonymous.
- **Patient specimens** are any human or animal material including, but not limited to, excreta, blood, blood components, tissue and tissue fluids, being shipped for purposes of diagnosis.
- **Biological Products** are products derived from living organisms, which are manufactured and distributed in accordance with the requirements of governmental authorities.
- **Regulated Medical Waste** is waste or reusable material that contains an infectious substance and is generated in the diagnosis, treatment, research or immunization of humans or animals, research pertaining to diagnosis, or treatment or immunization or production of biological products.

Classification

For the purposes of shipping, biological materials fit into one or more of the following categories:

- **Category A infectious substances** – includes agents listed on [IATA Table 3.3.A](#)
- **Category B infectious substances** – includes diagnostic specimens and infectious materials not included in Category A
- **Exempt Human (Animal) Specimen** – patient specimens that have a “minimal likelihood” of containing pathogens
- **Genetically modified organisms and microorganisms** – organisms and microorganisms whose genetic material has been purposely altered
- **Biological products** – materials which are manufactured and packaged in accordance with the requirements of appropriate national authorities and transported for the purposes final packaging or distribution, and use for personal health care by medical professionals or individuals

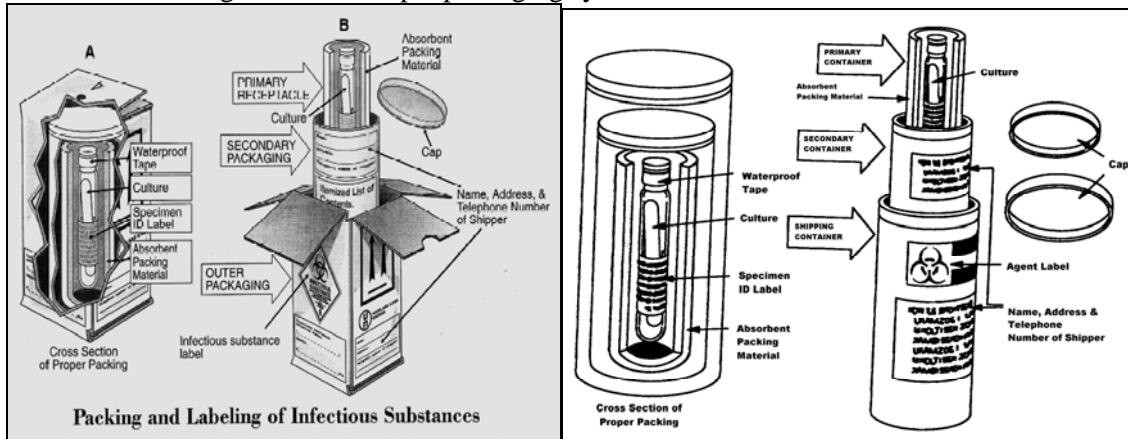
Packaging

All biological materials must be packaged to withstand leakage of contents, shocks, pressure changes and other conditions incident to normal handling and transportation. Contents should not leak to the outside of the shipping container, even if leakage of the primary container occurs.

Specific packaging requirements are determined by which shipping category the biological material(s) fall in to. Generally, a triple packaging system is employed with

- A watertight **primary receptacle**
- A watertight **secondary receptacle**
- **Absorbent material** in between primary and secondary packagings (enough to absorb the entire contents of the primary receptacle)
- A rigid **outer packaging** of adequate strength for its use

Below are two diagrams of the triple packaging system.



Category A Packaging Sample

Category B Packaging Sample

Packaging Volume < 50mL

- Place biological material in a securely closed, water tight primary container (test tube, vial, etc.).
- Wrap primary container in absorbent material (enough to absorb the entire amount of biological material).
- Enclose the primary container and absorbent in a secondary, durable, watertight container. (Several primary containers may be enclosed in a single secondary container as long as the total volume of material does not exceed 50mL.
- Enclose the set of primary and secondary receptacles in an outer shipping container constructed of fiberboard, cardboard, wood or other material of equal strength.
- If packaging with dry ice, see *Packaging with Dry Ice* section below.

Packaging Volume ≥ 50mL

- Follow the requirements for lesser volumes outlined above
- Place shock absorbent material at top, bottom and sides between the secondary and outer shipping containers. (This material should at least equal the amount of absorbent materials placed between the primary and secondary container).
- Ensure single primary receptacles contain no more than 1L of material; however, two or more primary receptacles (combined volumes not exceeding 1L) may be placed in a single secondary container. The maximum amount of etiologic agent that may be enclosed within a single outer shipping container must not exceed 4L.

Packaging with Dry Ice

- Place dry ice between the secondary and outside containers.
- Place shock absorbent material to prevent the secondary container from becoming loose inside the outer container as the dry ice sublimates.
- Outer receptacle must be designed and constructed to permit the release of carbon dioxide gas and to prevent a build-up of pressure that could rupture the packaging.

