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<b>Submitted by:</b> Darren R. Moss	<b>Title:</b> Biological Safety Manager	<b>Dept./Area:</b> EHS

**OFFICE OF ENVIRONMENTAL HEALTH AND SAFETY  
BIOLOGICAL SAFETY MANUAL**

## Overview

Biological safety or biosafety is the application of knowledge, techniques and equipment to prevent personal, laboratory and environmental exposure to potentially infectious agents or other biohazards. Biosafety defines the containment conditions under which these materials can be safely manipulated. The objective of containment is to confine biohazards and to reduce the potential exposure of the laboratory worker, persons outside of the laboratory, and the environment to biohazardous agents. It can be accomplished through the following means:

Primary Containment - the protection of personnel and the immediate laboratory environment through good microbiological technique (laboratory practice) and the use of appropriate safety equipment.

Secondary Containment - the protection of the environment external to the laboratory from exposure to biohazardous materials or other biohazards through a combination of facility design and operational practices.

This manual was established to provide guidance in accomplishing the following goals:

Protect personnel from exposure to infectious agents;

Prevent environmental contamination;

Provide an environment for high quality research while maintaining a safe work place, and;

Comply with applicable federal, state and local requirements.

### REQUIREMENTS

It is the employee's right to have access to information about the known physical and health hazards of potentially infectious and hazardous materials in his/her work areas and to receive adequate training to work safely with or around these substances.

The Biosafety Manual will be readily available to employees through their Principle Investigator or Primary Supervisor and is accessible from the University of Alabama Environmental Health and Safety Office Web Site: [ehs.ua.edu](http://ehs.ua.edu)

### REVIEW / REVISION

The Biosafety Manual shall be reviewed and updated at least annually and whenever necessary to reflect new or modified tasks and procedures and to reflect new or revised procedures.

All change requests to this manual should be submitted to the Biosafety Manager:  
Darren Moss, [dross@fa.ua.edu](mailto:dross@fa.ua.edu)

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# I. BIOSAFETY PROGRAM

## A. INTRODUCTION

This Biological Safety Manual has been developed by the Office of Environmental Health and Safety (EHS) to reflect the policies of the Biological Safety Program. EHS administers the Biological Safety Program on behalf of the administration of the IBC, the VP of Research, and the VP of Financial Affairs. EHS advises the Principle Investigators (PI) and the institution on regulatory requirements and safety best practices. The Biological Safety Program is a part of the Laboratory Safety Program and may also be subject to other programs administered by EHS or The University of Alabama, including Medical Waste Management, the Bloodborne Pathogen Policy, the Chemical Hygiene Plan and the Plan for the Prevention of the Transmission of Tuberculosis.

The Institutional Biological Safety Committee (IBC) was initially established to meet requirements established by the NIH. This committee is now responsible for the oversight of all projects, research and teaching involving any type of biological materials.

In general the handling and manipulation of biological agents and/or recombinant DNA requires the use of various precautionary procedures and measures. This manual will provide assistance in the evaluation and control of biohazardous agents. It will also establish a framework for institutional monitoring. The success of the program is largely dependent upon the Principle Investigator who serves as the Laboratory Biological Safety Officer; however, all employees and students must be active participants in biological safety to ensure the program goals are achieved.

References for this document include Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, the NIH Guidelines for Research Involving Recombinant DNA Molecules, and the World Health Organization's Laboratory Biosafety Manual - Third Edition.

Anyone working with any type of Biological Material must register with the Biological Safety Program using the appropriate registration form and complete all appropriate training.

## B. REGISTRATION AND APPROVAL

All work involving Class 1 biohazardous agents shall be registered with the IBC. All proposed work must be submitted to EHS on a Biological Use Authorization (BUA) Form. EHS will review and inspect lab and storage spaces. If approved, EHS will send letter to P.I. and report

review of application to IBC. If EHS can not immediately approve, the BUA form will be submitted to IBC for full review.

All work involving Class 2 biohazardous agents must be reviewed and approved by the IBC. EHS will do pre-review and inspection prior to IBC meeting; records of inspection will be part of IBC review. The Biological Use Authorization (BUA) registration form must be used and accompanied by written protocols.

Currently Class 3&4 work is not allowed at UA. Researchers wishing to work at Class 3 should contact the BSM Darren Moss at [dross@fa.ua.edu](mailto:dross@fa.ua.edu).

All work involving recombinant DNA must be reviewed by the IBC prior to work registration regardless of NIH classification.

Once work is registered or approved, any changes require new documentation and new forms must be forwarded to ORC for review by IBC.

All registrations must be updated and reviewed annually.

## C. INVENTORY

Detailed inventory records must be maintained by the PI for all agents or biological materials used and/or maintained in their lab areas. These records must include the full identity of the strains, their origins and the vendor/originator of the material (ie, ATCC, Dr. Smith at UCLA), their storage location, and the assigned biosafety level. Templates for inventory management are available through EHS on the EHS website, [ehs@ua.edu](mailto:ehs@ua.edu), under Research Safety/Biosafety. These must be submitted to EHS twice yearly, in January and July.

## D. INSTITUTIONAL BIOSAFETY COMMITTEE (IBC or IBSC)

Biosafety is a cooperative effort between the University of Alabama and its employees. The ORC, the Biosafety Officer, Principal Investigators and laboratory personnel must work in concert to minimize the risk of injury and illness associated with research involving potentially hazardous biological materials.

The Biosafety Program is managed through oversight provided by the IBC and the Biosafety Officer. The IBC is responsible for implementation of biosafety policies throughout the University.

### Institutional Biosafety Committee – Members

The Vice President for Research, with assistance from the Biosafety Officer and the Office of Research Compliance, recruits and nominates IBC members. Committee members are formally appointed in writing to three-year terms.

## RESPONSIBILITIES

Each personnel or lab involved in the use of biohazardous materials has a defined degree of responsibility in the Biosafety Program. Failure of any personnel to recognize this responsibility or to comply with established procedures is cause for disciplinary action.

### Institutional Biosafety Committee (IBC)

*NOTE: For the purposes of this document, we refer to the Institutional Biological Safety Committee / IBSC as the IBC. IBC is an institutional committee created under the [NIH Guidelines](#) to review research involving recombinant DNA.*

Develop policy and procedures which provide guidance for activities involving potentially biohazardous materials.

Ensure that all biosafety policies, practices and facilities meet regulatory requirements and follow University-accepted practice.

Ensure that an inventory of potentially biohazardous materials and toxins are maintained with EHS and updated biannually.

Review and/or approve risk assessments for specific biohazardous agents. When warranted, ask whether the scientific aims of the proposed research cannot be sought by means involving materials of lower biohazard potential. Also, when deemed appropriate bring to the attention of the VP of Research the risks associated with a particular experiment.

Review research proposals (BUA) involving potentially biohazardous materials or toxins.

Review adequacy of facilities, SOPs, along with PI and lab personnel training.

## E. INSTITUTIONAL BIOSAFETY OFFICER

The institution shall appoint a Biological Safety Officer if it engages in large-scale research or production activities involving viable organisms containing recombinant DNA molecules. The institution shall appoint a Biological Safety Officer if it engages in recombinant DNA research at BL3 or BL4. The Biological Safety Officer shall be a voting member of the Institutional Biosafety Committee. The Biological Safety Officer's duties include, but are not be limited to:

- Implementing the requirements of the Biological Safety Program.
- Periodic inspections to ensure that laboratory standards are rigorously followed.
- Reporting to the Institutional Biosafety Committee and the institution any significant problems, violations of the *NIH Guidelines*, violations of UA policy or practices or any



significant research-related accidents or illnesses of which the Biological Safety Officer becomes aware. Unless the Biological Safety Officer determines that a report has already been filed by the Principle Investigator.

- Developing emergency plans for handling accidental spills and personnel contamination for biohazardous material or recombinant DNA research.
- Investigating all accidents or incidents involving biological materials or occurring in labs approved for biological materials.
- Providing advice on laboratory security.
- Providing technical advice to Principle Investigators and the Institutional Biosafety Committee on research safety procedures.
- Developing written policies and procedures as necessary to support the Biosafety Program.

## F. TRAINING

Training of personnel and students is an integral part of any program. Principle Investigators (PIs) must complete the Basic Biosafety training and must verify that everyone working in their area completes this training program. Basic Biosafety training is provided by EHS, via the Skill Soft Academy Program

Persons working UA labs will receive lab specific training on the hazards in their area from the PI or their designee. Persons who work with Class 2 or 3 biohazardous agents shall receive procedure and agent specific training from their PI. This shall be provided before being allowed to work in the laboratory and shall include emergency procedures, decontamination, biosafety principles and other information related to specific organisms or hazards which may be present in the lab. Information contained in the appendices of this manual may be used for training. It is the sole responsibility of the principle investigator to see that all personnel receive all required training.

## G. NON COMPLIANCE

It is the responsibility of the PI and all persons who work with biohazardous agents to follow the guidelines of the Biological Safety Program and to utilize safe procedures. Violations shall be documented by the BSO or other EHS personnel communicated to the IBC. The responsible PI shall be informed in writing of the nature of the non-compliance, ways to implement correction and results of failure to comply. In the event of a situation determined to be immediately hazardous, the BSO may suspend the privileges of the responsible PI or take other actions necessary to protect the health of individuals or the safety of University facilities without prior notification to the IBC. In this case, ORC and the Chair of the IBC will be notified as soon as possible once the situation is secure and will be included in all subsequent communications.

As the committee responsible for biological safety, the IBC may suspend projects, revoke approvals or take other punitive actions deemed appropriate. Actions taken by the IBC shall be reported to the responsible PI, Department Chair and Vice President for Research. This situation

could arise when a PI willingly or negligently violates University policies or established guidelines which govern the use of biohazardous agents.

## II. BIOSAFETY

### A. BIOSAFETY LEVELS

The primary risk criteria used to define the four ascending levels of containment, referred to as biosafety levels 1 through 4, are infectivity, severity of disease, transmissibility, and the nature of the work being conducted. Another important risk factor for agents that cause moderate to severe disease is the origin of the agent, whether indigenous or exotic. Each level of containment describes the microbiological practices, safety equipment and facility safeguards for the corresponding level of risk associated with handling a particular agent. The basic practices and equipment are appropriate for protocols common to most research and clinical laboratories. The facility safeguards help protect non-laboratory occupants of the building and the public health and environment. Taken from the BMBL 5<sup>th</sup> Ed.

### B. RECOMBINANT DNA (rDNA) / SYNTHETIC DNA

Recombinant DNA (rDNA) work is addressed under the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules. The purpose of the *NIH Guidelines* is to specify practices for constructing and handling: (i) recombinant deoxyribonucleic acid (DNA) molecules and (ii) organisms and viruses containing recombinant DNA molecules. In the context of the *NIH Guidelines*, recombinant DNA molecules are defined as either: (i) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell or (ii) molecules that result from the replication of those described in (i) above. Synthetic DNA segments which are likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) are considered as equivalent to their natural DNA counterpart. If the synthetic DNA segment is not expressed *in vivo* as a biologically active polynucleotide or polypeptide product, it is exempt from the *NIH Guidelines*. Genomic DNA of plants and bacteria that have acquired a transposable element, even if the latter was donated from a recombinant vector no longer present, are not subject to the *NIH Guidelines* unless the transposon itself contains recombinant DNA.

As a condition for NIH funding of recombinant DNA research, institutions shall ensure that such research conducted at or sponsored by the institution, irrespective of the source of funding, shall comply with the *NIH Guidelines*. Information concerning noncompliance with the *NIH Guidelines* may be brought forward by any person. It should be delivered to both NIH/OBA and the relevant institution. The institution, generally through the Institutional Biosafety Committee, shall take appropriate action. The institution shall forward a complete report of the incident recommending any further action to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985, 301-496-9838/301-496-9839 (fax) (for non-USPS mail, use zip code 20817). In cases where NIH proposes to suspend, limit, or terminate financial assistance because of noncompliance with the

*NIH Guidelines*, applicable Department of Health and Human Services and Public Health Service procedures shall govern.

## C. BIOSECURITY

Biosafety and biosecurity are related, but not identical, concepts. Biosafety programs reduce or eliminate exposure of individuals and the environment to potentially hazardous biological agents. Biosafety is achieved by implementing various degrees of laboratory control and containment, through laboratory design and access restrictions, personnel expertise and training, use of containment equipment, and safe methods of managing infectious materials in a laboratory setting.

The objective of 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. While the objectives are different, biosafety and biosecurity measures are usually complementary.

Biosafety and biosecurity programs share common components. Both are 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.

Biosafety and biosecurity program risk assessments are performed to determine the appropriate levels of controls within each program. Biosafety looks at appropriate laboratory procedures and practices necessary to prevent exposures and occupationally-acquired infections, while biosecurity addresses procedures and practices to ensure that biological materials and relevant sensitive information remain secure. (Taken from the BMBL 5<sup>th</sup> Ed)

## III. ANIMAL SAFETY

### A. VERTEBRATE ANIMAL BIOSAFETY LEVEL CRITERIA

If experimental animals are used, institutional management must provide facilities and staff and establish practices which reasonably assure appropriate levels of environmental quality, safety and care. See BMBL, 5<sup>th</sup> edition, for a summary of recommended biosafety levels for activities in which experimentally or naturally infected animals are used.

Anytime animals are used in experimentation, approval by the Institutional Animal Care and Use Committee (IACUC) is also required prior to beginning any lab work.

### B. ZOONOSIS

#### Overview of Zoonotic Diseases

Humans may be susceptible to infectious diseases that affect animals. Infections of animals may sometimes produce severe disease in humans even when the animals themselves show little, if

any, sign of illness. A pathogen in the normal flora of a healthy animal may cause a serious disorder in a person exposed to it because the animal has developed resistance to these microorganisms, whereas humans with no previous exposure to the agent lack this protective immunity. Therefore, one should always be aware of possible consequences when working with each species of animal and take precautions to minimize the risk of infection. In the event that lab personnel become ill with a fever or some other sign of infection, it is important to follow UA policy.

The *Working Safely with Laboratory Animals* document located on the EHS web site provides general guidance for working with laboratory animals. This document is based on the National Research Council's document title "Occupational Health and Safety in the Care and Use of Research Animals".

## IV. EXPOSURE CONTROL

### A. ENGINEERING CONTROLS

Engineering controls are methods of controlling employee exposures by modifying the source or reducing and controlling the quantity of contaminants released into the work environment. Examples include biological safety cabinets, fume hoods, glove boxes, and local exhaust. Engineering controls are the preferred control measure for reducing potentially hazardous exposures.

#### Ventilation

Ventilation Controls are engineering controls intended to minimize employee exposure to infectious agents, hazardous chemicals or toxic substances by removing air contaminants from the work site. There are two main types of ventilation controls:

General (Dilution) Exhaust: is where you have a room or building-wide system which supplies air from the outside and removes it at the same rate.

Local Exhaust or Filtration: a ventilated, enclosed work space intended to capture, contain and exhaust or filter harmful or dangerous fumes, vapors and particulate matter. In the case of hazardous chemicals this includes a fume hood. In the case of infectious agents biosafety cabinets should be used.

#### Biological Safety Cabinets (BSCs)

BSCs are designed to provide personnel, environmental and product protection when appropriate practices and procedures are followed. Three kinds of biological safety cabinets (BSCs), designated as Class I, II and III have been developed to meet various research and clinical needs. Biological safety cabinets use high efficiency particulate air (HEPA) filters in their exhaust and/or supply systems and are intended to be used when handling infectious, toxic or sensitizing materials.

BSCs should not be confused with other laminar flow devices or "clean benches"; in particular, horizontal flow cabinets, which direct air towards the operator. These benches protect the product but do not protect the operator. Laboratory personnel should be trained in the correct use of biological safety cabinets to ensure that personnel and product protection (where applicable) is maintained. If issues arise contact EHS technician, Mitch Yerby- myerby@ua.f.edu

When properly used in research involving the manipulation of biohazardous agents, biological safety cabinets are effective in containing and controlling particulates and aerosols, and complement good laboratory practices and procedures. The correct location, installation, and certification of the biological safety cabinet is critical to containing infectious aerosols.

