

Biosafety Manual

for

University of Maryland

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Review and Approval Authority

Prepared and Edited by:

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Biosafety Officer

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_____ Date: _____
Vice President for Administrative Affairs

Approved as UMCP Policy:

_____ Date: _____
President

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IMPORTANT TELEPHONE NUMBERS

EMERGENCY TELEPHONE NUMBER

Fire, Police, Rescue, Emergency Medical Service

9-1-1

ASSISTANCE TELEPHONE NUMBERS

University Health Center

Occupational Health Clinic

(31)4-8172

Urgent Care Clinic

(31)4-8162

Department of Environmental Safety

(40)5-3960

Biosafety Officer

(40)5-3975

Assistant Biosafety Officer

(40)5-6513

Radiation Safety Officer

(31)4- 8336

USEFUL WEB SITES

NIH Guidelines for Research Involving Recombinant DNA Molecules:

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

Biosafety in Microbiological and Biomedical Laboratories:

<http://www.cdc.gov/od/ohs/biosfty/bmb15/bmb15toc.htm>

NIH Office of Biotechnology Activities:

<http://www4.od.nih.gov/oba>

CDC Select Agents Program:

<http://www.cdc.gov/od/sap/index.htm>

USDA/APHIS Select Agents Program:

http://www.aphis.usda.gov/programs/ag_selectagent/index.html

CDC Permit to Import or Transport Etiologic Agents:

<http://www.cdc.gov/od/ohs/biosfty/imprtper.htm>

USDA/APHIS Permit to Import or Transport Livestock Pathogens:

http://www.aphis.usda.gov/animal_health/permits/

USDA/APHIS Permit to Field Test, Import, or Transport Genetically Modified Organisms

http://www.aphis.usda.gov/brs/regulatory_activities.html

University of Maryland Form for Registration of Biological Materials

<https://des.umd.edu/research/login.cfm>

Selection, Installation, and Use of Biological Safety Cabinets

<http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm> (updated version is appendix A in 5th ed.)

BMBL: (<http://www.cdc.gov/od/ohs/biosfty/bmb15/sections/AppendixA.pdf>)

POLICY STATEMENT

I. Purpose

This is a statement of official University of Maryland policy to establish the process for compliance with the following documents:

- A. *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*, current edition;
- B. *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, current edition.

II. Policy

The University of Maryland is actively committed to preserving the health and safety of its students, staff, and faculty, and to protecting the environment and the community. It is recognized that use of potentially pathogenic microorganisms and organisms containing recombinant DNA (rDNA) is necessary in many university research and teaching laboratories. To ensure the safe handling of these organisms, the University requires compliance with the *NIH Guidelines* and with the recommendations in *BMBL*. Compliance with other applicable federal, state, and local regulations is also required.

III. Responsibilities

The Principal Investigator (PI) is directly and primarily responsible for the safe operation of the laboratory. His/her knowledge and judgment are critical in assessing risks and appropriately applying the recommendations in this manual. However, safety is a shared responsibility among all of the laboratory staff. Many resources exist to assist the PI with these responsibilities, including the Biological and Chemical Hygiene Committee (BACH), the Institutional Biosafety Committee (IBC), and the Department of Environmental Safety (DES).

- A. DES shall:
 - (1) Prepare the Biosafety Manual, with revisions as necessary;
 - (2) Distribute the Manual to each faculty member who works with biological materials;
 - (3) Investigate accidents involving infectious agents;
 - (4) Collect and dispose of Biological Waste (also referred to as Biological, Pathological and Medical Waste - or BPMW);
 - (5) Provide or coordinate biosafety training as requested;
 - (6) Assist investigators with risk assessment; and
 - (7) Administer all elements of the Biosafety Program, assist faculty with submission of registrations to the IBC, and maintain registration files.