Marking and Labeling

Labeling for all packages must include on the outside container:

- Sender and recipient's full name and address
- Proper shipping name and UN ID number of the shipped material
- Proper labels (dependent on classification of shipped materials)
- "*CARGO AIRCRAFT ONLY*" label (if amount of material exceeds 50mL or 50g)
- Class 9 label (if using dry ice)

More markings and labeling may have to be employed for packages, depending on the classification of the material being shipped, such as 24-hour emergency numbers. Consult the Infectious Materials Shipping Training presentation, available on Blackboard, or consult with EH&S.

SHIPPING AND TRANSPORTATION

Registered Mail (or Equivalent)

Per 42 CFR 72.3(f), the following etiologic agents must be shipped using registered mail or an equivalent system, which provides the sender with immediate notification of receipt:

- *Coccidioides immitis*
- Ebola virus
- *Francisella* (Pasteurella) tularensis
- Hemorrhagic fever agents including, but not limited to, Crimean hemorrhagic fever (Congo), Junín, Machupo viruses and Korean hemorrhagic fever viruses
- Herpesvirus simiae (B virus)
- *Histoplasma capsulatum*
- Lassa virus
- Marburg virus
- *Pseudomonas mallei*
- *Pseudomonas pseudomallei*
- Tick-borne encephalitis virus complex including, but not limited to, Russian spring-summer encephalitis, Kyasanur forest disease, Omsk Hemorrhagic fever and Central European encephalitis viruses, Variola minor and Variola major
- Variola major, Variola minor and Whitepox viruses
- *Yersinia* (*Pateurella*) *pestis*

Commercial Carriers

For shipments of biological materials, internationally or domestically, follow the International Air Transport Association (IATA) *Dangerous Goods Regulations*. Within the IATA regulations are the specific carrier's (FedEx, UPS, DHL, etc.) requirements. (Receipt of shipment notice is not required since the shipment is traceable through specific carrier). Follow the specific carrier's requirements in the IATA regulations and contact the carrier's dangerous goods agent prior to shipment for any additional packaging and labeling requirements.

Damaged Packages

Do not accept any leaking or damaged packages. When evidence of leakage or any other damage to packages bearing an Infectious Agents/Biomedical Material label is discovered, the carrier must promptly isolate the package and notify the Director for the Centers for Disease Control and Prevention (CDC) at

1600 Clifton Road NE
Atlanta GA 30333
Telephone: (404) 633-5313

Notice of Delivery Failure

In the event that a package sent by Eastern Virginia Medical School is not received by the recipient within 5 days following the anticipated delivery of the package, the sender must notify

Biosafety Branch - Centers for Disease Control and Prevention (CDC)
Telephone: (404) 639-7233

Importation/Exportation of Etiologic Agents

Importation of infectious agents, etiologic agents and vectors that may contain these agents is governed by federal regulation. In general, an importation permit is required for any infectious agent known to cause disease to man. This includes, but is not limited to, bacteria, viruses, rickettsia, parasites, yeasts and molds. In some instances, an agent that is suspected of causing human disease also requires a permit.

Any import coming within the above provisions are controlled by the U.S. Public Health Service and will not be released from custody prior to receipt by the District Director of U.S. Customs of a permit issued by the Director of the CDC (42 CFR 71.54). "Etiologic Agent Importation Permits" are issued by the CDC only to the importer, who must be located in the United States.

To obtain an Etiologic Agent Importation Permit, an application must be submitted to the CDC. Applications are found at <http://www.cdc.gov/od/eaipp/importApplication/> or by contacting the CDC directly. Applications can be submitted through both mail and fax. The contact information for CDC Import Permit Program is:

Centers for Disease Control and Prevention
1600 Clifton Road NE
Mailstop A-46
Atlanta GA 30333
Phone: 404-718-2077
Fax: 404-718-2093

PIs transferring or receiving select biological agents of human disease, Select Agents, must be registered with the CDC and each transfer of a select agent must be documented. More information on the CDC's Select Agent Program can be found at <http://www.selectagents.gov/index.html>.

Other Permits

U.S Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) permits are required for the importation or domestic shipping of infectious agents of livestock, poultry and other animal diseases and any materials that might contain these agents. Information for APHIS permits is found at

Animals: http://www.aphis.usda.gov/import_export/animals/animal_import/animal_imports.shtml

Plants: http://www.aphis.usda.gov/import_export/plants/plant_imports/index.shtml

Export of infectious materials may require license from the Department of Commerce (DoC). Exporters of a wide variety of etiologic agents of human, plant and animal diseases, including genetic material and products that might be used for culture of large amounts of agents will require an export license. Information for exporting may be obtained from the DoC Bureau of Industry and Security at <http://www.bis.doc.gov/>.