All BSCs shall be inspected annually and certified by trained and accredited service personnel according to the NSF (National Sanitation Foundation) Standard 49, Annex F. Inspection and re-certification is required if the cabinet is relocated or after any repairs, filter changes etc.

For general guidance on the safe and effective use of BSCs refer to the CDC\NIH document Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets available from BMBL 5<sup>th</sup> edition ([http://www.cdc.gov/od/ohs/biosfty/primary\\_containment\\_for\\_biohazards.pdf](http://www.cdc.gov/od/ohs/biosfty/primary_containment_for_biohazards.pdf))

NOTE: Before selecting any BSC for purchase, contact EHS for work-specific assessment and selection criteria.

### Chemical Fume Hoods

Chemical Fume Hoods are an important engineering control used to prevent exposure to hazardous materials. In conjunction with sound laboratory techniques, a chemical fume hood serves as an effective means for capturing toxic, carcinogenic, offensive, or flammable vapors or other airborne contaminants that would otherwise be released to the general laboratory atmosphere.

### Safety Showers

Safety showers provide an immediate water drench of an affected person. Standards for location, design and maintenance of safety showers are outlined in the Chemical Hygiene Plan.

### Eyewash Stations

Eyewash stations are required in all laboratories where injurious or corrosive chemicals are used or stored and where employees perform tasks that might result in splashes of potentially biohazardous materials.

Standards for location, design and maintenance of emergency eyewash facilities are outlined in the Chemical Hygiene Plan.

## B. ADMINISTRATIVE CONTROLS

Administrative Controls are methods of controlling employee exposures to infectious agents by adherence to appropriate work practices and by written procedures or policies. Examples include standard operating procedures or programs, training, signage, manuals and guidance documents. They should all be approved by IBC with the enforcement responsibility resting with the P.I.

## C. PERSONAL PROTECTIVE EQUIPMENT (PPE)

PPE is used to protect personnel from contact with hazardous materials and infectious agents. Appropriate clothing may also protect the experiment from contamination. Personal protective devices and safety equipment must be provided to all lab personnel under the appropriate circumstances and lab personnel have the responsibility of properly using the equipment.

## D. RECOMMENDED WORK PRACTICES

When pipetting, use the following precautions:

1. Mouth pipetting is strictly prohibited. Mechanical pipetting aids must be used.
2. Confine pipetting of biohazardous or toxic fluids to a biosafety cabinet if possible. If pipetting is done on the open bench, use absorbent pads or paper on the bench.
3. Respiratory protection may need to be considered depending on the agent in use.
4. Always use cotton-plugged pipettes when pipetting biohazardous or toxic fluids.
5. Never prepare any kind of biohazardous mixtures by suction and expulsion through a pipette, this can create aerosols.
6. Biohazardous materials should not be forcibly discharged from pipettes. Use “to deliver” pipettes rather than those requiring “blowout.”
7. Do not discharge biohazardous material from a pipette at a height. Whenever possible allow the discharge to run down the container wall.
8. Place contaminated, reusable pipettes horizontally in a pan containing enough liquid disinfectant to completely cover them.
9. If needed, autoclave the pan and pipettes as a unit before processing them for reuse.
10. Discard contaminated Pasteur pipettes in an appropriate size sharps container with appropriate biohazard label.

11. When work is performed inside a biosafety cabinet, all pans or sharps containers for contaminated glassware should be placed inside the cabinet as well while in use.

## Syringes and Needles

Syringes and hypodermic needles are dangerous objects that need to be handled with extreme caution to avoid accidental injection and aerosol generation. Generally, the use of syringes and needles should be restricted to procedures for which there is no other alternative. The use of “safe” needles is highly recommended. Do not use a syringe and needle as a substitute for a pipette.

When using syringes and needles with biohazardous or potentially infectious agents (see

1. Dispose of ALL needles and syringes (used or unused) in appropriate sharps containers. Do not discard syringes and needles into laboratory waste receptacles or pans containing pipettes or glassware.
2. Do not overfill sharps containers (2/3 filled = full).
3. DO NOT RECAP NEEDLES.
4. DO NOT BEND, CUT, REMOVE OR BREAK NEEDLES. Throw intact needle/syringes into a sharps container for disposal.
5. Use “safe” needles/syringes or sharps when appropriate.
6. Use needle-locking syringes or disposable syringe-needle units in which the needle is an integral part of the syringe or syringes which involve safe needle technology.
7. Plan your work to avoid quick and unnecessary movements while working with syringes.
8. Where appropriate, fill or immerse syringes in disinfectant prior to disposing into sharps container. Syringes and needles that are autoclaved prior to disposal or preparation for washing can be autoclaved in a pan of disinfectant solution.
9. Use separate containers for disposable and non-disposable syringes and needles to eliminate the need to sort later.
10. Wear gloves during all manipulations for general safety.
11. Examine glass syringes for chips and cracks, needles for barbs and plugs prior to sterilization and before use.
12. Only use glass syringes as a last resort. Disposable syringes and needles and/or safe needle/syringe technology is preferred.
13. Fill the syringe carefully to minimize air bubbles and frothing of the inoculum.



14. Work in a biosafety cabinet whenever possible.
15. Expel excess air, liquid and bubbles from the syringe vertically into a cotton pledget moistened with proper disinfectant, or into a small bottle of sterile cotton.
16. If you are filling from a test tube, avoid contaminating the hub of the needle, this may result in transfer of infectious material to the hands.
17. When inoculating animals be sure that your hands are "BEHIND" the needle to avoid punctures.
18. Ensure that the animal is properly secured and restrained prior to inoculating. Be alert for any unexpected movements of the animal.

### Cryostats

Frozen sections of unfixed human tissue or animal tissue infected with an etiologic agent pose a risk because freezing tissue usually does not inactivate infectious agents. Freezing propellants under pressure should not be used for frozen sections as they may cause spattering of droplets of infectious material.

Gloves should be worn during preparation of frozen sections. When working with biohazardous material in a cryostat, the following is recommended:

1. Consider the contents of the cryostat to be contaminated and decontaminate it frequently with 70% ethanol or any other disinfectant suitable for the agent(s) in use.
2. Consider trimmings and sections of tissue that accumulate in the cryostat to be potentially infectious and remove them during decontamination.
3. Defrost and decontaminate the cryostat with an appropriate disinfectant once a week and immediately after tissue known to contain an etiologic agent is cut.
4. Handle microtome knives with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.
5. Solutions used to stain potentially infected frozen sections should be considered contaminated.

### Centrifuge Equipment

Hazards associated with centrifugation include mechanical failure and the creation of aerosols. To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer's instructions. Users should be properly trained and operating instructions including safety precautions should be prominently posted on the unit.



Aerosols are created by practices such as filling centrifuge tubes, removing supernatant, and re-suspending sedimented pellets. The greatest aerosol hazard is created if a tube breaks during centrifugation. Use of glass tubes should be avoided wherever possible. To minimize the generation of aerosols when centrifuging biohazardous material, the following procedures should be followed:

1. Use sealed tubes and safety buckets that seal with O-rings. Before use, inspect tubes, O-rings and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil, parafilm, etc. to cap centrifuge tubes because it may detach or rupture during centrifugation.
2. Fill and open centrifuge tubes, rotors and accessories in a BSC. Avoid overfilling of centrifuge tubes so that closures do not become wet. After tubes are filled and sealed, wipe them down with disinfectant.
3. Always balance buckets, tubes and rotors properly before centrifugation.
4. Do not decant or pour off supernatant. Use a vacuum system with appropriate in-line reservoirs and filters.
5. Work in a BSC when re-suspending sedimented material. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.
6. Small low - speed centrifuges may be placed in a BSC during use to reduce the aerosol escape. High-speed centrifuges pose additional hazards. Precautions should be taken to filter the exhaust air from vacuum lines, to avoid metal fatiguing resulting in disintegration of rotors and to use proper cleaning techniques and centrifuge components. Manufacturer's recommendations must be meticulously followed to avoid metal fatigue, distortion and corrosion.
7. Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials. Celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They distort on boiling and can be highly explosive in an autoclave. If celluloid tubes must be used, IBC approved chemical disinfectants are necessary for decontamination.

#### Blenders, Ultrasonic Disrupters, Grinders and Lyophilizers

The use of any of these devices results in considerable aerosol production. Blending, cell-disrupting and grinding equipment should be used in a BSC when working with biohazardous materials.

## Safety Blenders

Safety blenders, although expensive, are designed to prevent leakage from the bottom of the blender jar, provide a cooling jacket to avoid biological inactivation, and to withstand sterilization by autoclaving. If blender rotors are not leak-proof, they should be tested with sterile saline or dye solution prior to use with biohazardous material. The use of glass blender jars is not recommended because of the breakage potential. If they must be used, glass jars should be covered with a polypropylene jar to prevent spraying of glass and contents in the event the blender jar breaks. A towel moistened with disinfectant should be placed over the top of the blender during use. Before opening the blender jar, allow the unit to rest for at least one minute to allow the aerosol to settle. The device should be decontaminated promptly after use.

## Lyophilizers and Ampoules

Depending on lyophilizer design, aerosol production may occur when material is loaded or removed from the lyophilizer unit. If possible, sample material should be loaded in a BSC. The vacuum pump exhaust should be filtered to remove any hazardous agents. After lyophilization is completed, all surfaces of the unit that have been exposed to the agent should be disinfected. If the lyophilizer is equipped with a removable chamber, it should be closed off and moved to a BSC for unloading and decontamination.

Handling of cultures should be minimized and vapor traps should be used wherever possible.

Opening ampoules containing liquid or lyophilized infectious culture material should be performed in a BSC to control the aerosol produced. Gloves must be worn. To open, nick the neck of the ampoule with a file, wrap it in disinfectant soaked towel, hold the ampoule upright and snap it open at the nick.

Reconstitute the contents of the ampoule by slowly adding liquid to avoid Aerosolization of the dried material. Mix the container. Discard the towel and ampoule top and bottom as biohazardous waste.

Ampoules used to store biohazardous material in liquid nitrogen have exploded causing eye injuries and exposure to the infectious agent. The use of polypropylene tubes eliminates this hazard. These tubes are available dust free or pre-sterilized and are fitted with polyethylene caps with silicone washers. Heat sealable polypropylene tubes are also available.

## UV Lights

The CDC, NIH and NSF agree that UV lamps are neither recommended nor required in Biological Safety Cabinets (BSC). Criteria are not even available from NSF to evaluate the performance of the UV lights within a biological safety cabinet. Numerous factors affect the activity of the germicidal effect of UV light, which require regular cleaning, maintenance and monitoring to ensure germicidal activity.

Retrofitting any equipment (e.g. UV lights) into a biological safety cabinet may alter the air flow characteristics of the cabinet and invalidate any manufacturer warranty and is not recommended.

It is the current opinion of the American Biological Safety Association that UV lights are not recommended for use in Biological Safety Cabinet.

#### Vacuum Lines

Vacuum lines shall be protected with liquid disinfectant traps and High Efficiency Particulate Air (HEPA) filters or filters of equivalent or superior efficiency. Filters must be checked routinely and maintained or replaced as necessary.

#### Housekeeping

Good housekeeping in laboratories as well as all work area is essential to reduce potential personnel exposures and protect the integrity of biological experiments. Routine housekeeping shall be relied upon to ensure work areas are free of significant sources of contamination. Housekeeping procedures should be based on the highest degree of risk to which personnel and experimental integrity may be subjected.

Laboratory personnel are responsible for maintaining the cleanliness of laboratory benches, equipment and areas that require specialized technical knowledge.

Additional laboratory housekeeping concerns include:

1. Keep the laboratory neat and free of clutter. Surfaces should be clean and free of infrequently used chemicals, glassware and equipment. Access to sinks, eyewash stations, emergency showers, exits, and fire extinguishers shall not be blocked.
2. Proper disposal of all unwanted chemicals, biological and non hazardous waste is essential.
3. The workplace must be free of physical hazards. Aisles and corridors should be free of tripping hazards. Attention should be paid to electrical safety, especially as it relates to the use of extension cords (long-term use is not allowed), proper grounding of equipment, and avoidance of electrical hazards in wet areas.
4. All laboratory equipment must be cleaned and a hazard tag completed and attached before being released for repair or maintenance. See UA Decommission Guide for instructions.

#### Methods of Decontamination

Decontamination is defined as the reduction of microorganisms to an acceptable level. This means, that viable microorganisms are still present. Methods applied to reach this goal can vary and most often include disinfection or sterilization. In contrast, sterilization is defined as the

complete killing of all organisms present. Depending on the circumstances and tasks, decontamination of a surface (e.g., lab bench) is accomplished with a disinfectant, while decontamination of biomedical waste is done by sterilization in an autoclave.

In order to select the proper method and tools, it is important to consider, for example, the following aspects:

- Type of biohazardous agents, concentration and potential for exposure;
- Physical and chemical hazards to products, materials, environment and personnel.

Physical and chemical means of decontamination fall into four main categories: Heat, Liquid Chemicals, Vapors and Gases, and Radiation.

Disinfection is normally accomplished by applying liquid chemicals or wet heat during boiling or pasteurization. To sterilize, vapors, gases (e.g., ethylene oxide), radiation, and wet heat (steam sterilization in an autoclave) are used. Some liquid chemicals are also applied for sterilization, if used in the right concentration and contact time. For a description of different methods and chemicals available for disinfecting refer to appendix 4.

#### Potential Laboratory Hazards

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

#### Recommended Practices

1. Human and other primate cells at minimum should be handled using Biosafety Level 2 practices and containment;
2. All work should be performed in a biosafety cabinet;
3. All material should be decontaminated by autoclaving or disinfection before discarding;
4. All lab personnel working with human cells and tissues shall be enrolled in the Bloodborne Pathogens Training, and work under the policies and guidelines established by the Potentially Infectious Material/Bloodborne Pathogens Program. This includes being offered the Hepatitis B vaccine.

5. See the Potentially Infectious Material/Bloodborne Pathogens Program for more information (ehs.ua.edu)
6. Propagation and/or manipulation of *Mycobacterium tuberculosis* and *M. bovis* cultures in the laboratory or animal room must be performed at BSL-3. At this time UA does not perform BSL-3 lab work Contact the Biosafety Officer for guidance.

#### Guidelines for Clinical Laboratories

Clinical laboratories receive clinical specimens with requests for a variety of diagnostic services. The infectious nature of this material is largely unknown. In most circumstances, the initial processing of clinical specimens and identification of microbial isolates can be done safely at BSL-2.

A primary barrier, such as a biological safety cabinet, should be used:

1. Anticipation that splashing, spraying or splattering of clinical materials may occur.
2. Initial processing of clinical specimens where it is suggested that an agent transmissible by infectious aerosols may be present (e.g., *M. tuberculosis*), to protect the integrity of the specimen or where presence of infection is known (flu, strep, etc.)

All laboratory personnel who handle human source materials shall have the Bloodborne Pathogens Training as outlined in the Potentially Infectious Material/Bloodborne Pathogens Program. “Universal Precautions” need to be followed when handling human blood, blood products, body fluids or tissues. See link [Bloodborne Universal Precautions](#)

#### E. CDC SELECT AGENTS PROGRAM

At this time, UA does not maintain a permit for work with Select Agents. If work is to be performed with any of the following contact EHS.

<http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20List.html>

#### F. SHIPPING OR TRANSPORT OF BIOLOGICAL AGENTS

The transfer of biological agents from campus may be subject to the regulatory requirements of EPA, TSCA, DOT, CDC or USDA or other federal or state regulatory agency. Additionally, Material Transfer Agreements may be required prior to transferring any biological materials. Material Transfer Agreements (MTA) describe the terms under which University researchers can share materials, typically for research or evaluation purposes. These agreements are administered by the Office of Technology Transfer (OTT). Intellectual property rights can be endangered if materials are used without a proper MTA. Before transferring any biological agent, contact EHS and OTT so that transport compliance can be satisfied. Also, see appendix 7

Ordering procedures are posted on EHS website. Approval is required based upon IBC review. Material must have an approved BUA to be ordered.

## G. EMERGENCY PROCEDURES

All persons who work in the lab shall be trained in emergency procedures by their PI. Basic emergency procedures are as follows:

- To evacuate and secure the area if necessary.
- To notify the PI and BSO as soon as possible.
- To assess the type of spill and hazard.
- To determine the most effective and least hazardous method of remediation.
- To decontaminate the area, waste and equipment utilized.