- B. PIs shall:
 - (1) Assess the risks of their experiments;
 - (2) Ensure the safe operation of their laboratory;
 - (3) Train laboratory personnel in safe work practices;
 - (4) Comply with all applicable state and federal regulations and guidelines;
 - (5) Register the following experiments with the IBC or DES, as required:
 - (a) recombinant DNA activities;
 - (b) work with infectious agents;

- (c) experiments involving the use of human blood or other potentially infectious materials, such as unfixed human tissues, primary human cell lines, and certain body fluids; and animal and plant pathogens
- C. The BACH Committee shall work cooperatively with researchers, faculty, and staff to promote biological and chemical safety on the campus.
- D. The IBC shall:
 - (1) Review rDNA research conducted at or sponsored by the University for compliance with the *NIH Guidelines*, and approve those research projects that are found to conform with the *NIH Guidelines*;
 - (2) Review research involving infectious agents conducted at or sponsored by the University for compliance with the guidelines in *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, and approve those research projects that are found to conform with the recommendations in *BMBL*;
 - (3) Notify the PI of the results of the IBC's review and approval;
 - (4) Report any significant problems with or violations of the *NIH Guidelines* and any significant research-related accidents or illness to the appropriate Institutional official and to the NIH Office of Biotechnology DNA Activities (OBA) within 30 days; and
 - (5) Follow the guidelines for membership defined by NIH, with the additional requirement of one representative from the University of Maryland Animal Care and Use Committee, and a plant pathologist from USDA as appropriate.
- E. The University Health Center (UHC) shall:
 - (1) Provide medical evaluation, as required by the OSHA Bloodborne Pathogens Standard (CFR 1910.1030), and as recommended in the *BMBL* and *NIH Guidelines*; and
 - (2) Provide vaccinations, as required.
- F. Laboratory personnel shall:
 - (1) Comply with safety recommendations for the work being performed; and
 - (2) Report accidents or injuries to the PI.

IV. Information

DES will assist any Department in providing training and guidance for implementation of this policy. Registration forms and the publications referenced in Section I above are available through the DES web site at <http://www.des.umd.edu>.

CLASSIFICATION OF POTENTIALLY INFECTIOUS AGENTS

Procedures and facilities involved in protecting laboratory workers, the public, and the environment from laboratory biological hazards are governed by federal and state regulations and guidelines. Many granting agencies require that grant recipients certify that they adhere to both the guidelines and the regulations.

MICROORGANISMS

The National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) publish guidelines for work with infectious microorganisms. The publication, entitled *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, recommends that work be done using one of four levels of containment: Biosafety Level 1 (BSL1), BSL2, BSL3 and BSL4 (see next chapter). The *NIH Guidelines* (Appendix B) classifies pathogenic agents into one of four risk groups according to specific criteria. It is University of Maryland policy that all laboratories adhere to these NIH/CDC guidelines.

Microorganisms capable of causing infection in humans

Investigators must register any project involving a pathogenic agent with the IBC and receive its approval before work is begun. Following receipt of the completed Registration Document by DES, the laboratory will be surveyed by the Biosafety Officer (BSO) to ascertain that it meets the containment requirements listed in *BMBL* for the agent being studied. If the lab meets the requirements, the work will be reviewed and approved or disapproved by the IBC. Online registration is available at <http://www.des.umd.edu>.

Genetically Engineered Microorganisms

Work with all genetically engineered organisms must comply with the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. These guidelines classify recombinant DNA experiments into four levels of containment (BSL1, BSL2, BSL3, and BSL4) based on the hazard of the microorganism and the procedures and quantities being used. Additionally, the United States Department of Agriculture (USDA) requires permits for field testing of genetically engineered plants. It is University of Maryland policy that all laboratories follow these guidelines.

Registration Document

Each PI is responsible for registering all recombinant DNA experiments, including those exempt from *NIH Guidelines*. Online registration is available at <http://www.des.umd.edu>. The BSO audits all laboratories where BSL2 or BSL3 containment is required, and all BSL1 laboratories which are subject to the *NIH Guidelines*.

Review and Approval of Experiments

The IBC, which oversees recombinant DNA research at the University of Maryland, or the BSO will review and approve the registration.

a. Experiments covered by the *NIH Guidelines*

Many experiments involving rDNA molecules require registration and approval by the IBC before work may be initiated. Experiments that require IBC approval before initiation include those that involve:

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

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

- ✓ the formation of recombinant DNA molecules containing no more than 2/3 of the genome of any eukaryotic virus propagated in tissue culture.
- ✓ recombinant DNA-modified whole plants, and/or recombinant DNA-modified organisms associated with whole plants, except those that fall under Section III-A, III-B, III-C, or III-E of the *Guidelines*.
- ✓ the generation of transgenic rodents that require BSL1 containment.

b. Experiments exempt from the *NIH Guidelines*

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

- ✓ use rDNA molecules that are not in organisms or viruses.
- ✓ consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.
- ✓ consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means.
- ✓ consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).
- ✓ consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent.
- ✓ do not present a significant risk to health or the environment as determined by the NIH Director, with the advice of the Recombinant DNA Advisory Committee (RAC), and following appropriate notice and opportunity for public comment.
- ✓ contain less than one-half of any eukaryotic viral genome propagated in cell culture.
- ✓ use *E. coli* K12, *Saccharomyces cerevisiae*, or *Bacillus subtilis* host-vector systems, unless genes from Risk Group 3 or 4 pathogens or restricted animal pathogens are cloned into these hosts.
- ✓ involve the purchase or transfer of transgenic rodents for experiments that require BSL1 containment.