APPENDIX A: SELECT AGENTS AND TOXINS

HHS AND USDA SELECT AGENTS AND TOXINS 7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73

HHS SELECT AGENTS AND TOXINS

Abrin
Botulinum neurotoxins*
Botulinum neurotoxin producing species of *Clostridium**
Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence X₁CCX₂PACGX₃X₄X₅X₆CX₇)¹
Coxiella burnetii
Crimean-Congo haemorrhagic fever virus
Diacetoxyscirpenol
Eastern Equine Encephalitis virus³
Ebola virus*
*Francisella tularensis**
Lassa fever virus
Lujo virus
Marburg virus*
Monkeypox virus³
Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
Ricin
Rickettsia prowazekii
SARS-associated coronavirus (SARS-CoV)
Saxitoxin
South American Haemorrhagic Fever viruses:
 Chapare
 Guanarito
 Junin
 Machupo
 Sabia
Staphylococcal enterotoxins A,B,C,D,E subtypes
T-2 toxin
Tetrodotoxin
Tick-borne encephalitis complex (flavi) viruses:
 Far Eastern subtype
 Siberian subtype
Kyasanur Forest disease virus
Omsk hemorrhagic fever virus
Variola major virus (Smallpox virus)*
Variola minor virus (Alastrim)*
*Yersinia pestis**

OVERLAP SELECT AGENTS AND TOXINS

Bacillus anthracis *
Bacillus anthracis Pasteur strain
Brucella abortus
Brucella melitensis
Brucella suis
*Burkholderia mallei**
*Burkholderia pseudomallei**
Hendra virus
Nipah virus
Rift Valley fever virus
Venezuelan equine encephalitis virus³

USDA SELECT AGENTS AND TOXINS

African horse sickness virus
African swine fever virus
Avian influenza virus³
Classical swine fever virus
Foot-and-mouth disease virus*
Goat pox virus
Lumpy skin disease virus
*Mycoplasma capricolum*³
*Mycoplasma mycoides*³
Newcastle disease virus^{2,3}
Peste des petits ruminants virus
Rinderpest virus*
Sheep pox virus
Swine vesicular disease virus

USDA PLANT PROTECTION AND QUARANTINE (PPQ) SELECT AGENTS AND TOXINS

Peronosclerospora philippinensis (*Peronosclerospora sacchari*)
Phoma glycinicola (formerly *Pyrenochaeta glycinis*)
Ralstonia solanacearum
Rathayibacter toxicus
Sclerophthora rayssiae
Synchytrium endobioticum
Xanthomonas oryzae