The class of biohazardous agent which is involved in the spill or accident will dictate the extent to which emergency procedures are utilized.

All lab personnel should be aware of locations for fire extinguishers, fire pull stations and evacuation placards.

## H. WASTE DISPOSAL

It is the responsibility of the lab supervisor to indicate how potentially infectious waste has been treated. If waste has been pretreated by autoclaving, it becomes "NORMAL WASTE" and should be labeled to indicate it has been autoclaved. Autoclaved waste can be disposed of by routine housekeeping practices. However, if infectious waste is to be untreated, it must be placed in Biohazard bags or sharps containers and managed as "MEDICAL WASTE" in accordance with the UA Medical Waste Manual on EHS website.

Practices for the disposal of waste of potentially infectious material must be dictated by the Classification of Biological Agents on the basis of Hazard. For Class 1 and Class 2 agents, biohazardous waste may be handled either of two ways:

1. Pretreated Waste - Decontamination (by autoclaving) before disposal. According to ADEM 335-13-7-08, infectious waste treated in the following manner may then be handled as ordinary trash.
  - A. Autoclaves should be equipped to continuously monitor and record temperature and pressure during the entire length of each cycle.
  - B. Each bag or container shall be exposed to a minimum temperature of 250 degrees F and at least 15 pounds of pressure for 30 minutes.
  - C. Each sterilizer shall be evaluated for effectiveness under full loading by an approved method (e.g. Biological indicator spore tests) at least once for each 40 hours of general operation.

- D. Sterilizers utilized for waste treatment shall not be utilized for sterilization of equipment, food or other related items.
- E. A written log or other means of documentation shall be maintained for the autoclave used for decontamination of infectious waste. It shall include:
- The date, duration time, and operator for each cycle.
  - Approximate weight or volume of medical waste treated during each cycle.
  - The temperature and pressure maintained during each cycle.
  - Method used for confirmation of temperature and pressure.
  - Dates and results of calibration and maintenance.
2. Untreated Waste - Non-autoclaved media that has utilized human body fluids or tissue or non-autoclaved discarded specimens of any potentially infectious material shall be collected in labeled biohazard bags and managed as medical waste. These procedures are described in the UA Medical Waste Manual on the EHS website.
3. For any other material, contact EHS to confirm proper disposal procedures.

# APPENDIX 1

## *DEFINITIONS AND ACRONYMS*

ABSL - Animal Biosafety Level (See Biosafety Level below).

ACF - Animal Care Facility

ADEM - Alabama Department of Environmental Management

Antisepsis - The application of a liquid antimicrobial chemical to living tissue to prevent growth or destroy potentially infectious organisms.

BBP - (See Blood-borne Pathogen).

Biohazard - (See Biohazardous Materials).

Biohazardous Materials - Hazardous biological materials and organisms, including: a) infectious organisms (bacteria, fungi, parasites, prions, rickettsias, viruses, etc), which can cause disease in healthy humans and/or significant environmental or agricultural impact; b) human or primate tissues, fluids, cells, or cell culture; c) recombinant DNA; and d) animals known to be vectors of zoonotic diseases.

Biosafety Cabinet - (Biological Safety Cabinet) - A device enclosed (except for necessary exhaust purposes) on three sides and top and bottom, designed to draw air inward by means of mechanical ventilation, operated with insertion of only the hands and arms of the user, and in which virulent pathogens are used. Biosafety Cabinets are classified as: Class I - Provides personnel and environmental protection but no product protection, the exhaust is HEPA filtered; Class II - Provides personnel, product and environmental protection; Class III - Totally enclosed (glove box) ventilated cabinet with gas-tight construction.

Biosafety Level - Combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and are based on the potential hazards imposed by the agents used and for the laboratory function and activity. Biosafety Level I provides the least stringent containment conditions and Biosafety Level 4 the most stringent.

Blood-borne Pathogen (BBP) - Microorganisms that are present in human/primate blood, tissues or fluids and can cause disease in humans. These pathogens include (but are not limited to) hepatitis B virus (HBV) and human immunodeficiency virus (HIV).

BSC - (See Biosafety Cabinet).

BSL - Biosafety Level.

BSO - Biological Safety Officer

CDC - Centers for Disease Control and Prevention.

Containment - Safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure to laboratory workers, other persons and the environment to potentially hazardous agents.

Decontamination - Routinely required step of destroying/inactivating microorganisms in microbiological laboratories to protect laboratory workers and prevent contamination of their work. This is the destruction or removal of microorganisms to some lower level, but not



necessarily total destruction. Sterilization, disinfection and antisepsis are all forms of decontamination.

Disinfection - Implies the use of antimicrobial agents to inanimate objects (e.g., work surfaces, equipment, etc) to destroy all organisms that could pose a potential hazard to humans or compromise the integrity of the experiment.

DNA - Deoxyribonucleic Acid.

DOT - Department of Transportation.

Exposure Control Plan (ECP) - A written document of practices and procedures, required equipment and facilities designed to eliminate or minimize employee exposure to infectious agents or biohazardous materials.

EHS - Office of Environmental Health and Safety

Etiologic Agent - Biological agent or agents that causes disease in humans or animals.

Gene Therapy - The delivery of exogenous DNA to mammalian cells to cause the expression of this material thereby altering the cells phenotypically.

HBV - Hepatitis B Virus.

HEPA - High Efficiency Particulate Air Filter has an efficiency of 99.97% for particles of 0.3 microns or larger. Biological Safety Cabinets filter air through one of more sets of HEPA filters.

HIV - Human Immunodeficiency Virus.

IACUC - Institutional Animal Care and Use Committee

Infectious Agents - Microorganisms capable of producing an infection and disease in a healthy human.

Infectious Substance - (Same as Etiologic agent).

IBSC - Institutional Biological Safety Committee.

IBSO - Institutional Biological Safety Officer.

Laminar Flow Hoods - Cabinets which are designed to protect only the product. These cabinets blow air into the face of the worker. Use of these cabinets for any biological organism is discouraged.

PI -The faculty member primarily responsible for the space in which work is being performed.

NIH - National Institutes of Health.

NIOSH - National Institute for Occupational Safety and Health.

OSHA - Occupational Safety and Health Administration.

Pathogen - Microorganism or substance capable of producing disease.

PPE - Personal Protective Equipment.

PI - Laboratory Biological Safety Officer

Recombinant DNA (rDNA) - a) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or b) molecules that result from replication of those described in a) above.

RNA - Ribonucleic Acid.

Sterilization - The process of destroying or removing all living organisms and viruses, usually by steam or gas autoclaving.

Universal Precautions - Refers to the infection-control method in which all human/primate blood, tissues, and/or fluids are treated as if they are known to be infectious.

USDA - United States Department of Agriculture.

Zoonotic Diseases - Diseases communicable from animals to humans under natural conditions.

## APPENDIX 2

### **FORMS**

Autoclave Log

Biological Inventory Template

Biosafety Self Audit

Biological Use Authorization

Chemical Disposal Guidelines

Decommissioning Guidelines for Laboratories

Decommissioning Hazard Tag

Decommissioning Hazard Tag Guide

Decommissioning Safe and Compliant Room Tag

Documentation of Participation in Training Program (for Bloodborne Pathogen Program)

Hepatitis B Vaccination Series

Hepatitis B Vaccination Decline to Accept Form

IBC Project Review Flowchart

Lab Signage Form

Lab Inspection Form

Medical Evaluation Form – Respiratory Protection

Model Exposure Control Plan (for Bloodborne Pathogens and Potentially Infectious Material)

Respiratory Protection Assessment FlowChart

Risk Appraisal Survey (for Bloodborne Pathogens and Potentially Infectious Material)

Sharps Evaluation and Sharps Injury Log (for Bloodborne Pathogens and Potentially Infectious Material)

SOP Template

Student/NonEmployee Injury Report

## APPENDIX 3

### ***BIOLOGICAL SAFETY CABINETS***

Biological Safety Cabinets and other primary containment devices are an essential component of conducting biomedical research. As a primary safety barrier, the effectiveness of the BSC is limited by the techniques employed by the researcher (e.g. good microbiological techniques), an understanding of how the cabinet functions, and the location of the biological safety cabinet within the facility. As a general rule, keep biosafety cabinets away from doors, high traffic areas and supply diffusers. EHS can provide consultation and guidance on the selection, operation and use that meet your specific research needs.

A comprehensive description of BSC types, performance characteristics, and applications can be found in the publication *Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets* available online at: <http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm>.

#### **BSC Certifications**

BSCs are required to be certified annually or when moved or when repaired. EHS Lab Safety Program provides for the annual certification of BSCs.

If you have any questions about the changes in the program or if you discover that your cabinet is past due for its annual certification, contact Marcy Huey at [mhuey@ua.edu](mailto:mhuey@ua.edu) or 348-5912.

#### ***Why must my BSC be certified?***

BSC are the primary containment device used to protect the worker, product and environment from exposure to biohazardous agents. The accepted standard is the **NSF Title 49**, which overall, serves to validate the design, operation, and testing of biological safety cabinets. The purpose of testing and certification is to ensure the balance of inflow and exhaust air, the distribution of air onto the work surface, and the integrity of the cabinet.

#### ***How often must my BSC be certified?***

BSC must be certified at the time of installation and annually thereafter. The BSC must be re-certified after it has been moved or when repaired (e.g., HEPA filter replacement).

#### **Who schedules certifications?**

EHS maintains a centralized database of all BSCs at UA. The database is used by EHS to coordinate annual certification of BSCs with the appropriate vendor prior to the BSC recertification due date; however, laboratory personnel are ultimately responsible for ensuring that BSCs are certified prior to expiration. Please contact EHS (348-5319) if you would like to verify that a vendor has been scheduled to recertify your BSC.

#### **What is covered?**

Testing and certification services are paid for by EHS. At the completion of services, vendors will submit an invoice directly to EHS for processing and payment.

Decontamination Services for lab moves/relocations. To better ensure when/if decontamination is necessary to safely move or relocate a BSC, contact EHS for a hazard assessment. If it is required, EHS will work with the PI and an approved vendor to determine what type of decontamination is required and to schedule decontamination services. This should be addressed in connection with the EHS Lab Decommissioning Protocols.

**Note: gas changes decontamination for moves, repairs and other services including HEPA filter changes are not covered** by EHS (see below).

Certification Required after every move.

What is not covered?

Repairs, expendable parts (e.g. HEPA filter changes, motors, light tubes, etc.), and labor charges.

If a gas decon is necessary for repairs, a move or a HEPA filter change, the lab must cover this cost. Vendors may request a PO up front for services not covered.

**Note: Vendor prices will vary and therefore, it is essential to communicate with the vendor contact to provide quotes for associated costs.**

*Policy on use of uncertified biosafety cabinets*

If a biosafety cabinet is not certified (either via certification expiration or certification failure), it must not be used as a primary safety device. From a safety perspective, an uncertified biosafety cabinet with the blowers running has the potential to disseminate potentially harmful material throughout the environment. Any work with pathogens or potentially infectious materials - even those considered Risk Group 1 - should not be performed in an uncertified cabinet.

Occasionally, there are requests to work with non-pathogenic material (e.g. plant or certain animal tissue cultures) in an uncertified cabinet. This should only be considered if the situation can be carefully managed. The cabinet should have a warning sign, and occupants of the laboratory should be trained on the limitations and potential hazards of using the equipment incorrectly. Such use is strongly discouraged in laboratories where pathogens or potentially infectious materials are also present since the potential for and risks of using the equipment incorrectly are increased.

If you have an uncertified biological safety cabinet and wish to use it for work with biological material, please contact EHS (348-5319). We will work with you to develop a risk assessment to determine if the proposed use would qualify for an exemption from the certification requirement.

***Who do I contact about getting a BSC?***

Contact EHS to determine if a BSC is needed and if one might be currently available for use/relocation. Installation of a new BSC may require a Project Initiation Request (PIR) submitted to Construction Administration.

***What type of BSC do I need?***

The type of BSC needed is determined by the type of material that you wish to use in the BSC. Contact EHS (348-5912) if you are unsure about the type of BSC you need.

A **Class II, A2** Biosafety Cabinet is recommended and the most common type of BSC in use, as it is appropriate for most biohazardous work applications. Class II BSC provide personnel protection from biohazardous materials using HEPA filtered air prior to release into the room, in addition to providing product protection (to maintain sterility). Information on specific classes and types of Biosafety Cabinets can be found at

<http://www.cdc.gov/od/ohs/biosfty/bmbl5/sections/AppendixA.pdf>

***Can I use a clean bench for biohazardous work?***

Laminar Flow Cabinets, sometimes referred to as Clean Benches, may be used for work with materials that are non-infectious. **No infectious work (human cell lines, infectious microorganisms, toxins, or animal work) should be performed in Laminar Flow Cabinets.** They are not recommended as they provide only product protection, and in effect, blow air from the cabinet into the user's face providing no personnel protection.

### *Chemicals in a BSC*

BSCs should not be used in place of a chemical fume hood. Volatile or toxic chemicals should not be used in unducted Class II Type A cabinets since vapor build-up inside the cabinet presents a fire or explosion hazard. In addition, this type of cabinet recirculates air to the cabinet work space and exhausts into the room, potentially exposing the operator and other room occupants to toxic chemical vapors via the air flow.

### *Gas connections, gas burners and alcohol lamps in the BSC*

Gas connections to a BSC are not permitted without a written and approved (by the BSO and IBC) SOP for specialized, limited duration work. Note that routine “flaming” is not considered adequate justification.

### *Open flames in BSCs*

Create turbulence in the airflow, compromising protection of both the worker and the work  
Present a potential fire or explosion hazard, especially when using a gas burner in conjunction with ethanol

Cause excessive heat build-up, which may damage HEPA filters and compromise the cabinet’s integrity

May inactivate the manufacturer’s warranty

### *Ultraviolet (UV) lamp usage*

UV lamps are not required or recommended in BSCs. There is a potential for exposure to UV radiation above recommended limits if working in or near a BSC with a UV light in use. In addition, the germicidal activity of UV lights is limited by a number of factors, many of which are difficult to control. These include the ability of the light to penetrate the cabinet air flow, relative humidity levels >70%, ambient temperature, cleanliness of the bulb, and age of the bulb (should be measured every 6 months for intensity). In the event a UV light is included as part of the cabinet, it should be interlocked so that the light will only activate with the sash fully closed.

### ***Should my BSC be hard ducted or thimble connected to the building exhaust system?***

For most applications, it is not necessary to connect a BSC to the building ventilation system. However, certain types of Class II cabinetry require a hard-duct connection to the building ventilation system. These include Class II Type B1 and B2 cabinets and are only recommended in specific situations involving the use of certain volatile chemicals or radioactive materials.

Contact EHS (348-5912) for information on when a hard-ducted cabinet is necessary.

Class II A2 cabinets are designed to be “convertible” units and can be connected to the ventilation system using a thimble connection designed with an air gap. This is recommended only in certain situations that merit connection to the HVAC system. Contact EHS at 348-5912 and Facilities Management before having any BSC connected (or disconnected) to the building exhaust system.

Note: In most cases, a recirculating Class II A2 cabinet is the most appropriate choice for UA researchers. We recommend these be connected to the building ventilation system to provide maximum flow in research and teaching activities.

### ***What if I need to move my BSC? Or dispose of it?***

In many cases, the BSC must be decontaminated prior to being moved, stored, or disposed of in order to protect personnel from any potential exposure to biohazardous materials.

EHS can assist with contracting a vendor to decontaminate a BSC. Once decontaminated, EHS will certify that the unit is ready to be moved and will provide the lab with a sticker indicating that the BSC is safe to move. (Lab Decommissioning Program)

Contact Surplus Property to move the unit once it has been cleared by EHS. If moving to a new location, the BSC must be re-certified in the new location prior to use.

### *Working Safely in the BSC*

Biosafety cabinets are designed to be operated 24 hours a day. For frequent work with BSL-2 agents in the BSC, it is recommended that blowers remain on at all times.

If it is necessary to turn off the blower, allow sufficient time to purge airborne contaminants from the work area (Centers for Disease Control recommends a minimum of 5 minutes before and 5 minutes after work, taking into account sufficient time for settling of aerosols).