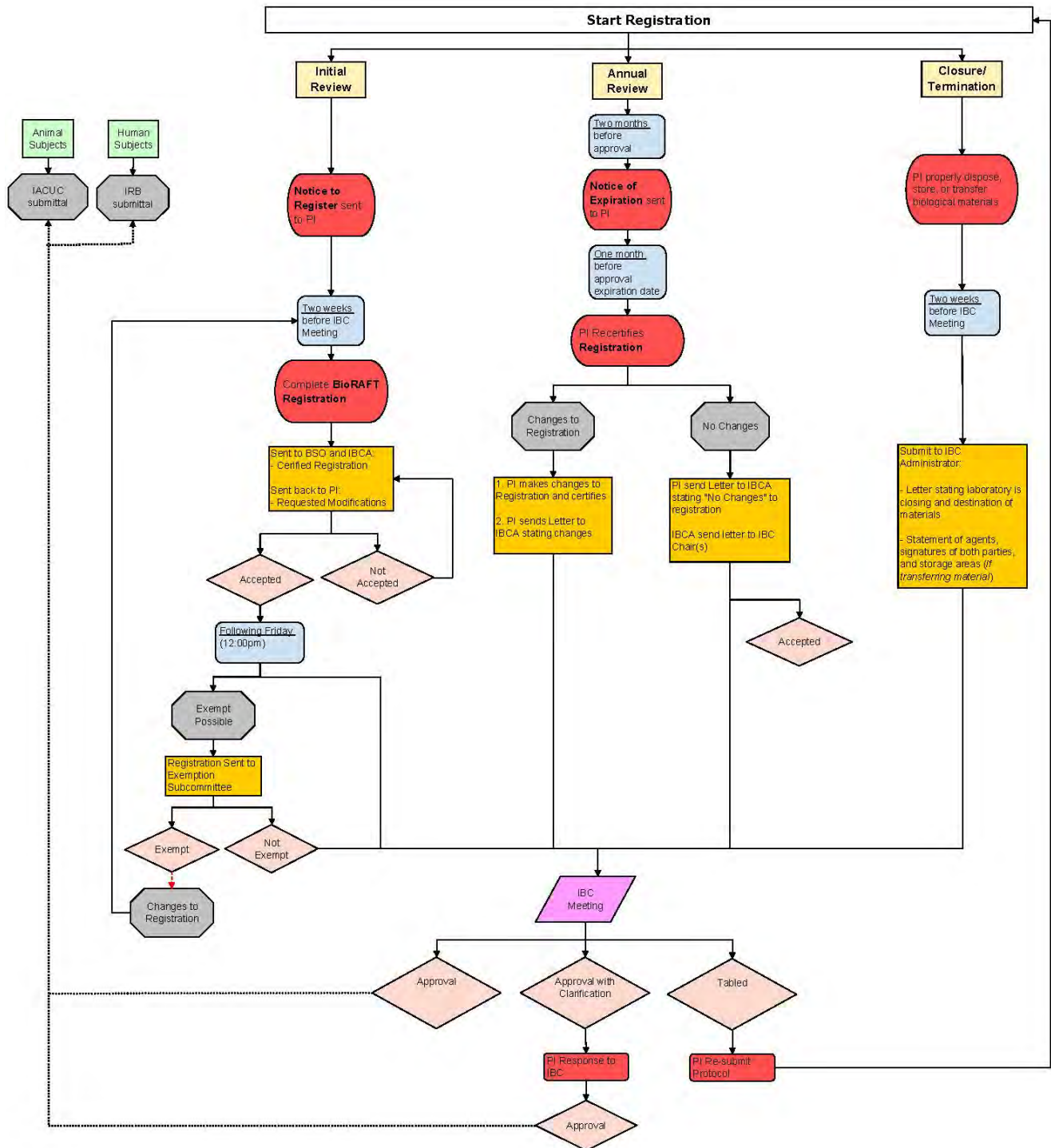
*Denotes Tier 1 Agent

¹ C = Cysteine residues are all present as disulfides, with the 1st and 3rd Cysteine, and the 2nd and 4th Cysteine forming specific disulfide bridges; The consensus sequence includes known toxins α-MI and α-GI (shown above) as well as α-GIA, Ac1.1a, α-CnIA, α-CnIB; X₁ = any amino acid(s) or Des-X; X₂ = Asparagine or Histidine; P = Proline; A = Alanine; G = Glycine; X₃ = Arginine or Lysine; X₄ = Asparagine, Histidine, Lysine, Arginine, Tyrosine, Phenylalanine or Tryptophan; X₅ = Tyrosine, Phenylalanine, or Tryptophan; X₆ = Serine, Threonine, Glutamate, Aspartate, Glutamine, or Asparagine; X₇ = Any amino acid(s) or Des X and; "Des X" = "an amino acid does not have to be present at this position." For example if a peptide sequence were XCCHPA then the related peptide CCHPA would be designated as Des-X.

² A virulent Newcastle disease virus (avian paramyxovirus serotype 2) has an intracerebral pathogenicity index in day-old chicks (*Gallus gallus*) of 0.7 or greater or has an amino acid sequence at the fusion (F) protein cleavage site that is consistent with virulent strains of Newcastle disease virus. A failure to detect a cleavage site that is consistent with virulent strains does not confirm the absence of a virulent virus.

³ Select agents that meet any of the following criteria are excluded from the requirements of this part: Any low pathogenic strains of avian influenza virus, South American genotype of eastern equine encephalitis virus, west African clade of Monkeypox viruses, any strain of Newcastle disease virus which does not meet the criteria for virulent Newcastle disease virus, all subspecies *Mycoplasma capricolum* except subspecies *capripneumoniae* (contagious caprine pleuropneumonia), all subspecies *Mycoplasma mycoides* except subspecies *mycoides* small colony (Mmm SC) (contagious bovine pleuropneumonia), any subtypes of Venezuelan equine encephalitis virus except for Subtypes IAB or IC, and Vesicular stomatitis virus (exotic); Indiana subtypes VSV-IN2, VSV-IN3, provided that the individual or entity can verify that the agent is within the exclusion category.

APPENDIX B: IBC SUBMITTAL PROCESS



APPENDIX C: CONTAMINATED LINENS POLICY

Background

Although soiled laundry may harbor large numbers of pathogenic microorganisms, the risk of actual disease transmission from soiled laundry is negligible. In general, soiled laundry should be handled as little as possible and with minimum agitation to prevent gross microbial contamination of the air and of persons handling the laundry.

For Biological Safety Level 2 facilities, including clinical areas, decontamination of laundry prior to transport to a commercial laundry facility is not necessary. Commercial laundry facilities use water temperatures of at least 160°F and 50-150 ppm of chlorine bleach to remove significant quantities of microorganisms from grossly contaminated laundry.

This Standard Operating Procedure for blood or OPIM contaminated laundry is written in accordance with guidelines of the Centers for Disease Control and Prevention ([CDC](#)), the National Institutes of Health ([NIH](#)), the Occupational Safety and Health Administration ([OSHA](#)), and the *Biosafety in the Microbiological and Biomedical Laboratories, 5th edition* ([BMBL](#)).

Definitions

- Biological Safety Level 2 Facilities (BSL-2)

BSL-2 facilities are suitable for work involving agents or materials that pose moderate hazards to personnel and the environment. Human cells, tissues, and body fluids are considered BSL-2 materials. Standard microbiological practices should be observed when handling BSL-2 materials, including utilizing proper PPE.

- Laundry

Any article made of cloth or cloth-like material, including but not limited to bed sheets, lab coats, street clothing, and scrubs.