Minimize other activities in the room (e.g., rapid movement, open/closing room doors, etc.) to avoid disrupting the cabinet air barrier.

Laboratory coats are worn buttoned over street clothing; gloves are worn to provide hand protection.

Before beginning work, the investigator must adjust the stool height so that his/her face is above the front opening.

Plastic-backed absorbent toweling can be placed on the work surface (but not on the front or rear grille openings). This toweling facilitates routine cleanup and reduces splatter and aerosol formation during an overt spill.

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

Place necessary materials in the BSC before beginning work. This serves to minimize the number of arm-movement disruptions across the air barrier of the cabinet. All materials must be placed as far back in the cabinet as practical, toward the rear edge of the work surface and away from the front grille of the cabinet.

The front grille must not be blocked with research notes, discarded plastic wrappers, pipeting devices, etc.

Aspirator suction flasks must contain an appropriate disinfectant, and a High Efficiency Particulate Air (HEPA) in-line filter. This combination will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment. Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution such as bleach, into the flask to kill the microorganisms as they are collected. Once inactivation occurs, liquid materials can be disposed of as noninfectious waste.

Horizontal pipette discard trays containing an autoclave bag or an appropriate chemical disinfectant should be used within the cabinet. Upright pipette collection containers placed on the floor outside the cabinet or autoclavable biohazard collection bags taped to the outside of the cabinet should not be used. The frequent inward/outward movement needed to place objects in these containers is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection.

All operations should be performed on the work surface at least four (4) inches from the inside edge of the front grille.

Active work should flow from the clean to contaminated area across the work surface. Bulky items such as biohazard bags, discard pipette trays and suction collection flasks must be placed to one side of the interior of the cabinet.

Use of glass Pasteur pipettes is discouraged. Glass pipettes should be replaced with safer alternatives (i.e., plastic) as recommended by the U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Institutes of Health in *Biosafety in Microbiological and Biomedical Laboratories, 5th Edition* and by *The World Health Organization Biosafety Manual*. Contact EHS for more information on safer alternatives.

Open flames (i.e., Bunsen burners) are **rarely necessary** in the near microbe-free environment of a biological safety cabinet and are an artifact left over from usage of A1 cabinets (e.g., provided only personnel, not produce protection) several decades ago. An open flame creates turbulence that disrupts the pattern of HEPA-filtered air supplied to the work surface. If approved, microincinerators (electric) are the best alternative for use in the BSC.

Use of ultraviolet light (UV) in the BSC is strongly discouraged. UV bulbs in the BSC must be cleaned and monitored regularly, as dust and debris inhibit effectiveness as well as gradual degradation of the UV bulb over time and should never be used as a primary or sole means of disinfecting the unit. Therefore, chemical surface disinfection must be the primary means of decontaminating the BSC.

**Clean Up:** Upon completion of work, the final surface decontamination of the cabinet must include a wipe-down of the interior surfaces. Investigators must remove their gloves and gowns in a manner to prevent contamination of unprotected skin and aerosol generation and wash their hands as the final step in safe microbiological practice. Investigators must determine the appropriate method of decontaminating materials that will be removed from the BSC at the conclusion of the work.



## APPENDIX 4

### *Decontamination, Sterilization, Disinfection and Antisepsis* *From: McGill Laboratory Biosafety Manual – Copyright 2014*

Refer also to Laboratory Safety Principles and Practices, Second Edition Editors Diane O. Fleming et al. American Society for Microbiology Press, p 219-237

#### Sterilization and Disinfection in the Laboratory

It is important to distinguish between sterilization and disinfection. Whereas sterilization results in destruction of all forms of microbial life, disinfection results in destruction of specific pathogenic microorganisms. A more detailed description of disinfection levels can be found in the Glossary at the back of this manual.

#### 6.1 Microbial Resistance to Physical and Chemical Agents

Microorganisms vary in their resistance to destruction by physical or chemical means. A disinfectant that destroys bacteria may be ineffective against viruses or fungi. There are differences in susceptibility between gram-negative and gram-positive bacteria, and sometimes even between strains of the same species. Bacterial spores are more resistant than vegetative forms, and non-enveloped, non-lipid-containing viruses respond differently than do viruses which have a lipid coating. Information on the susceptibility of a particular microorganism to disinfectants and physical inactivation procedures can be found in the material safety data sheet (MSDS) for that agent. MSDSs provide additional details such as health hazards associated with the microorganism, mode of transmission, containment requirements and spill response procedures. The Environmental Safety Office has available, and can provide to individuals, MSDSs on a number of infectious microorganisms.

#### 6.2 Physical Sterilants and Disinfectants

##### 6.2.1 Heat Sterilization and Decontamination

Generally, sterilization is best achieved by physical methods such as steam or dry heat, which are less time-consuming and more reliable than chemical germicides. A summary of physical agents which employ heat for control of microorganisms can be found in Table 2. Of these physical procedures, steam autoclaving is the most practical option for the majority of laboratories for both sterilization and decontamination purposes.



TABLE 2 - Outline of the properties of heat decontamination methods. For everyday laboratory purposes, autoclaving is the preferred method, unless the item cannot withstand the heat and/or moisture of autoclaving.

	Principle/Conditions	Advantages	Disadvantages	Uses
Dry Heat	Thermal inactivation: destroys by oxidation	Non-corrosive Simple design and principle	Less effective than moist heat; requires longer times and/or higher temperatures	Materials that are damaged by, or are impenetrable to, moist heat
Hot Air Oven	<ul style="list-style-type: none"> <li>• 160-180°C for 2-4 hours</li> </ul>	<ul style="list-style-type: none"> <li>• penetrates water-insoluble materials (e.g., grease and oil)</li> <li>• less corrosive to metals and sharp instruments than steam</li> </ul>	<ul style="list-style-type: none"> <li>• slow diffusion, penetration</li> <li>• loading, packing critical to performance</li> <li>• not suitable for reusable plastics</li> </ul>	<ul style="list-style-type: none"> <li>• anhydrous materials, such as oils, greases and powders</li> <li>• laboratory glassware, instruments</li> <li>• closed containers</li> </ul>
Red-heat Flame	<ul style="list-style-type: none"> <li>• oxidation to ashes (burning)</li> </ul>	<ul style="list-style-type: none"> <li>• rapid</li> </ul>	<ul style="list-style-type: none"> <li>• initial contact with flame can produce a viable aerosol</li> <li>• possibility of accidental fire</li> </ul>	<ul style="list-style-type: none"> <li>• inoculating loops, needles</li> </ul>
Incineration	<ul style="list-style-type: none"> <li>• oxidation to ashes (burning)</li> <li>• 1-60 minutes: temperatures may exceed 1000°C</li> </ul>	<ul style="list-style-type: none"> <li>• reduces volume of waste by up to 95%</li> </ul>	<ul style="list-style-type: none"> <li>• improper use may lead to emission of pathogens in smoke</li> <li>• requires transport of infectious waste</li> <li>• excess plastic (&gt;20%) content reduces combustibility</li> </ul>	<ul style="list-style-type: none"> <li>• for decontamination of waste items prior to disposal in landfill</li> </ul>
Moist Heat	Irreversible	More rapid		

	coagulation of (microbial) proteins	and more effective than dry heat		
Pasteurization	<ul style="list-style-type: none"> <li>heating to below boiling point (generally 77°C) for up to 30 minutes</li> </ul>	<ul style="list-style-type: none"> <li>can be used on heat sensitive liquids and medical devices</li> <li>low cost</li> </ul>	<ul style="list-style-type: none"> <li>not reliably sporicidal</li> </ul>	<ul style="list-style-type: none"> <li>milk and dairy products</li> <li>some heat-sensitive medical equipment</li> </ul>
Tyndallization (Fractional Sterilization)	<ul style="list-style-type: none"> <li>heating to 80-100°C for 30 mins on successive days, with incubation periods in between</li> </ul>	<ul style="list-style-type: none"> <li>resistant spores germinate and are killed on the second and third days</li> </ul>	<ul style="list-style-type: none"> <li>time consuming</li> <li>not reliably sporicidal</li> </ul>	<ul style="list-style-type: none"> <li>heat sensitive materials such as bacteriologic media, solutions of chemicals, biological materials</li> </ul>
Boiling	<ul style="list-style-type: none"> <li>maximum temperature obtainable is approximately 100°C 10-30 mins</li> </ul>	<ul style="list-style-type: none"> <li>minimal equipment required</li> </ul>	<ul style="list-style-type: none"> <li>cumbersome: not practical for everyday lab use</li> <li>not reliably sporicidal</li> </ul>	<ul style="list-style-type: none"> <li>small instruments and equipment</li> </ul>
autoclaving	<ul style="list-style-type: none"> <li>steam under pressure</li> <li>121°C/15 psi for 15-90 mins (gravity displacement autoclave)</li> <li>132°C/27 psi for 4-20 minutes (pre-vacuum autoclave)</li> </ul>	<ul style="list-style-type: none"> <li>minimal time required</li> <li>most dependable sterilant for lab use</li> </ul>	<ul style="list-style-type: none"> <li>loading and packing critical to performance</li> <li>shielding dirt must first be removed</li> <li>maintenance and quality control essential</li> <li>damages heat-sensitive times</li> </ul>	<ul style="list-style-type: none"> <li>penetration of sterile glassware, media and instruments</li> <li>decontamination of reusable supplies and equipment</li> <li>decontamination of infectious waste</li> </ul>

## 6.2.2 Other Physical Agents of Sterilization and Disinfection

### 6.2.2.1 Ultraviolet Light (Germicidal Lamps)

The light (approximately 260 nm wavelength) emitted by UV lamps is germicidal, and can be used to reduce the number of pathogenic microorganisms on exposed surfaces and in air.

However, UV light has poor penetrating power; accumulations of dust, dirt, grease or clumps of microorganisms may shield microorganisms from the direct exposure required for destruction.

UV light presents skin and eye burn hazard, and factors such as lamp age and poor maintenance can reduce performance. For safe and reliable use of germicidal lamps:

Clean the bulb at least every 2 weeks; turn off power and wipe with an alcohol-moistened cloth.

Blue light output is not an indication of the lamp's effectiveness; measure radiation output at least twice yearly with a UV meter or replace the bulb when emission declines to 70% of its rated output.

Post warning signs to discourage personnel from entering areas where there is potential exposure to UV light.

Wear UV protective goggles, caps, gowns and gloves in rooms with UV installations.

#### 6.2.2.2 Miscellaneous Physical Methods

The procedures listed below are included for the reader's interest:

Infrared radiation: used for heat treatment of small metal and glass items.

Microwaves: used for treatment of liquids, nonmetallic objects, and biohazardous waste.

Gamma irradiation: disrupts DNA and RNA in living organisms, and is used by hospital and laboratory suppliers for materials that do not tolerate heat and pressure (i.e., autoclaving) or chemical treatments.

Membrane filtration: physically removes particulates (e.g., microorganisms) from heat-sensitive pharmaceutical and biological fluids. The size of the particles removed is determined by the pore size of the filter membrane.

### 6.3 Chemical Sterilants and Disinfectants

Instruments or materials which cannot withstand sterilization in a steam autoclave or dry-air oven can be sterilized with a gas such as ethylene oxide or a broad spectrum liquid chemical germicide. Chemical decontamination of surfaces may also be necessary for very large or fixed items. Since liquid chemical germicides generally require high concentrations and several hours of exposure time for sterilization purposes, they are usually used for disinfection rather than for sterilization purposes. The majority of chemical disinfectants have toxic properties: follow the manufacturer's directions for use and wear the appropriate personal protective equipment (e.g., gloves, eye protection, apron), especially when handling stock solutions.

Choice of a chemical germicide for use on contaminated equipment, supplies, laboratory surfaces or biohazardous waste depends upon a number of factors, including:

number and nature of microbes to be destroyed (e.g., spores vs vegetative cells, bacteria vs viruses),

type and configuration of item to be disinfected (fissures, crevices and enclosures may shield organisms),

purpose of treatment (e.g., disinfection vs sterilization),

interaction with other active chemicals,

whether the item is covered with soil which might inactivate the disinfectant,

contact time required for disinfection,

toxicity to individuals, culture systems, environment, residual toxicity on items,

pH, temperature, hardness of available dilution water,

cost.

Direct contact between germicide and microorganism is essential for disinfection.

Microorganisms can be shielded within air bubbles or under dirt, grease, oil, rust or clumps of microorganisms. Agar or proteinaceous nutrients and other cellular material can, either directly (through inactivation of the germicide) or indirectly (via physical shielding of microorganisms) reduce the efficacy of some liquid germicides.

No one chemical germicide is effective for all disinfection or sterilization purposes. A summary of chemical germicides, their use, effective concentrations, advantages and disadvantages can be found in Tables 3, 4A and 4B.

TABLE 3 - Summary of concentrations used, contact times, advantages and disadvantages and uses of some of the halogen-releasing chemical germicides. The wide ranges of effective concentrations and contact times cited are due to a number of factors, including the interdependence of time and concentration, the variability in resistance of different microorganisms, the amount of organic material present and the desired effect (e.g., low-level vs high-level disinfection)

	Effective Concentrations, Contact Times	Advantages	Disadvantages	Examples of Uses
Chlorine Compounds: Sodium hypochlorite solution <sup>1</sup> (liquid bleach)	<ul style="list-style-type: none"> <li>• 100-10,000 ppm (.01-1%) free chlorine</li> <li>• 10-60 minutes (&gt;= 3,000 ppm for broad spectrum)</li> </ul>	<ul style="list-style-type: none"> <li>• broad spectrum</li> <li>• inexpensive</li> <li>• widely available</li> <li>• bactericidal at low temperature</li> </ul>	<ul style="list-style-type: none"> <li>• toxic, corrosive to skin and metals</li> <li>• unstable at optimum effective pH of 6</li> <li>• inactivated by organic matter</li> <li>• deteriorates under light and heat: shelf life of dilutions is less than 1 week</li> </ul>	<ul style="list-style-type: none"> <li>• general disinfectant</li> <li>• waste liquids</li> <li>• surface decontamination</li> <li>• emergency spill clean up</li> <li>• instrument disinfection</li> </ul>
Calcium hypochlorite <sup>2</sup> granules, powder, tablets	<ul style="list-style-type: none"> <li>• as for liquid bleach</li> </ul>	<ul style="list-style-type: none"> <li>• as for liquid bleach but more stable</li> </ul>	<ul style="list-style-type: none"> <li>• as for liquid bleach above, except shelf life is longer</li> </ul>	<ul style="list-style-type: none"> <li>• as for liquid bleach</li> </ul>
NaDCC <sup>3</sup> (Sodium dichloroisocyanurate) powder, granules, tablets	<ul style="list-style-type: none"> <li>• as for liquid bleach</li> </ul>	<ul style="list-style-type: none"> <li>• more stable than hypochlorites</li> <li>• stable at pH 6.0</li> </ul>	<ul style="list-style-type: none"> <li>• toxic, corrosive</li> <li>• inactivated by organic matter</li> </ul>	<ul style="list-style-type: none"> <li>• as for liquid bleach</li> </ul>
Chloramine-T <sup>4</sup> (Sodium tosylchloramide) powder or tablets	<ul style="list-style-type: none"> <li>• as for liquid bleach</li> </ul>	<ul style="list-style-type: none"> <li>• more stable, less affected by organic matter than hypochlorites</li> <li>• longer activity than</li> </ul>	<ul style="list-style-type: none"> <li>• deteriorates under humidity, light and heat</li> </ul>	<ul style="list-style-type: none"> <li>• as for liquid bleach</li> </ul>

		hypochlorites		
Chlorine dioxide <sup>5</sup>	<ul style="list-style-type: none"> <li>• demand-release of chlorine dioxide in situ</li> </ul>	<ul style="list-style-type: none"> <li>• longer activity than other chlorine compounds</li> <li>• less corrosive, less toxic than other chlorine compounds</li> <li>• effective at pH 6-10</li> </ul>	<ul style="list-style-type: none"> <li>• aqueous solutions decompose under light</li> </ul>	<ul style="list-style-type: none"> <li>• instrument disinfection</li> <li>• gas sterilization of germ-free animal chambers</li> </ul>
Iodine Preparations: Iodophors <sup>6</sup>	<ul style="list-style-type: none"> <li>• 30-1,000 ppm (.003-.1%) free iodine</li> <li>• 10-30 minutes</li> </ul>	<ul style="list-style-type: none"> <li>• broad spectrum</li> <li>• germicidal over a wide pH range</li> <li>• generally nonstaining, less toxic and less irritating than aqueous or alcoholic iodine solutions</li> </ul>	<ul style="list-style-type: none"> <li>• not consistently sporicidal</li> <li>• efficacy reduced by organic matter</li> <li>• some iodophor solutions support growth of <i>Pseudomonas</i><sup>7</sup></li> </ul>	<ul style="list-style-type: none"> <li>• germicidal soaps and antiseptics</li> <li>• surface decontamination</li> <li>• work surface wipedown</li> <li>• instrument disinfection</li> </ul>

Notes:

<sup>1</sup> a 1/10 dilution of 5.25% bleach provides 5,250 ppm available chlorine

<sup>2</sup> "high tested" provides 70-72% available chlorine; chlorinated lime or bleaching powder provides approximately 35% available chlorine

<sup>3</sup> approximately 60% available chlorine

<sup>4</sup> approximately 25% available chlorine

<sup>5</sup> To avoid shipping of this extremely reactive product, reagents ("base" and "activator") from commercially available kits are mixed with water to generate chlorine dioxide immediately prior to use

<sup>6</sup> 10% povidone-iodine provides 1% available iodine

<sup>7</sup> An iodophor stock solution may actually be a less effective germicide than its dilution. For example, a full-strength (10%) solution of povidone-iodine provides approximately 10 times less free available iodine than a 1/100 dilution. Iodophors must be used at the manufacturer's recommended concentrations.

TABLE 4A Summary of recommended concentrations, contact times, advantages and disadvantages of non-halogen chemical germicides. The wide ranges of effective concentrations and contact times cited reflect the interdependence of time and concentration as well as factors such as resistance of the particular class or strain of target microorganism(s) and desired effect. Also, some germicides are available in combinations (e.g., glutaraldehyde/phenol or peracetic acid/alcohol mixtures) which are synergistic whereby the components in combination produce a greater antimicrobial effect than the sum of their individual effects.

	Effective Concentrations and Contact Times	Advantages	Disadvantages	Examples of Laboratory Uses
Alcohols	<ul style="list-style-type: none"> <li>• 70-80% ethanol</li> <li>• 60-95% isopropanol</li> <li>• 10-30 minutes</li> </ul>	<ul style="list-style-type: none"> <li>• low toxicity</li> <li>• rapid action</li> <li>• low residue</li> <li>• non-corrosive</li> </ul>	<ul style="list-style-type: none"> <li>• rapid evaporation limits contact time</li> <li>• flammable, eye irritant</li> <li>• may damage rubber, plastic, shellac</li> <li>• ineffective against bacterial spores</li> </ul>	<ul style="list-style-type: none"> <li>• skin disinfectant (antiseptic)</li> <li>• surface decontamination</li> <li>• benchtop, cabinet wipedown</li> </ul>
Phenolic Compounds	<ul style="list-style-type: none"> <li>• 400-50,000 ppm (.05-1.5%)</li> <li>• 10-30 minutes</li> </ul>	<ul style="list-style-type: none"> <li>• tolerant of organic load, "hard" dilution water</li> <li>• leaves an active residue (may be desirable on some surfaces)</li> <li>• biodegradable</li> </ul>	<ul style="list-style-type: none"> <li>• pungent odour, corrosive, some forms toxic</li> <li>• not sporicidal; limited activity against viruses</li> <li>• leaves a residual film (undesirable in culture systems)</li> <li>• may support growth of bacteria<sup>1</sup></li> </ul>	<ul style="list-style-type: none"> <li>• instruments and equipment disinfection</li> <li>• disinfection of floors and other surfaces</li> <li>• antiseptic soaps and lotions</li> </ul>
Quaternary Ammonium Compounds	<ul style="list-style-type: none"> <li>• 500-15,000 ppm (.05-1.5%)</li> <li>• 10-30 minutes</li> </ul>	<ul style="list-style-type: none"> <li>• combined detergent and germicidal activity</li> </ul>	<ul style="list-style-type: none"> <li>• non sporicidal, limited activity against viruses,</li> </ul>	<ul style="list-style-type: none"> <li>• surface decontamination</li> <li>• equipment wipedown</li> </ul>

		<ul style="list-style-type: none"> <li>• stable</li> <li>• working dilutions have low toxicity</li> </ul>	<p>mycobacteria</p> <ul style="list-style-type: none"> <li>• most formulations not readily biodegradable</li> <li>• may support growth of bacteria<sup>2</sup></li> </ul>	<ul style="list-style-type: none"> <li>• antiseptic formulations available</li> <li>• floors and walls</li> </ul>
Hydrogen Peroxide	<ul style="list-style-type: none"> <li>• 3-30% aqueous solution</li> <li>• 10-60 minutes</li> <li>• 6% for 30 minutes may kill spores</li> </ul>	<ul style="list-style-type: none"> <li>• rapid action</li> <li>• no residue</li> <li>• low toxicity</li> <li>• environmentally safe</li> </ul>	<ul style="list-style-type: none"> <li>• limited sporicidal activity</li> <li>• corrosive to some metals</li> <li>• potentially explosive at high concentrations</li> <li>• stock solutions irritating to skin and eyes</li> </ul>	<ul style="list-style-type: none"> <li>• surface decontamination</li> <li>• instruments and equipment</li> </ul>
Peracetic Acid (PAA)	<ul style="list-style-type: none"> <li>• .001-.3% aqueous solution</li> <li>• gas phase: 2-4%</li> <li>• 5-120 minutes</li> </ul>	<ul style="list-style-type: none"> <li>• broad spectrum</li> <li>• sporicidal at low temperatures</li> <li>• can tolerate organic load</li> <li>• rapid action</li> <li>• nontoxic decomposition products</li> <li>• leaves no residue</li> </ul>	<ul style="list-style-type: none"> <li>• pungent odor</li> <li>• corrosive to some metals</li> <li>• shelf life of dilutions is less than 1 week</li> <li>• stock solutions irritating to skin and eyes</li> <li>• stock must be protected from heat, light</li> <li>• gas phase: respiratory irritant, fire hazard above 55°C</li> </ul>	<ul style="list-style-type: none"> <li>• instruments and equipment</li> <li>• gas phase sterilization of chambers for germ-free animals</li> </ul>

Table 4B - Summary of recommended concentrations, contact times, advantages and disadvantages of non-halogen chemical germicides. The wide ranges of effective concentrations and contact times cited reflect the interdependence of time and concentration as well as factors such as resistance of the particular class or strain of target microorganism(s) and desired effect. Also, some germicides are available in combinations (e.g., glutaraldehyde/phenol or peracetic acid/alcohol mixtures) which are synergistic whereby the components in combination produce a greater antimicrobial effect than the sum of their individual effects.

	Effective Concentrations and Contact Times	Advantages	Disadvantages	Examples of Laboratory Uses
Aldehydes:				
Glutaraldehyde	<ul style="list-style-type: none"> <li>• 0.5-2.5% alkalized aqueous solution</li> <li>• 2-30 mins; up to 12 hours to kill all spores</li> </ul>	<ul style="list-style-type: none"> <li>• broad spectrum</li> <li>• does not corrode metal</li> <li>• can tolerate organic load</li> </ul>	<ul style="list-style-type: none"> <li>• expensive</li> <li>• pH, temperature dependent</li> <li>• pungent odor</li> <li>• toxic: skin, eye, respiratory tract irritant</li> <li>• activated solutions have less than 2-week shelf life</li> </ul>	<ul style="list-style-type: none"> <li>• cold sterilant and fixative</li> <li>• surface decontamination</li> <li>• instruments, equipment, glassware</li> </ul>
Formalin (37% aqueous formaldehyde)	<ul style="list-style-type: none"> <li>• 3-27% formalin (1-10% formaldehyde) in 70-90% alcohol</li> <li>• 10-30 minutes</li> </ul>	<ul style="list-style-type: none"> <li>• broad spectrum</li> <li>• inexpensive</li> <li>• does not corrode metal</li> <li>• can tolerate organic load</li> </ul>	<ul style="list-style-type: none"> <li>• pungent odor</li> <li>• skin, eye and respiratory tract irritant</li> <li>• potential carcinogen (animal studies)</li> <li>• may require 24 hrs or more to kill all spores</li> </ul>	<ul style="list-style-type: none"> <li>• cold sterilant and fixative</li> <li>• surface decontamination</li> <li>• instruments and equipment</li> </ul>
Formaldehyde (gas)	<ul style="list-style-type: none"> <li>• 1-3 hours</li> </ul>	<ul style="list-style-type: none"> <li>• as for formalin</li> <li>• effective penetration</li> </ul>	<ul style="list-style-type: none"> <li>• as for formalin</li> <li>• flammable</li> <li>• poor penetration of covered surfaces</li> </ul>	<ul style="list-style-type: none"> <li>• on site decontamination of biological safety cabinet HEPA filters</li> <li>• enclosed areas</li> </ul>



Ethylene Oxide Gas	<ul style="list-style-type: none"> <li>• 50-1200 mg/L</li> <li>• 1-12 hours</li> </ul>	<ul style="list-style-type: none"> <li>• broad spectrum</li> <li>• no heat or moisture evolved</li> <li>• penetrates packaging materials</li> </ul>	<ul style="list-style-type: none"> <li>• flammable, reactive</li> <li>• toxic: potential carcinogen and mutagen</li> <li>• some sterilized items may need more than 24 hours for outgassing</li> </ul>	<ul style="list-style-type: none"> <li>• heat or moisture sensitive supplies, instruments and equipment</li> </ul>
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## APPENDIX 5

***Biosafety in Microbiological and Biomedical Laboratories***  
<http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>

Table 1 Summary of recommended biosafety levels for infectious agents

Biosafety level	Agents	Practices	Safety Equipment (primary barriers)	Facilities (secondary barriers)
1	Not known to cause disease in healthy adults.	Standard Microbiological Practices	None required	Open bench top sink required
2	Associated with human disease, hazard = auto-inoculation, ingestion, mucous membrane exposure	BSL-1 practice plus: <ul style="list-style-type: none"> <li>◆ Limited access</li> <li>◆ Biohaz. warning sign</li> <li>◆ Sharps precautions</li> <li>◆ Bio Manual defining any needed waste decontamination or med. Surveillance policies.</li> </ul>	Primary barriers= class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPE: lab coats, gloves, face protection as needed.	BSL-1 plus: recommend autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences.	BSL-2 practices plus: Controlled access Decontamination of all waste Decontamination of lab clothing before laundering <ul style="list-style-type: none"> <li>◆ Baseline serum</li> </ul>	Primary barriers = class I or II BSCs or other physical containment devices used for all manipulations of agents; PPEs: protective lab clothing; gloves; respiratory protection as needed	BSL-2 plus: <ul style="list-style-type: none"> <li>◆ Physical separation from access corridors</li> <li>◆ Self-closing, double door access</li> <li>◆ Exhausted air not recirculated</li> <li>◆ Negative airflow into lab.</li> </ul>
4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission.	BSL3 practices plus: <ul style="list-style-type: none"> <li>◆ Clothing change before entering</li> <li>◆ Shower on exit</li> <li>◆ All material decontaminated on exit from facility.</li> </ul>	Primary barriers =All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit.	BSL 3 plus: <ul style="list-style-type: none"> <li>◆ Separate building or isolated zone</li> <li>◆ Dedicated supply/exhaust, vacuum and decon systems</li> <li>◆ Other requirements outlined in the text.</li> </ul>

## BIOSAFETY LEVEL 1

Includes Class 1 Agents - Agents of minimal hazard under ordinary conditions of handling.

Biosafety Level 1 is suitable for work involving well-characterized agents that are not known to consistently cause disease in immunocompetent adult humans and that present minimal potential hazard to laboratory personnel and the environment. However, many agents not ordinarily associated with disease processes in humans are opportunistic pathogens and may cause infection in the young, the aged, immunocompromised individuals, and after accidental inoculation or exposure, such as a splash into the eyes. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

The following standard practices, safety equipment, and facility requirements apply to BSL-1.

### *A. Standard Microbiological Practices*

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory. All UA research labs should be restricted to lab personnel and approved visitors only.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. It is the policy of The University of Alabama that non-infectious sharps and broken glass should be collected in a puncture resistant container, sealed, labeled, and taken to the nearest dumpster location associated with each building. Housekeeping staff does not handle any type of broken glass. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
  - a. Careful management of needles and other sharps is of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
  - b. Used disposable needles and syringes must be carefully placed in conveniently located clearly labeled puncture-resistant containers used for sharps disposal.
  - c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
  - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with disinfectant appropriate for the agent or project. Disinfectants should be noted on the project registration.

8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an approved effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport.

a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use and the name and phone number of the laboratory supervisor or other responsible personnel. Door signage is provided by EHS and shows signs and symptoms of exposure.

10. An effective integrated pest management program is required. 11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance. (see also F. Training)

#### *B. Special Practices*

None required.

#### *C. Safety Equipment (Primary Barriers and Personal Protective Equipment)*

1. Special containment devices or equipment, such as biological safety cabinets (BSCs), are not generally required.

2. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

3. Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.

4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment, typically performed by the lab PI and reviewed by the BSO. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers should:

a. Change gloves when contaminated, when glove integrity is compromised, or when otherwise necessary.

b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

#### *D. Laboratory Facilities (Secondary Barriers)*

1. Laboratories should have doors for access control.

2. Laboratories must have a sink for hand washing.

3. The laboratory should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
  - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. Laboratories with windows that open to the exterior should be fitted with screens.

#### D. BIOSAFETY LEVEL 2

Includes Class 2 Agents - Agents of ordinary potential hazard.

Biosafety Level 2 practices, equipment and facilities are applicable to clinical, diagnostic and teaching laboratories. Biosafety level 2 is appropriate when work is done with any human-derived blood, body fluids or tissues where the presence of an infectious agent may be unknown. (Refer to Bloodborne Pathogen Policy for specific details.) Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that: 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in Biological Safety Cabinets (BSCs) or other physical containment equipment. The following standard and special practices, safety equipment, and facility requirements apply to BSL-2.

##### A. *Standard Microbiological Practices*

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory. All UA research labs should be restricted to lab personnel and approved visitors only.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. It is the policy of The University of Alabama that non-infectious sharps and broken glass should be collected in a puncture resistant container, sealed, labeled, and taken to the nearest dumpster location associated with each building. Housekeeping staff does not handle any type of broken glass. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

- a. Careful management of needles and other sharps is of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
- b. Used disposable needles and syringes must be carefully placed in conveniently located clearly labeled puncture-resistant containers used for sharps disposal.
- c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
- d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. All decontamination methods must be included in the lab safety registration forms and reviewed by the Institutional Biosafety Committee. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
  - a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
  - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Door signage is provided by EHS and shows signs and symptoms of exposure.
10. An effective integrated pest management program is required.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance. (see also M. Training)

#### *B. Special Practices*

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements. These are dependent upon the materials being used and the experimental procedures.
2. Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
3. When necessary labs should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.



6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should have a routine disinfection schedule. Equipment should also be disinfected after spills, splashes, or other potential contamination.
  - a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
  - b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

*C. Safety Equipment (Primary Barriers and Personal Protective Equipment)*

1. Properly maintained BSCs, other appropriate personal protective equipment, or other physical containment devices must be used whenever:
  - a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
  - b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
2. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas, e.g., cafeteria, library, and administrative offices. Dispose of protective clothing appropriately or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.
3. Eye and face protection (goggles, masks, face shields or other splatter guards) are used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
  - a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
  - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
  - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

*D. Laboratory Facilities (Secondary Barriers)*

UA IBC requires that all new construction comply with the most recent NIH design guidelines, <http://orf.od.nih.gov/PoliciesAndGuidelines/BiomedicalandAnimalResearchFacilitiesDesignPoliciesandGuidelines/Pages/policy-index.aspx>, to the extent possible and applicable.