- OPIM

Other Potentially Infectious Materials. This includes but is not limited to sputum, semen, cerebrospinal fluid, and any body fluid contaminated with blood.

- PPE

Personal Protective Equipment. Specialized clothing or equipment worn by employees for protection against health and safety hazards. Includes gloves, lab coats, aprons, goggles, and respirators.

- Soiled

In this document, refers to contamination of laundry by blood and/or OPIM.

Procedures

In the event laundry, whether in the laboratory or clinical setting, becomes soiled, the following procedures shall be followed:

1. DO NOT sort, rinse, or soak laundry in the location of use. Employees are not to take contaminated laundry home for any reason.
2. While wearing appropriate PPE, remove the contaminated piece of laundry.
3. Place laundry in an appropriately labeled bag or container at the location where it was used.
 - a. Bag or container must be leak-proof and labeled with the Biohazard symbol.
 - b. *Note:* Commercial laundry bags from a contracted vendor are appropriately labeled and are coated in a liquid-resistant material to prevent leaks.
4. If laundry is wet and presents a reasonable likelihood of leakage to the exterior of a single container, it must be placed in a second container (or “double-bagged”).
5. After all soiled laundry has been placed in the appropriate container(s), dispose of used PPE in the medical waste container.
6. All laundry shall be sent to a commercial laundry facility for decontamination and cleaning at no expense to the employee.

References

- *Laundry: Washing Infected Material* by CDC. <http://www.cdc.gov/HAI/prevent/laundry.html>
- *Biosafety in the Microbiological and Biomedical Laboratories, 5th ed.* <http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>

APPENDIX D: EMERGENCY FREEZER POLICY

Introduction

The Environmental Health and Safety Department is dedicated to assisting EVMS research personnel in the safety and efficiency of their work. As part of this endeavor, EH&S is responsible for an Emergency Freezer Policy.

The Emergency Freezers are utilized as *temporary* cold storage equipment in cases of an individual laboratory's emergency need. This policy will provide

- Standard Operating Procedures for the utilization of the Emergency Freezers
- Emergency procedures in cases of equipment failure
- Contact information for those responsible for the Emergency Freezers

Scope

This storage freezer policy has been established by the Eastern Virginia Medical School Environmental Health & Safety Department in order to provide the clinical and research segments safe and secure cold storage in emergency circumstances.

These shared freezers will be maintained and operated in accordance with the procedures outlined in this policy. Each Principal Investigator (PI) and research laboratory that utilizes these freezers should be familiar with this information and is responsible for the proper implementation of these policies. Utilization of the freezer implies agreement to abiding by this policy.

Materials

There are restrictions on the types of materials acceptable for storage in the Emergency Freezers. The following materials will *not* be accepted for storage:

- Radioactive materials
- Improperly contained materials
- Materials from non-research related laboratories and departments
- Materials deemed unsafe by the Environmental Health & Safety Department

EH&S reserves the right to refuse the storage of any materials for safety or security reasons. Unauthorized storage of materials in the freezers is not permitted and will result in the disposal of the materials by EH&S.

Responsibilities

Environmental Health & Safety

EH&S is responsible for access control of the Emergency Freezers. EH&S is also responsible for updating and maintaining a contents inventory for each Emergency Freezer.

EVMS Police & Public Safety

EVMS Police Dispatch will monitor the freezer alarms and notify the responsible parties of problems.

Physical Facilities

A Preventative Maintenance Contract on the Emergency Freezers will be initiated and maintained between the EVMS Physical Facilities Department and an outside service provider. Repairs to the freezers will also be the responsibility of the Physical Facilities Department.

Procedures for Material Storage

The following procedures will be used when short-term cold storage is deemed necessary.

- The PI (or their designee) must contact EH&S to request storage of their materials. Contact can be made by phone, email or in the EH&S Office (Lewis Hall 2132).
- EH&S personnel will unlock the Emergency Freezer for the PI (or their designee).
- All containers stored in the Emergency Freezer must be properly labeled with the PIs name and the contents of the container. Unlabeled materials will be discarded by EH&S.
- The PI (or their designee) must complete, sign and date a Description of Emergency Freezer Materials form. A *Retention Date* will also be recorded on the form informing the PI of the last date of storage for the material. This form will be retained by EH&S and will be stored for 6 months after the removal of the materials from the freezer.

Duration of Storage

Material may be stored in the emergency freezers for *no more than 60 calendar days* from the storage date indicated on the Description of Materials Form. Approximately 10 days before the 60-day deadline, the PI will be contacted to remove their materials from the Emergency Freezer.

If the PI does not remove their material after notification and makes no plans with EH&S, the materials will be returned to their laboratory by EH&S.

Materials Tracking

The materials contained in the Emergency Freezers will be tracked by EH&S. Each freezer will be entered into the BioRAFT Management System with their contents continuously updated. A hard-copy of the contents will be updated monthly and kept in the EH&S Office as well.