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies. Accessibility is determined by each department in compliance with approved procedures from Access Control.
2. Laboratories must have a sink for hand washing. The sink may be manually activated, hands-free, or automatically operated. It should be located near the exit door.
3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
  - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines should be protected with liquid disinfectant traps.
8. An eyewash station must be readily available.
9. There are no specific requirements for ventilation systems. However, NIH guidelines state that planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
10. HEPA filtered exhaust air from a Class II BSC can be safely recirculated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection per latest version of NSF 49 Standard. Provisions to assure proper safety cabinet performance and air system operation must be verified. Contact EHS for guidance on purchasing, training, and usage of all Biological Safety Cabinets.
11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

**E. BIOSAFETY LEVEL 3**

Class 3 Agents - Agents that cause disease which may have serious or lethal consequences. Agents involving special hazards, or agents derived from outside the United States, that require a federal permit for importation, unless they are specified for higher classification. This class includes pathogens that require special conditions for containment. Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease



through the inhalation route of exposure. 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.

All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices.

A BSL-3 laboratory has special engineering and design features.

The following standard and special safety practices, equipment, and facility requirements apply to BSL-3.

#### *A. Standard Microbiological Practices*

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory. Unauthorized personnel are not allowed into the lab area and approved visitors must be escorted at all times.

2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

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

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

6. Perform all procedures to minimize the creation of splashes and/or aerosols.

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. All decontamination methods must be included in the lab safety registration forms and approved by the Institutional Biosafety Committee. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). Depending on where the decontamination will be performed, the following methods should be used prior to transport:

a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

- b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Door signage is provided by EHS and shows signs and symptoms of exposure.
10. An effective integrated pest management program is required.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

#### *B. Special Practices*

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements. It is the responsibility of the PI and lab personnel to inform visitors entering the area of any hazards or additional requirements.
2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents. This must be documented.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
  - a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
  - b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal

protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor must be used.

### *C. Safety Equipment (Primary Barriers and Personal Protective Equipment)*

1. All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.
2. Workers in the laboratory wear protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.
3. Eye and face protection (goggles, mask, face shield or other splash guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers:
  - a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
  - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
  - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. Eye, face, and respiratory protection must be used in rooms containing infected animals.

### *D. Laboratory Facilities (Secondary Barriers)*

1. Laboratory doors must be self-closing and have locks in accordance with the institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Laboratory access is restricted. Access to the laboratory is through two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.
2. Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.
3. The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.
  - a. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.
  - b. Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.

c. Ceilings should be constructed, sealed, and finished in the same general manner as walls.

Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment.

4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.

a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

5. All windows in the laboratory must be sealed.

6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

7. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.

8. An eyewash station must be readily available in the laboratory.

9. A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.

a. Laboratory personnel must be able to verify directional airflow. A visual monitoring device, which confirms directional airflow, must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.

b. The laboratory exhaust air must not re-circulate to any other area of the building.

c. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.

HEPA filter housings should have gas-tight isolation dampers, decontamination ports, and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer’s recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.

11. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, or other validated decontamination method).

12. Equipment that may produce infectious aerosols must be contained in primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.

13. Facility design consideration should be given to the best method for decontaminating large pieces of equipment before removal from the laboratory.

14. Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following: an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices, such as biometrics.

15. The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.

#### F. BIOSAFETY LEVEL 4

Class 4 Agents - Agents that require the most stringent conditions for their containment because they are extremely hazardous to laboratory personnel or may cause serious epidemic diseases. Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or re-designate the level. Class 4 agents may not be used at the University of Alabama. Contact EHS for more information.

See Table 1 in Appendix 5 for summary of recommended biosafety levels for infectious agents.

## APPENDIX 6

### *Biohazard Spill Clean-Up Procedures*

Since spills of biological materials will happen, it is important to be prepared prior to dealing with the problem. Laboratories working with biohazards should have a basic biological spill kit ready to use at all times. For most instances the basic kit can be assembled with materials already used in the laboratory. Although it is preferable to have the contents of the spill kit in one location, as long as the materials are easily accessible and their location communicated to everyone in the lab, prior assembly might not be necessary. However, ready assembled spill kits are available through various vendors. EHS can assist you with identifying a proper spill kit.

#### Basic Biological Spill Kit:

Disinfectant (e.g., bleach 1:10 dilution, prepared fresh)

Absorbent Material (e.g., paper towels)

Waste Containers (e.g., biohazard bags, sharps containers)

Personal Protective Equipment (e.g., lab coat, gloves, eye and face protection)

Mechanical Tools (e.g., forceps, dustpan and broom)

The following procedures are provided as a guideline to biohazardous spill clean-up and will need to be modified for specific situations. As with any emergency situation, stay calm, call 911 if necessary, and proceed with caution. Call EHS at 348-5905 if further assistance is required, especially if the spill outgrows the resources in the laboratory.

#### Spill Inside the Laboratory (BL-2, RG-2)

Clear spill area of all personnel. Wait for any aerosols to settle before entering spill area.

Remove any contaminated clothing and place in biohazard bag for further decontamination processing. Don a disposable gown or lab coat, safety goggles and gloves.

Have a complete biological spill kit ready to go before you start the clean-up.

Initiate cleanup with disinfectant as follows:

Cover spill with paper towels or other absorbent material containing disinfectant.

Encircle the spill with disinfectant (if feasible and necessary), being careful to minimize aerosolization.

Decontaminate and remove all items within spill area.

Remove broken glassware with forceps or broom and dustpan and dispose in sharps container.

Do not pick up any contaminated sharp object with your hands.

Remove paper towels and any other absorbent material and dispose in biohazard bags.

Apply disinfectant to the spill area and allow for at least 10 minutes contact time to ensure germicidal action of disinfectant.

Remove disinfectant with paper towels or other absorbent material and dispose of in biohazard bag.

Wipe off any residual spilled material and reapply disinfectant before final clean-up.

Wipe equipment with equipment compatible disinfectant (e.g., non-corrosive). Rinse with water if necessary.

Place disposable contaminated spill materials in biohazard bags for autoclaving.

Place contaminated reusable items in biohazard bags, or heat resistant pans or containers with lids before autoclaving.

Reopen area to general use only after spill clean-up and decontamination is complete.



Inform all personnel and laboratory supervisor about the spill and successful clean-up as soon as possible.

#### Spill Inside the Biological Safety Cabinet (BL-2, RG-2)

Have a complete biological spill kit ready to go before you start the clean-up.

Wear lab coat, safety goggles and gloves during clean-up.

Allow cabinet to run during clean-up.

Initiate cleanup with disinfectant as follows:

Soak up spilled material with disposable paper towels (work surface and drain basin) and apply disinfectant with a minimum of 10 minutes contact time.

Wipe up spillage and disinfectant with disposable paper towels.

Wipe the walls, work surface and any equipment in the cabinet with a disinfectant soaked paper towel.

Discard contaminated disposable materials in biohazard bag(s) and autoclave before discarding as waste.

Place contaminated reusable items in biohazard bags, or heat resistant pans or containers with lids before autoclaving and further clean-up.

Expose non-autoclavable materials to disinfectant, 10 minutes contact time, before removal from the BSC.

Remove protective clothing used during cleanup and place in a biohazard bag for decontamination.

Run cabinet at least 10 minutes after clean-up and before resuming work.

Inform all users of the BSC as well as the laboratory supervisor about the spill and successful clean-up as soon as possible.

#### Spill Outside the Laboratory, During Transport on Campus.

Always transport biohazardous materials in an unbreakable well-sealed primary container placed inside a leak proof, closed and unbreakable secondary container, labeled with the biohazard symbol (plastic cooler, bio-specimen pack, etc.).

Should a spill of BL-2 material occur in the public, contact EHS at 348-5905. Do not attempt to clean up the spill without the proper personal protective equipment and spill clean-up material.

Should the spill occur inside a car, leave the vehicle, close all doors and windows, and contact EHS at 348-5905 for assistance.

## APPENDIX 7

### *Shipping/Receiving Biological Material*

Transportation rules and regulations are designed to protect the shipper, carrier, public, receiver, and the environment from the point of origin to the destination. The correct and legal transport of dangerous goods is the direct responsibility of the shipper. Anyone shipping hazardous materials, including infectious or potentially infectious material, is subject to regulations promulgated by DOT, IATA, and the country of origin (for international shipments). Training is required for anyone involved in the transport of dangerous goods. EHS maintains certifications to ship all classes of hazardous materials. For more information or for assistance with a shipment, contact EHS at 348-5912.

Note: Importing any biological materials into the US generally requires an import permit from the United States Department of Agriculture and the Centers for Disease Control and Prevention. For certain species, other branches of the government may be involved. The shipper is responsible for obtaining all appropriate permits and for assuring that shipments are packaged and handled according to current regulations. If a UA lab supervisor expects receipt of a package of infectious materials, it is prudent that he/she ask for documentation that these items have been addressed.

#### **PACKAGING AND TRANSPORTATION OF BIOLOGICAL MATERIALS ON AND OFF SITE**

All biological materials shall be packaged and transported in a way that maintains the integrity of the material during normal transport conditions and thus helps to ensure the safety of employees, the public and the environment.

#### **Transportation BETWEEN buildings and floors on buildings:**

When transporting diagnostic and clinical specimens (both human and animal samples), biohazardous materials and recombinant DNA molecules the following guidelines should be followed:

Samples need to be packaged in a sealed, leak proof primary container (e.g., plastic screw-top conical tube), which is securely positioned in a secondary leak proof and closable container (e.g., cooler, ice chest). The secondary container shall have a clearly visible biohazard symbol on the outside.

A list of contents as well as emergency information (e.g., PI phone number) needs to be accompanying the material (e.g., attached to the cooler in a plastic pouch).

#### **Transportation and shipment OFF Site:**

The transportation and shipment over public roadways of diagnostic and clinical specimens, biological products, infectious agents and recombinant DNA molecules is regulated by national and international transportation rules. This includes specific procedures for the correct packing and packaging of these materials, necessary documentation and labeling and permits. For more information about specific shipment requirements, contact the Biosafety Officer.

The use of private cars for transport of biological materials is strictly prohibited.



## APPENDIX 8

### ***PREVENTING TRANSMISSION OF ZOO NOTIC DISEASES Risks for Those Who Handle Animals and Their Tissues***

Hazards associated with handling animals fall into three categories:

Physical injuries can occur from bites or scratches (rodents, rabbits, dogs, cats, swine, primates and others), kicks or other direct injuries such. The key to prevention of these types of injuries is proper training of personnel by the animal care staff or other qualified individuals.

Allergic hazards can be associated with breathing or contacting allergens found in animal dander or urine. Though some persons are much more susceptible than others, all employees can reduce their risk by wearing protective clothing (such as safety glasses, respirators, gloves and a lab jacket) when handling animals. Additional precautions may be posted on the animal room door.

There is the potential for transmission of zoonotic diseases between animals and humans. Although zoonotic diseases are not common in modern laboratory facilities, the prevention, detection and eradication of zoonotic diseases from the animal facility is a primary concern of the entire animal care staff. The risk for zoonotic diseases may be increased in farm situations. Remember that infected tissues, any body secretion/excretion as well as the living animals can frequently transmit zoonotic diseases.

## APPENDIX 9

### POTENTIALLY INFECTIOUS MATERIAL/BLOODBORNE PATHOGEN POLICY

The potentially infectious material policy affects a variety of areas on campus. Many employees and students may perform tasks that are covered by this policy. By definition covered tasks involves much more than working only with human blood. If an employee or student works with or is potentially exposed to the following materials then they are covered by this policy.

- Blood which included human blood, human blood components and products made from human blood.
- Human body fluids including: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures and any body fluid visibly contaminated with blood.
- Any unfixed tissue or organ (other than intact skin) from a human living or dead.
- Cell, tissue or organ cultures from humans or experimental animals unless known to be free of bloodborne pathogens.
- Blood, organs, or other tissues from experimental animals unless known to be free of bloodborne pathogens.
- Culture medium unless known to be free of bloodborne pathogens.

Procedures and specifications for working with or handling potentially infectious material are covered in the Bloodborne Pathogen/Potentially Infections Material Program document. This program encompasses persons who work in jobs or as a part of their academic instruction may reasonably be exposed to those materials. This could include police officers, housekeepers, emergency response personnel, health care providers, research laboratory students or employees and others.

The basic elements of the program include:

- Determine if employees or students are at risk.
- Establish an exposure control plan.
- Implement the use of universal precautions.
- Identify and use engineering controls, work practice controls and personal protective equipment.
- Make available hepatitis B vaccinations.
- Make available post exposure evaluation and follow up to those who experience an exposure incident.
- Use labels and signs to communicate hazards.
- Provide training.

For further information as it relates to research or academic laboratories or functions contact EHS at 348-5905 or email

Darren Moss [dross@ehs.ua.edu](mailto:dross@ehs.ua.edu)

Andrea Davidson [adavidson@ehs.ua.edu](mailto:adavidson@ehs.ua.edu)

For information as it relates to Facilities or service areas contact EHS at 348-5905 or email Tammy Trimm [ttrimm@bama.ua.edu](mailto:ttrimm@bama.ua.edu)

## APPENDIX 10

### *Medical Waste Disposal*

Within 90 days of the effective date of this Division, each generator of medical waste shall prepare, maintain, and update as necessary a written plan to ensure proper management of medical waste. This plan must be made available to the Department upon request. This plan shall address the following if applicable to the generators:

a) Type of Medical waste generated: animal, blood, and body fluids, microbiological, pathological, sharps, surgical and medical waste.

b) Proper segregation, packaging and labeling procedures of untreated medical waste intended for off-site transportation: Sharps are placed in puncture proof containers. All RCRA and other non-medical waste is placed on containers other than those designated for medical waste or in the case of RCRA waste handled as appropriate.

c) Treatment method to be utilized on-site: N/A

d) Transporter of any untreated medical waste transported off-site:

Name: West Med

Address: 27 Industrial Park Drive

Woodstock, AL 35188

Telephone: (205)938-3333

Contact: George West

e) Storage Facilities utilized: West Med (same as d) f) Treatment/processing facilities utilized: West Med (same as d)

ADEM Treatment Facility Permit: TRT5120710-0401

g) Disposal Facilities: West Med (same as d)

ADEM Transporter Permit Number: TRN2139201

DOT Identification Number:

h) Frequency of medical waste removed off site: weekly

i) Training of Employees:

a) Steps taken to minimize the exposure of employees to infection agents: UA employees are provided gloves, masks, and other PPE as needed. All employees have received training concerning Bloodborne pathogens and medical waste management.

b) Individual responsible for training: Supervisor of generating area.

j) Each generator of medical waste shall notify the Alabama Department of Environmental Management in writing within 90 days from the effective date of the new regulations (October 2, 1990). This notification shall address the following:

a) Name and address of generator.

b) Name and telephone of responsible person.

c) Street address, including nearest city, of generator's facility.

d) A person who will be in charge of the generation of medical waste after the effective date of the Division must submit the above notification and prepare a written Medical Waste Management Plan prior to initiating any waste generation, treatment, transportation, or disposal activity.

For more information regarding medical waste management on campus contact EHS at 348-5905 or

Jeff Hallman [jhallman@bama.ua.edu](mailto:jhallman@bama.ua.edu)

Tammy Trimm [ttrimm@bama.ua.edu](mailto:ttrimm@bama.ua.edu)

# APPENDIX 11

## RECOMBINANT DNA (rDNA) And SYNTHETIC DNA

Recombinant DNA (rDNA) work is addressed under the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules. The purpose of the *NIH Guidelines* is to specify practices for constructing and handling: (i) recombinant deoxyribonucleic acid (DNA) molecules and (ii) organisms and viruses containing recombinant DNA molecules. In the context of the *NIH Guidelines*, recombinant DNA molecules are defined as either: (i) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell or (ii) molecules that result from the replication of those described in (i) above. Synthetic DNA segments which are likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) are considered as equivalent to their natural DNA counterpart. If the synthetic DNA segment is not expressed *in vivo* as a biologically active polynucleotide or polypeptide product, it is exempt from the *NIH Guidelines*. Genomic DNA of plants and bacteria that have acquired a transposable element, even if the latter was donated from a recombinant vector no longer present, are not subject to the *NIH Guidelines* unless the transposon itself contains recombinant DNA.