Emergency Procedures

Emergency Freezer Alarm

The Emergency Freezer Alarms are set to contact EH&S Police and Public Safety Dispatch in the event of temperature or power interruption.

In the event of temperature-control failure for more than 36 hours, EH&S will have the emergency freezer repair initiated or will move the contents to another freezer. EH&S reserves the right to extend the 36 hour window based on a risk assessment of unforeseen circumstances (such as in cases of hurricanes, floods, etc.).

Laboratory Freezer Malfunction

In the instance of a research laboratory malfunction, approved materials may be stored in the Emergency Freezer.

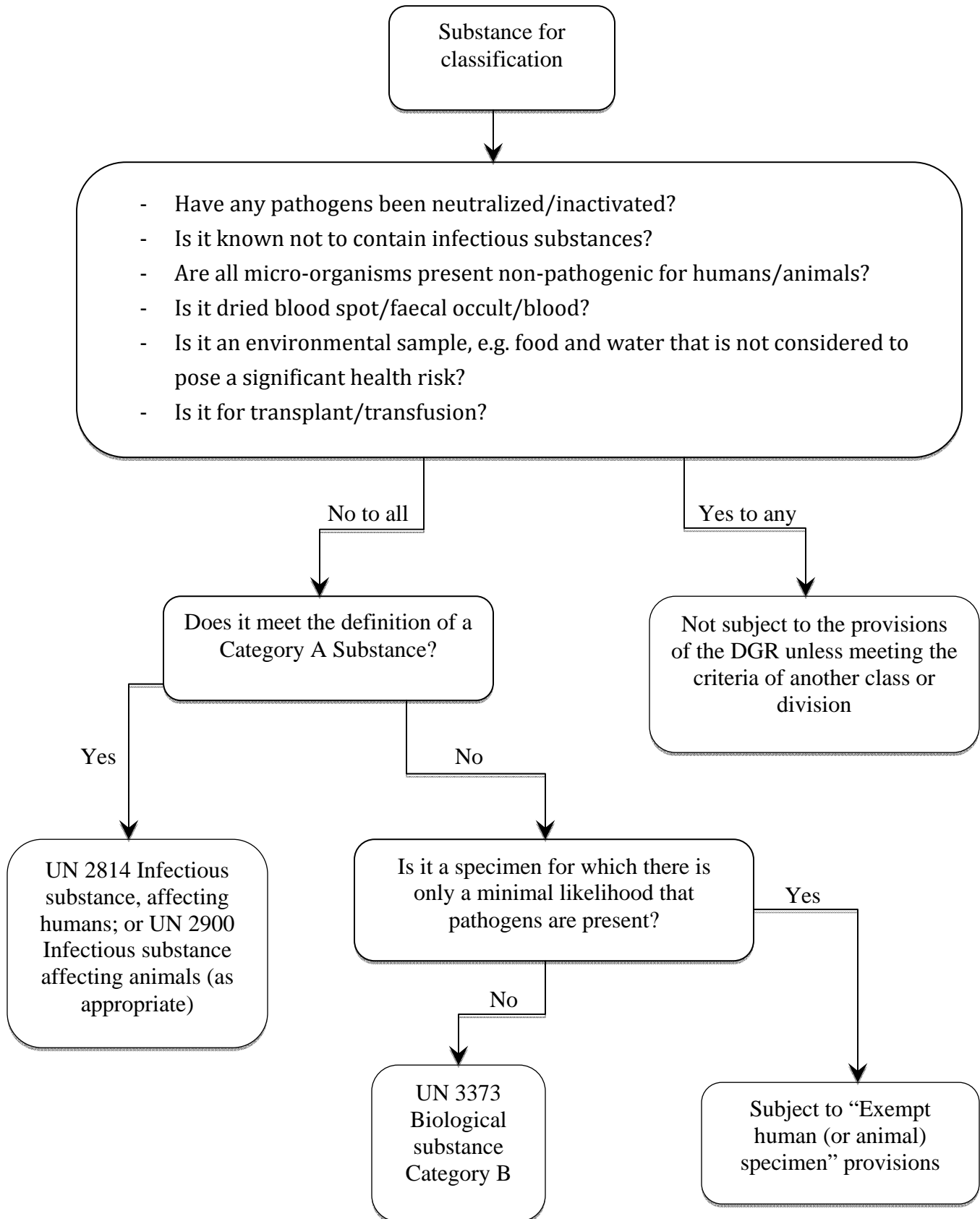
EH&S will have up to 24 hours to respond to a request of material storage from a laboratory. Until a response from EH&S is given, the research laboratory should keep the malfunctioning freezer's doors closed in order to conserve its temperature.

For an emergency after normal business hours, a lab must communicate with Police Dispatch (446-5199) regarding the need for material storage. Dispatch will then contact EH&S personnel to coordinate unlocking the Emergency Freezers.

In severe cases of storage space needed (such as long-term power failures), EH&S recommends the use of Dry Ice from an outside vendor until power is restored.

APPENDIX E: IATA INFECTIOUS SHIPPING GUIDE

Classification Flowchart



APPENDIX F: IATA TABLE 3.3.A

Indicative Examples of Infectious Substances Included in Category A in Any Form Unless Otherwise Indicated
(3.3.2)

UN Number and Proper Shipping Name	Micro-organism
UN 2814 Infectious substance affecting humans	<i>Bacillus anthracis</i> (cultures only) <i>Brucella abortus</i> (cultures only) <i>Brucella melitensis</i> (cultures only) <i>Brucella suis</i> (cultures only) <i>Burkholderia mallei</i> - <i>Pseudomonas mallei</i> -Glanders (cultures only) <i>Burkholderia pseudomallei</i> - <i>Pseudomonas pseudomallei</i> (cultures only) <i>Chlamydia psittaci</i> -avian strains (cultures only) <i>Clostridium botulinum</i> (cultures only) <i>Coccidioides immitis</i> (cultures only) <i>Coxiella burnetii</i> (cultures only) Crimean-Congo hemorrhagic fever virus Dengue virus (cultures only) Eastern equine encephalitis virus (cultures only) <i>Escherichia coli</i> , verotoxigenic (cultures only) Ebola virus Flexal virus <i>Francisella tularensis</i> (cultures only) Guanarito virus Hantaan virus Hantavirus causing hemorrhagic fever with renal syndrome Hendra virus Hepatitis B virus (cultures only) Herpes B virus (cultures only) Human immunodeficiency virus (cultures only) Highly pathogenic avian influenza virus (cultures only) Japanese Encephalitis virus (cultures only) Junin virus Kyasanur Forest disease virus Lassa virus Machupo virus Marburg virus Monkeypox virus <i>Mycobacterium tuberculosis</i> (cultures only) Nipah virus Omsk hemorrhagic fever virus <i>Poliovirus</i> (cultures only) Rabies virus (cultures only)

UN Number and Proper Shipping Name	Micro-organism
	<p><i>Rickettsia prowazekii</i> (cultures only) <i>Rickettsia rickettsia</i> (cultures only) Rift Valley fever (cultures only) <i>Russian spring-summer encephalitis virus</i> (cultures only) Sabia virus <i>Shigella dysenteriae type 1</i> (cultures only) <i>Tick-borne encephalitis virus</i> (cultures only) Variola virus Venezuelan equine encephalitis virus (cultures only) <i>West Nile virus</i> (cultures only) <i>Yellow fever virus</i> (cultures only) <i>Yersinia pestis</i> (cultures only)</p>
UN 2900 Infectious substances affecting animals	<p>African swine fever virus (cultures only) Avian paramyxovirus Type 1-Velogenic Newcastle disease virus (cultures only) Classical swine fever virus (cultures only) Foot and mouth disease virus (cultures only) Lumpy skin disease virus (cultures only) <i>Mycoplasma mycoides</i>-Contagious bovine pleuropneumonia (cultures only) Peste des petits ruminants virus (cultures only) Rinderpest virus (cultures only) Sheep-pox virus (cultures only) Goatpox virus (cultures only) Swine vesicular disease virus (cultures only) Vesicular stomatitis virus (cultures only)</p>

APPENDIX G: LENTIVIRUS VECTOR SUPPLEMENT

INTRODUCTION

Lentiviral vector constructs have proven to be very productive in terms of transduction due to their ability to infect both replicating and non-replicating cells, including stem cells. Lentiviral vectors are becoming the vectors of choice for short-interfering RNA (siRNA) delivery. The increased use of lentiviral vector constructs in established and novel research applications makes it essential for laboratory workers to understand and protect themselves from related exposure hazards.

The purpose of this document is to provide principal investigators (PIs), laboratory technicians, and IBC members with information regarding risk assessment, proper work methods, containment levels, suitable engineering controls options, and personal protective equipment for development of research protocols that effectively reduce the risk of occupational exposure to lentivirus vector.

BACKGROUND

Third generation lentiviral vectors are usually created in a transient transfection system in which a cell line is transfected with multiple plasmid expression systems. These include:

- (1) The transfer vector plasmid (portions of the HIV provirus)
- (2) The [packaging plasmid or construct](#)
- (3) A plasmid with the heterologous envelop gene (*env*) of a different virus

The multiple plasmid components of the vector are put into a packaging cell which is then inserted into the HIV shell. These so-called split-configuration packaging cell lines require multiple recombination events to generate replication competent lentiviruses (RCLs).

A number of features are incorporated in the latest vector designs to enhance biosafety. These features include:

- Transgene: Non-oncogene; Vector and packaging components are distributed onto multiple plasmids that contain very little, if any, overlap or homology
- Deletion of viral genes (number of HIV genes is reduced to three (gag, pol and rev))
- Non-native viral *env* used in packaging system
- No expression of Tat (essential for lentiviral replication)
- Deletion in the 3' LTR that results in "self-inactivation"

RISK FACTORS

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

- Potential for generation of replication-competent lentivirus (RCL)
- 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.