As a condition for NIH funding of recombinant DNA research, institutions shall ensure that such research conducted at or sponsored by the institution, irrespective of the source of funding, shall comply with the *NIH Guidelines*. Information concerning noncompliance with the *NIH Guidelines* may be brought forward by any person. It should be delivered to both NIH/OBA and the relevant institution. The institution, generally through the Institutional Biosafety Committee, shall take appropriate action. The institution shall forward a complete report of the incident recommending any further action to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985, 301-496-9838/301-496-9839 (fax) (for non-USPS mail, use zip code 20817). In cases where NIH proposes to suspend, limit, or terminate financial assistance because of noncompliance with the *NIH Guidelines*, applicable Department of Health and Human Services and Public Health Service procedures shall govern.

The policies on compliance are as follows: Section I-D-1. All NIH-funded projects involving recombinant DNA techniques must comply with the *NIH Guidelines*. Non-compliance may result in (i) suspension, limitation, or termination of financial assistance for the noncompliant NIH-funded research project and of NIH funds for other recombinant DNA research at the institution or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the institution. Section I-D-2. All non-NIH funded projects involving recombinant DNA techniques conducted at or sponsored by an institution that receives NIH funds for projects involving such techniques must comply with the *NIH Guidelines*. Noncompliance may result in (i) suspension, limitation, or termination of NIH funds for recombinant DNA research at the institution or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the institution.

The following is directly from the NIH Guidelines – all referenced forms and documents refer to NIH forms, not the UA required documentation:

### SECTION III. EXPERIMENTS COVERED BY THE *NIH GUIDELINES*

This section describes six categories of experiments involving recombinant DNA: (i) those that require Institutional Biosafety Committee (IBC) approval, RAC review, and NIH Director approval before initiation

(see [Section III-A](#)), (ii) those that require NIH/OBA and Institutional Biosafety Committee approval before initiation (see [Section III-B](#)), (iii) those that require Institutional Biosafety Committee and Institutional Review Board approvals and RAC review before research participant enrollment (see [Section III-C](#)), (iv) those that require Institutional Biosafety Committee approval before initiation (see [Section III-D](#)), (v) those that require Institutional Biosafety Committee notification simultaneous with initiation (see [Section III-E](#)), and (vi) those that are exempt from the *NIH Guidelines* (see [Section III-F](#)). Note: *If an experiment falls into Sections III-A, III-B, or III-C and one of the other sections, the rules pertaining to Sections III-A, III-B, or III-C shall be followed. If an experiment falls into Section III-F and into either Sections III-D or III-E as well, the experiment is considered exempt from the NIH Guidelines.* Any change in containment level, which is different from those specified in the *NIH Guidelines*, may not be initiated without the express approval of NIH/OBA (see [Section IV-C-1-b-\(2\)](#) and its subsections, *Minor Actions*).

### Section III-A. Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Director Approval Before Initiation

#### Section III-A-1. Major Actions under the *NIH Guidelines*

Experiments considered as *Major Actions* under the *NIH Guidelines* cannot be initiated without submission of relevant information on the proposed experiment to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax), the publication of the proposal in the *Federal Register* for 15 days of comment, review by RAC, and specific approval by NIH. The containment conditions or stipulation requirements for such experiments will be recommended by RAC and set by NIH at the time of approval. Such experiments require Institutional Biosafety Committee approval before initiation. Specific experiments already approved are included in [Appendix D](#), *Major Actions Taken under the NIH Guidelines*, which may be obtained from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

Section III-A-1-a. The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally (see [Section V-B](#), *Footnotes and References of Sections I-IV*), if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture, will be reviewed by RAC.

### Section III-B. Experiments That Require NIH/OBA and Institutional Biosafety Committee Approval Before Initiation

Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH/OBA. The containment conditions for such experiments will be determined by NIH/OBA in consultation with *ad hoc* experts. Such experiments require Institutional Biosafety Committee approval before initiation (see [Section IV-B-2-b-\(1\)](#), *Institutional Biosafety Committee*).

#### Section III-B-1. Experiments Involving the Cloning of Toxin Molecules with LD<sub>50</sub> of Less than 100 Nanograms per Kilogram Body Weight

Deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD<sub>50</sub> of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin). Specific approval has been given for the cloning in *Escherichia coli* K-12 of DNA containing genes coding for the biosynthesis of toxic molecules which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight. Specific experiments already approved under this section may be obtained from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

### Section III-C. Experiments that Require Institutional Biosafety Committee and Institutional Review Board Approvals and RAC Review Before Research Participant Enrollment

#### Section III-C-1. Experiments Involving the Deliberate Transfer of Recombinant DNA, or DNA or RNA Derived from Recombinant DNA, into One or More Human Research Participants

For an experiment involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into human research participants (human gene transfer), no research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) until the RAC review process has been completed (see [Appendix M-I-B](#), *RAC Review Requirements*).

In its evaluation of human gene transfer proposals, the RAC will consider whether a proposed human gene transfer experiment presents characteristics that warrant public RAC review and discussion (See [Appendix M-I-B-2](#)). The process of public RAC review and discussion is intended to foster the safe and ethical conduct of human gene transfer experiments. Public review and discussion of a human gene transfer experiment (and access to relevant information) also serves to inform the public about the technical aspects of the proposal, meaning and significance of the research, and any significant safety, social, and ethical implications of the research.

Public RAC review and discussion of a human gene transfer experiment may be: (1) initiated by the NIH Director; or (2) initiated by the NIH OBA Director following a recommendation to NIH OBA by: (a) three or more RAC members; or (b) a Federal agency other than NIH. After a human gene transfer experiment is reviewed by the RAC at a regularly scheduled meeting, NIH OBA will send a letter, unless NIH OBA determines that there are exceptional circumstances, within 10 working days to the NIH Director, the Principle Investigator, the sponsoring institution, and other DHHS components, as appropriate, summarizing the RAC recommendations.

For a clinical trial site that is added after the RAC review process, no research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) at the clinical trial site until the following documentation has been submitted to NIH OBA: (1) Institutional Biosafety Committee approval (from the clinical trial site); (2) Institutional Review Board approval; (3) Institutional Review Board-approved informed consent document; (4) curriculum vitae of the Principle investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.

In order to maintain public access to information regarding human gene transfer protocols (including protocols that are not publicly reviewed by the RAC), NIH OBA will maintain the documentation described in Appendices M-I through M-V. The information provided in response to [Appendix M](#) should not contain any confidential commercial information or trade secrets, enabling all aspects of RAC review to be open to the public.

Note: For specific directives concerning the use of retroviral vectors for gene delivery, consult [Appendix B-V-1](#), *Murine Retroviral Vectors*.

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

Prior to the initiation of an experiment that falls into this category, the Principle Investigator must submit a registration document to the Institutional Biosafety Committee which contains the following information: (i) the source(s) of DNA; (ii) the nature of the inserted DNA sequences; (iii) the host(s) and vector(s) to be used; (iv) if an attempt will be made to obtain expression of a foreign gene, and if so, indicate the protein that will be produced; and (v) the containment conditions that will be implemented as specified in the *NIH Guidelines*. For experiments in this category, the registration document shall be dated, signed by the Principle Investigator, and filed with the Institutional Biosafety Committee. The Institutional Biosafety Committee shall review and approve all experiments in this category prior to their initiation. Requests to



decrease the level of containment specified for experiments in this category will be considered by NIH (see [Section IV-C-1-b-\(2\)-\(c\)](#), *Minor Actions*).

**Section III-D-1. Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems (See [Section II-A](#), Risk Assessment)**

Section III-D-1-a. Experiments involving the introduction of recombinant DNA into Risk Group 2 agents will usually be conducted at Biosafety Level (BL) 2 containment. Experiments with such agents will usually be conducted with whole animals at BL2 or BL2-N (Animals) containment.

Section III-D-1-b. Experiments involving the introduction of recombinant DNA into Risk Group 3 agents will usually be conducted at BL3 containment. Experiments with such agents will usually be conducted with whole animals at BL3 or BL3-N containment.

Section III-D-1-c. Experiments involving the introduction of recombinant DNA into Risk Group 4 agents shall be conducted at BL4 containment. Experiments with such agents shall be conducted with whole animals at BL4 or BL4-N containment.

Section III-D-1-d. Containment conditions for experiments involving the introduction of recombinant DNA into restricted agents shall be set on a case-by-case basis following NIH/OBA review. A U.S. Department of Agriculture - [Animal and Plant Health Inspection Service](#) (USDA/APHIS) permit is required for work with plant or animal pathogens (see [Section V-G and V-M](#), *Footnotes and References of Sections I-IV*). Experiments with such agents shall be conducted with whole animals at BL4 or BL4-N containment.

**Section III-D-2. Experiments in Which DNA From Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems**

Section III-D-2-a. Experiments in which DNA from Risk Group 2 or Risk Group 3 agents (see [Section II-A](#), *Risk Assessment*) is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment. Experiments in which DNA from Risk Group 4 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment after demonstration that only a totally and irreversibly defective fraction of the agent's genome is present in a given recombinant. In the absence of such a demonstration, BL4 containment shall be used. The Institutional Biosafety Committee may approve the specific lowering of containment for particular experiments to BL1. Many experiments in this category are exempt from the *NIH Guidelines* (see [Section III-F](#), *Exempt Experiments*). Experiments involving the formation of recombinant DNA for certain genes coding for molecules toxic for vertebrates require NIH/OBA approval (see [Section III-B-1](#), *Experiments Involving the Cloning of Toxin Molecules with LD<sub>50</sub> of Less than 100 Nanograms Per Kilogram Body Weight*) or shall be conducted under NIH specified conditions as described in [Appendix F](#), *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*.

Section III-D-2-b. Containment conditions for experiments in which DNA from restricted agents is transferred into nonpathogenic prokaryotes or lower eukaryotes shall be determined by NIH/OBA following a case-by-case review (see [Section V-L](#), *Footnotes and References of Sections I-IV*). A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see [Section V-G](#), *Footnotes and References of Sections I-IV*).

**Section III-D-3. Experiments Involving the Use of Infectious DNA or RNA Viruses or Defective DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems**  
Caution: Special care should be used in the evaluation of containment levels for experiments which are likely to either enhance the pathogenicity (e.g., insertion of a host oncogene) or to extend the host range (e.g., introduction of novel control elements) of viral vectors under conditions that permit a productive



infection. In such cases, serious consideration should be given to increasing physical containment by at least one level.

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

Section III-D-3-a. Experiments involving the use of infectious or defective Risk Group 2 viruses (see [Appendix B-II](#), *Risk Group 2 Agents*) in the presence of helper virus may be conducted at BL2.

Section III-D-3-b. Experiments involving the use of infectious or defective Risk Group 3 viruses (see [Appendix B-III-D](#), *Risk Group 3 (RG3) - Viruses and Prions*) in the presence of helper virus may be conducted at BL3.

Section III-D-3-c. Experiments involving the use of infectious or defective Risk Group 4 viruses (see [Appendix B-IV-D](#), *Risk Group 4 (RG4) - Viral Agents*) in the presence of helper virus may be conducted at BL4.

Section III-D-3-d. Experiments involving the use of infectious or defective restricted poxviruses (see [Sections V-A and V-L](#), *Footnotes and References of Sections I-IV*) in the presence of helper virus shall be determined on a case-by-case basis following NIH/OBA review. A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see [Section V-G](#), *Footnotes and References of Sections I-IV*).

Section III-D-3-e. Experiments involving the use of infectious or defective viruses in the presence of helper virus which are not covered in [Sections III-D-3-a](#) through III-D-3-d may be conducted at BL1.

#### Section III-D-4. Experiments Involving Whole Animals

This section covers experiments involving whole animals in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals. For the latter, other than viruses which are only vertically transmitted, the experiments may *not* be conducted at BL1-N containment. A minimum containment of BL2 or BL2-N is required.

Caution - Special care should be used in the evaluation of containment conditions for some experiments with transgenic animals. For example, such experiments might lead to the creation of novel mechanisms or increased transmission of a recombinant pathogen or production of undesirable traits in the host animal. In such cases, serious consideration should be given to increasing the containment conditions.

Section III-D-4-a. Recombinant DNA, or DNA or RNA molecules derived therefrom, from any source except for greater than two-thirds of eukaryotic viral genome may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study (see [Section V-B](#), *Footnotes and References of Sections I-IV*). Animals that contain sequences from viral vectors, which do not lead to transmissible infection either directly or indirectly as a result of complementation or recombination in animals, may be propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study. Experiments involving the introduction of other sequences from eukaryotic viral genomes into animals are covered under [Section III-D-4-b](#), *Experiments Involving Whole Animals*. For experiments involving recombinant DNA-modified Risk Groups 2, 3, 4, or restricted organisms, see [Sections V-A, V-G, and V-L](#), *Footnotes and References of Sections I-IV*. It is

important that the investigator demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see [Section V-G](#), *Footnotes and References of Sections I-IV*).

Section III-D-4-b. For experiments involving recombinant DNA, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals, and not covered by [Sections III-D-1](#), *Experiments Using Human or Animal Pathogens (Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems)*, or [III-D-4-a](#), *Experiments Involving Whole Animals*, the appropriate containment shall be determined by the Institutional Biosafety Committee.

Section III-D-4-c. Exceptions under [Section III-D-4](#), *Experiments Involving Whole Animals*

Section III-D-4-c-(1). Experiments involving the generation of transgenic rodents that require BL1 containment are described under [Section III-E-3](#), *Experiments Involving Transgenic Rodents*.

Section III-D-4-c-(2). The purchase or transfer of transgenic rodents is exempt from the *NIH Guidelines* under [Section III-F](#), *Exempt Experiments* (see [Appendix C-VI](#), *The Purchase or Transfer of Transgenic Rodents*).

### Section III-D-5. Experiments Involving Whole Plants

Experiments to genetically engineer plants by recombinant DNA methods, to use such plants for other experimental purposes (e.g., response to stress), to propagate such plants, or to use plants together with microorganisms or insects containing recombinant DNA, may be conducted under the containment conditions described in [Sections III-D-5-a](#) through III-D-5-e. If experiments involving whole plants are not described in [Section III-D-5](#) and do not fall under [Sections III-A](#), [III-B](#), [III-D](#) or [III-F](#), they are included in [Section III-E](#).

NOTE - For recombinant DNA experiments falling under [Sections III-D-5-a](#) through III-D-5-d, physical containment requirements may be reduced to the next lower level by appropriate biological containment practices, such as conducting experiments on a virus with an obligate insect vector in the absence of that vector or using a genetically attenuated strain.

Section III-D-5-a. BL3-P (Plants) or BL2-P + biological containment is recommended for experiments involving most exotic (see [Section V-M](#), *Footnotes and References of Sections I-IV*) infectious agents with recognized potential for serious detrimental impact on managed or natural ecosystems when recombinant DNA techniques are associated with whole plants.

Section III-D-5-b. BL3-P or BL2-P + biological containment is recommended for experiments involving plants containing cloned genomes of readily transmissible exotic (see [Section V-M](#), *Footnotes and References of Sections I-IV*) infectious agents with recognized potential for serious detrimental effects on managed or natural ecosystems in which there exists the possibility of reconstituting the complete and functional genome of the infectious agent by genomic complementation *in planta*.

Section III-D-5-c. BL4-P containment is recommended for experiments with a small number of readily transmissible exotic (see [Section V-M](#), *Footnotes and References of Sections I-IV*) infectious agents, such as the soybean rust fungus (*Phakospora pachyrhizi*) and maize streak or other viruses in the presence of their specific arthropod vectors, that have the potential of being serious pathogens of major U.S. crops.

Section III-D-5-d. BL3-P containment is recommended for experiments involving sequences encoding potent vertebrate toxins introduced into plants or associated organisms. Recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD<sub>50</sub> of <100 nanograms per kilogram body weight fall under [Section III-B-1](#), *Experiments Involving the Cloning of Toxin Molecules*

with  $LD_{50}$  of Less than 100 Nanograms Per Kilogram Body Weight, and require NIH/OBA and Institutional Biosafety Committee approval before initiation.

Section III-D-5-e. BL3-P or BL2-P + biological containment is recommended for experiments with microbial pathogens of insects or small animals associated with plants if the recombinant DNA-modified organism has a recognized potential for serious detrimental impact on managed or natural ecosystems.