MODES OF TRANSMISSION

Lentivirus may be transmitted by:

- Penetration of the skin via puncture or absorption (through scratches, cuts, abrasions, dermatitis or other lesions)
- Mucous membrane exposure of the eyes, nose, and mouth

GENERAL CRITERIA FOR RISK ASSESSMENT

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

The potential for the generation of RCL from HIV-1 based lentivirus vectors depends upon several parameters:

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

CONTAINMENT

Generally, BSL-2 or BSL-2+ (enhanced BSL-2 containment including BSL-3 work practices and PPE) are often appropriate in the laboratory setting for higher generation lentivirus vectors with multiple safety features (four or more plasmids). Enhanced BSL-2 may include in addition to sharps safety, the use of PPE intended to reduce the potential for mucosal exposure to the vector (such as an N-95 respirator), especially if working with high volumes (>10L).

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 flash containing a 10% bleach solution.

PERSONAL PROTECTIVE EQUIPMENT

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

- Gloves (consider double-gloving depending on the procedures being performed)
- Lab Coat
- Goggles
- Face shield

DECONTAMINATION PROCEDURES

All materials that have come into contact with lentiviral vectors should be disinfected using a 10% bleach solution before disposal. Additionally, all work surfaces must be disinfected with a 10% solution of bleach once work is completed and at the end of the work day. (*Note: A 10 minute contact time is required for decontamination*)

WASTE DISPOSAL PROCEDURES

Non-Sharp Waste - All cultures, stocks, and cell culture materials must be disinfected and autoclaved prior to being disposed of into a double red bag-lined biohazard box.

Sharps Waste - All needles, syringes, razors, scalpels, Pasteur pipettes and pipette tips must be disposed of in an approved, puncture resistant sharps container. Sharps containers must not filled more than 2/3 of their capacity.

ANIMAL STUDIES

Some animals cannot support replication of infectious HIV-1; as a result, the potential for shedding of RCL is very low. It may be prudent to consider the biosafety issues associated with animal husbandry and housing after the initial injection separately from the inoculation itself, which may pose sharps hazards.

NIH recommends that the initial delivery of vector should be performed under BSL-2. It may be permissible to reduce the containment level at some point following vector delivery. An example is as follows: if there is no expectation of infection, the site of inoculation has been thoroughly cleansed, and the bedding changed, it may be acceptable to consider reducing containment from BSL-2 to BSL-1 within a few days (this time period is to be determined by the IBC and usually ranges between 1-7 days).

Stereostatic injections that require equipment that cannot fit in a biosafety cabinet, need to be taken into consideration during the risk assessment. BSL-2 + with stress on N-95 respirators may be suitable in this situation.

An important consideration is animals that have been grafted with human cells that are permissive for HIV-1 replication. These animals may require a higher level of containment.

*This document must be stored in the lab as a supplement to the lab-specific biosafety manual and it will be checked at the annual Environmental Health and Safety lab bio inspection. The vector-specific SOPs describing lentiviral work must be approved by IBC before lentivirus is used in the lab.

*The Principal Investigator is responsible for ensuring all lab personnel read this document and understand the information contained herein.

REFERENCES

3. *Biosafety Considerations for Research with Lentiviral Vectors*. National Institutes of Health – Office of Biotechnology Activities. http://oba.od.nih.gov/rdna_rac/rac_guidance_lentivirus.html
4. *Biosafety in the Microbiological and Biomedical Laboratories, 5th ed.* <http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>
5. *NIH Guidelines for Research Involving Recombinant DNA molecules*. National Institutes of Health – Office of Biotechnology Activities. http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

*This page must be signed by lab personnel and will be checked during annual lab inspections by EH&S

I am aware of the EVMS guidelines for working with lentivirus in the lab and my lab-specific Standard Operating Procedures involving lentiviral work.

Name	Signature	Date
_____	_____	_____
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ABBREVIATIONS

APHIS	Animal and Plant Health Inspection Service
BBP	Bloodborne Pathogens program
BMBL	<i>Biosafety in Microbiological and Biomedical Laboratories</i> , 5 th edition (CDC/NIH)
BSC	Biological Safety Cabinet
BSL	Biological Safety Level
BSO	Biological Safety Officer
CDC	Centers for Disease Control and Prevention
DEQ	Virginia Department of Environmental Quality
DHHS	Department of Health and Human Services
DoC	U.S. Department of Commerce
EH&S	Environmental Health and Safety Department
EVMS	Eastern Virginia Medical School
IBC	Institutional Biosafety Committee
IBCA	Institutional Biosafety Committee Administrator
IRB	Institutional Review Board
NIH	National Institute of Health
OPIM	Other potentially infectious materials
OSHA	Occupational Safety and Health Administration
PI	Principal Investigator
PPE	Personal Protective Equipment
rDNA	Recombinant DNA
USDA	United States Department of Agriculture
USPHS	United States Public Health Service