#### Section III-D-6. Experiments Involving More than 10 Liters of Culture

The appropriate containment will be decided by the Institutional Biosafety Committee. Where appropriate, Appendix K, *Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules*, shall be used. [Appendix K](#) describes containment conditions Good Large Scale Practice through BL3-Large Scale.

#### Section III-D-7. Experiments Involving Influenza Viruses

Experiments with influenza viruses generated by recombinant methods (e.g., generation by reverse genetics of chimeric viruses with reassorted segments, introduction of specific mutations) shall be conducted at the biosafety level containment corresponding to the risk group of the virus that was the source of the majority of segments in the recombinant virus (e.g., experiments with viruses containing a majority of segments from a RG3 virus shall be conducted at BL3). Experiments with influenza viruses containing genes or segments from 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968) and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1) shall be conducted at BL3 enhanced containment (see [Appendix G-II-C-5](#), Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses) unless indicated below.

Section III-D-7-a. Human H2N2 (1957-1968). Experiments with influenza viruses containing the H2 hemagglutinin (HA) segment shall be conducted at BL3 enhanced (see [Appendix G-II-C-5](#), Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses). Experiments with the H2 HA gene in cold-adapted, live attenuated vaccine strains (e.g., A/Ann Arbor/6/60 H2N2) may be conducted at BL2 containment provided segments with mutations conferring temperature sensitivity and attenuation are not altered in the recombinant virus. Experiments with Risk Group 2 influenza viruses containing genes from human H2N2 other than the HA gene can be worked on at BL2.

Section III-D-7-b. Highly Pathogenic Avian Influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1). Experiments involving influenza viruses containing a majority of genes and/or segments from a HPAI H5N1 influenza virus shall be conducted at BL3 enhanced containment, (see [Appendix G-II-C-5](#), Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses). Experiments involving influenza viruses containing a minority of genes and/or segments from a HPAI H5N1 influenza virus shall be conducted at BL3 enhanced unless a risk assessment performed by the IBC determines that they can be conducted safely at biosafety level 2 and after they have been excluded pursuant to [9 CFR 121.3\(e\)](#). OBA is available to IBCs to provide consultation with the RAC and influenza virus experts when risk assessments are being made to determine the appropriate biocontainment for experiments with influenza viruses containing a minority of gene/segments from HPAI H5N1. Such experiments may be performed at BL3 enhanced containment or containment may be lowered to biosafety level 2, the level of containment for most research with other influenza viruses. ([USDA/APHIS](#) regulations and decisions on lowering containment also apply.) In deciding to lower containment, the IBC should consider whether, in at least two animal models (e.g., ferret, mouse, Syrian golden hamster, cotton rat, non-human primates), there is evidence that the resulting influenza virus shows reduced replication and virulence compared to the parental RG3 virus at relevant doses. This should be determined by measuring biological indices appropriate for the specific animal model (e.g., severe weight loss, elevated temperature, mortality or neurological symptoms).

Section III-D-7-c. 1918 H1N1. Experiments involving influenza viruses containing any gene or segment from 1918 H1N1 shall be conducted at BL3 enhanced containment (see [Appendix G-II-C-5](#), Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses).

Section III-D-7-d. Antiviral Susceptibility and Containment. The availability of antiviral drugs as preventive and therapeutic measures is an important safeguard for experiments with 1918 H1N1, HPAI H5N1, and human H2N2 (1957-1968). If an influenza virus containing genes from one of these viruses is resistant to both classes of current antiviral agents, adamantanes and neuraminidase inhibitors, higher containment may be required based on the risk assessment considering transmissibility to humans, virulence, pandemic potential, alternative antiviral agents if available, etc.

Experiments with 1918 H1N1, human H2N2 (1957-1968) or HPAI H5N1 that are designed to create resistance to neuraminidase inhibitors or other effective antiviral agents (including investigational antiviral agents being developed for influenza) would be subject to [Section III-A-1](#) (Major Actions) and require RAC review and NIH Director approval. As per [Section I-A-1](#) of the NIH Guidelines, if the agent is a Select Agent, the NIH will defer to the appropriate Federal agency (HHS or USDA Select Agent Divisions) on such experiments.

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

Experiments not included in Sections [III-A](#), [III-B](#), [III-C](#), [III-D](#), [III-F](#), and their subsections are considered in [Section III-E](#). All such experiments may be conducted at BL1 containment. For experiments in this category, a registration document (see [Section III-D](#), *Experiments that Require Institutional Biosafety Committee Approval Before Initiation*) shall be dated and signed by the investigator and filed with the local Institutional Biosafety Committee at the time the experiment is initiated. The Institutional Biosafety Committee reviews and approves all such proposals, but Institutional Biosafety Committee review and approval prior to initiation of the experiment is not required (see [Section IV-A](#), *Policy*). For example, experiments in which all components derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes fall under [Section III-E](#) and may be conducted at BL1 containment.

#### Section III-E-1. Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus

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

#### Section III-E-2. Experiments Involving Whole Plants

This section covers experiments involving recombinant DNA-modified whole plants, and/or experiments involving recombinant DNA-modified organisms associated with whole plants, except those that fall under [Section III-A](#), [III-B](#), [III-D](#), or [III-F](#). It should be emphasized that knowledge of the organisms and judgment based on accepted scientific practices should be used in all cases in selecting the appropriate level of containment. For example, if the genetic modification has the objective of increasing pathogenicity or converting a non-pathogenic organism into a pathogen, then a higher level of containment may be appropriate depending on the organism, its mode of dissemination, and its target organisms. By contrast, a lower level of containment may be appropriate for small animals associated with many types of recombinant DNA-modified plants.

Section III-E-2-a. BL1-P is recommended for all experiments with recombinant DNA-containing plants and plant-associated microorganisms not covered in [Section III-E-2-b](#) or other sections of the *NIH Guidelines*. Examples of such experiments are those involving recombinant DNA-modified plants that are not noxious weeds or that cannot interbreed with noxious weeds in the immediate geographic area, and

experiments involving whole plants and recombinant DNA-modified non-exotic (see [Section V-M](#), *Footnotes and References of Sections I-IV*) microorganisms that have no recognized potential for rapid and widespread dissemination or for serious detrimental impact on managed or natural ecosystems (e.g., *Rhizobium* spp. and *Agrobacterium* spp.).

Section III-E-2-b. BL2-P or BL1-P + biological containment is recommended for the following experiments:

Section III-E-2-b-(1). Plants modified by recombinant DNA that are noxious weeds or can interbreed with noxious weeds in the immediate geographic area.

Section III-E-2-b-(2). Plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent (see [Section V-M](#), *Footnotes and References of Sections I-IV*).

Section III-E-2-b-(3). Plants associated with recombinant DNA-modified non-exotic microorganisms that have a recognized potential for serious detrimental impact on managed or natural ecosystems (see [Section V-M](#), *Footnotes and References of Sections I-IV*).

Section III-E-2-b-(4). Plants associated with recombinant DNA-modified exotic microorganisms that have no recognized potential for serious detrimental impact on managed or natural ecosystems (see [Section V-M](#), *Footnotes and References of Sections I-IV*).

Section III-E-2-b-(5). Experiments with recombinant DNA-modified arthropods or small animals associated with plants, or with arthropods or small animals with recombinant DNA-modified microorganisms associated with them if the recombinant DNA-modified microorganisms have no recognized potential for serious detrimental impact on managed or natural ecosystems (see [Section V-M](#), *Footnotes and References of Sections I-IV*).

### Section III-E-3. Experiments Involving Transgenic Rodents

This section covers experiments involving the generation of rodents in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic rodents). Only experiments that require BL1 containment are covered under this section; experiments that require BL2, BL3, or BL4 containment are covered under [Section III-D-4](#), *Experiments Involving Whole Animals*.

Section III-E-3-a. Experiments involving the breeding of certain BL1 transgenic rodents are exempt under Section III-F, Exempt Experiments (See [Appendix C-VII](#), Generation of BL1 Transgenic Rodents via Breeding).

### Section III-F. Exempt Experiments

The following recombinant DNA molecules are exempt from the *NIH Guidelines* and registration with the Institutional Biosafety Committee is not required (UA imposes additional requirements and registration with the IBC IS required):

Section III-F-1. Those that are not in organisms or viruses.

Section III-F-2. Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.

Section III-F-3. Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means.

Section III-F-4. Those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).



Section III-F-5. Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice of the RAC after appropriate notice and opportunity for public comment (see [Section IV-C-1-b-\(1\)-\(c\)](#), *Major Actions*). See [Appendices A-I](#) through A-VI, *Exemptions Under Section III-F-5--Sublists of Natural Exchangers*, for a list of natural exchangers that are exempt from the *NIH Guidelines*.

Section III-F-6. Those that do not present a significant risk to health or the environment (see [Section IV-C-1-b-\(1\)-\(c\)](#), *Major Actions*), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See [Appendix C](#), *Exemptions under Section III-F-6* for other classes of experiments which are exempt from the *NIH Guidelines*.

#### Section IV-B-2-a. Membership and Procedures

Section IV-B-2-a-(1). The Institutional Biosafety Committee must be comprised of no fewer than five members so selected that they collectively have experience and expertise in recombinant DNA technology and the capability to assess the safety of recombinant DNA research and to identify any potential risk to public health or the environment. At least two members shall not be affiliated with the institution (apart from their membership on the Institutional Biosafety Committee) and who represent the interest of the surrounding community with respect to health and protection of the environment (e.g., officials of state or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the community). The Institutional Biosafety Committee shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing [Appendix P](#), *Physical and Biological Containment for Recombinant DNA Research Involving Plants*, require prior approval by the Institutional Biosafety Committee. The Institutional Biosafety Committee shall include at least one scientist with expertise in animal containment principles when experiments utilizing [Appendix Q](#), *Physical and Biological Containment for Recombinant DNA Research Involving Animals*, require Institutional Biosafety Committee prior approval. When the institution conducts recombinant DNA research at BL3, BL4, or Large Scale (greater than 10 liters), a Biological Safety Officer is mandatory and shall be a member of the Institutional Biosafety Committee (see [Section IV-B-3](#), *Biological Safety Officer*). When the institution participates in or sponsors recombinant DNA research involving human research participants, the institution must ensure that: (i) the Institutional Biosafety Committee has adequate expertise and training (using *ad hoc* consultants as deemed necessary); (ii) all aspects of [Appendix M](#) have been appropriately addressed by the Principle Investigator; (iii) no research participant shall be enrolled (see definition of enrollment in [Section I-E-7](#)) in a human gene transfer experiment until the RAC review process has been completed (see [Appendix M-I-B](#), *RAC Review Requirements*); and (iv) final IBC approval is granted only after the RAC review process has been completed (see [Appendix M-I-B](#), *RAC Review Requirements*). Institutional Biosafety Committee approval must be obtained from the institution at which recombinant DNA material will be administered to human research participants (rather than the site involved in manufacturing gene transfer products).

Note: Individuals, corporations, and institutions not otherwise covered by the *NIH Guidelines*, are encouraged to adhere to the standards and procedures set forth in [Sections I](#) through IV (see [Section IV-D](#), *Voluntary Compliance*). The policy and procedures for establishing an Institutional Biosafety Committee under *Voluntary Compliance*, are specified in [Section IV-D-2](#), *Institutional Biosafety Committee Approval*).

Section IV-B-2-a-(2). In order to ensure the competence necessary to review and approve recombinant DNA activities, it is recommended that the Institutional Biosafety Committee: (i) include persons with expertise in recombinant DNA technology, biological safety, and physical containment; (ii) include or have available as consultants persons knowledgeable in institutional commitments and policies, applicable law, standards of professional conduct and practice, community attitudes, and the environment, and (iii) include at least one member representing the laboratory technical staff.

Section IV-B-2-a-(3). The institution shall file an annual report with NIH/OBA which includes: (i) a roster of all Institutional Biosafety Committee members clearly indicating the Chair, contact person, Biological Safety Officer (if applicable), plant expert (if applicable), animal expert (if applicable), human gene

therapy expertise or *ad hoc* consultant(if applicable); and (ii) biographical sketches of all Institutional Biosafety Committee members (including community members).

Section IV-B-2-a-(4). No member of an Institutional Biosafety Committee may be involved (except to provide information requested by the Institutional Biosafety Committee) in the review or approval of a project in which he/she has been or expects to be engaged or has a direct financial interest.

Section IV-B-2-a-(5). The institution, that is ultimately responsible for the effectiveness of the Institutional Biosafety Committee, may establish procedures that the Institutional Biosafety Committee shall follow in its initial and continuing review and approval of applications, proposals, and activities.

Section IV-B-2-a-(6). When possible and consistent with protection of privacy and proprietary interests, the institution is encouraged to open its Institutional Biosafety Committee meetings to the public.

Section IV-B-2-a-(7). Upon request, the institution shall make available to the public all Institutional Biosafety Committee meeting minutes and any documents submitted to or received from funding agencies which the latter are required to make available to the public. If public comments are made on Institutional Biosafety Committee actions, the institution shall forward both the public comments and the Institutional Biosafety Committee's response to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

#### Section IV-B-2-b. Functions

On behalf of the institution, the Institutional Biosafety Committee is responsible for:

Section IV-B-2-b-(1). Reviewing recombinant DNA research conducted at or sponsored by the institution for compliance with the *NIH Guidelines* as specified in [Section III](#), *Experiments Covered by the NIH Guidelines*, and approving those research projects that are found to conform with the *NIH Guidelines*. This review shall include: (i) independent assessment of the containment levels required by the *NIH Guidelines* for the proposed research; (ii) assessment of the facilities, procedures, practices, and training and expertise of personnel involved in recombinant DNA research; (iii) ensuring that all aspects of [Appendix M](#) have been appropriately addressed by the Principle Investigator; (iv) ensuring that no research participant is enrolled (see definition of enrollment in [Section I-E-7](#)) in a human gene transfer experiment until the RAC review process has been completed (see [Appendix M-I-B](#), *RAC Review Requirements*), Institutional Biosafety Committee approval (from the clinical trial site) has been obtained, Institutional Review Board approval has been obtained, and all applicable regulatory authorizations have been obtained; (v) for human gene transfer protocols selected for public RAC review and discussion, consideration of the issues raised and recommendations made as a result of this review and consideration of the Principle Investigator's response to the RAC recommendations; (vi) ensuring that final IBC approval is granted only after the RAC review process has been completed (see [Appendix M-I-B](#), *RAC Review Requirements*); and (vii) ensuring compliance with all surveillance, data reporting, and adverse event reporting requirements set forth in the *NIH Guidelines*.

Section IV-B-2-b-(2). Notifying the Principle Investigator of the results of the Institutional Biosafety Committee's review and approval.

Section IV-B-2-b-(3). Lowering containment levels for certain experiments as specified in [Section III-D-2-a](#), *Experiments in which DNA from Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems*.

Section IV-B-2-b-(4). Setting containment levels as specified in [Sections III-D-4-b](#), *Experiments Involving Whole Animals*, and [III-D-5](#), *Experiments Involving Whole Plants*.

Section IV-B-2-b-(5). Periodically reviewing recombinant DNA research conducted at the institution to ensure compliance with the *NIH Guidelines*.

Section IV-B-2-b-(6). Adopting emergency plans covering accidental spills and personnel contamination resulting from recombinant DNA research.

Note: The *Laboratory Safety Monograph* describes basic elements for developing specific procedures dealing with major spills of potentially hazardous materials in the laboratory, including information and references about decontamination and emergency plans. The NIH and the Centers for Disease Control and Prevention are available to provide consultation and direct assistance, if necessary, as posted in the *Laboratory Safety Monograph*. The institution shall cooperate with the state and local public health departments by reporting any significant research-related illness or accident that may be hazardous to the public health.

Section IV-B-2-b-(7). Reporting any significant problems with or violations of the *NIH Guidelines* and any significant research-related accidents or illnesses to the appropriate institutional official and NIH/OBA within 30 days, unless the Institutional Biosafety Committee determines that a report has already been filed by the Principle Investigator. Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

Section IV-B-2-b-(8). The Institutional Biosafety Committee may not authorize initiation of experiments which are not explicitly covered by the *NIH Guidelines* until NIH (with the advice of the RAC when required) establishes the containment requirement.

Section IV-B-2-b-(9). Performing such other functions as may be delegated to the Institutional Biosafety Committee under [Section IV-B-2](#), *Institutional Biosafety Committee*.