HUMAN BLOOD, UNFIXED TISSUE, AND CELL CULTURE

Please refer to the *Bloodborne Pathogens Exposure Control Plan* for detailed information on handling human material. Copies are available online at <http://www.des.umd.edu>.

Work with human material is regulated by the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard, 29 CFR, Part 1910.1030. Human blood, unfixed tissue, cell culture, and certain other body fluids are considered potentially infectious for bloodborne pathogens such as hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). All human clinical material should be presumed infectious and handled using BSL2 work practices. This concept is called Universal Precautions. Investigators are responsible for notifying DES of their use of human materials so training and immunization can be provided as required by OSHA.

PLANT AND ANIMAL PATHOGENS

The BACH Committee requires investigators to register their campus use of animal and plant pathogens with DES. The registration form for animal pathogens is available at the DES web site at <http://www.des.umd.edu>. Registration of plant pathogens may be completed by forwarding a copy of the USDA/APHIS permit to the BSO.

SELECT AGENTS

Select Agents are microorganisms and toxins that have potential for use by terrorists. The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 restricts their possession and use, and requires the University to collect and maintain information on the location and use on campus of any select agents or toxins. Please contact the BSO immediately if you currently possess or plan to acquire any of the agents listed in Appendix 4 and have not yet reported that fact. Failure to provide notice may result in civil and criminal liability for individual researchers and/or the University. If you have questions, you may contact the BSO, or visit the CDC's Select Agent web site (<http://www.cdc.gov/od/sap/index.htm>), which provides links to select agent program information.

NON-HUMAN PRIMATE (NHP) UNFIXED TISSUE & PRIMARY CELL CULTURE

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

Work with any NHP primary cell cultures or unfixed tissues must be registered with DES, and lab personnel must be trained in the safety procedures required for handling NHP material prior to beginning the research. Sharps use with these materials should be eliminated or restricted.

BIOSAFETY CONTAINMENT LEVELS

Four levels of biosafety are defined in the publication *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, published by the CDC and NIH. The levels, designated in ascending order by degree of protection provided to personnel, the environment, and the community, are combinations of laboratory practices, safety equipment, and laboratory facilities (see Appendices). Most microbiological work at the University of Maryland is conducted at BSL1 or BSL2 containment. There are no BSL4 laboratories at the University.

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

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.
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. Plasticware 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. 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. Agent information should be posted in accordance with the institutional policy.

Biosafety Level 2 practices, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low. Hepatitis B virus, HIV, the salmonellae, and *Toxoplasma* spp. are representative of microorganisms assigned to this containment level. BSL-2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. (Laboratory personnel working with human-derived materials should refer to the OSHA *Bloodborne Pathogen Standard 2* for specific required precautions).

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or in devices such as a BSC or safety centrifuge cups. Personal protective equipment should be used as appropriate, such as splash shields, face protection, gowns, and gloves.

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

Biosafety Level 3 practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii* are representative of the microorganisms assigned to this level. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols.

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

Biosafety Level 4 practices, safety equipment, and facility design and construction are applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Agents with a close or identical antigenic relationship to BSL-4 agents also should be handled at this level. When sufficient data are obtained, work with these agents may continue at this level or at a lower level. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at BSL- 4.

The primary hazards to personnel working with BSL-4 agents are respiratory exposure to infectious aerosols, mucous membrane or broken skin exposure to infectious droplets, and autoinoculation. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals, pose a high risk of exposure and infection to laboratory personnel, the community, and the environment.

The laboratory worker's complete isolation from aerosolized infectious materials is accomplished primarily by working in a Class III BSC or in a full-body, air-supplied positive-pressure personnel suit. The BSL-4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems to prevent release of viable agents to the environment.

The laboratory director is specifically and primarily responsible for the safe operation of the laboratory. His/her knowledge and judgment are critical in assessing risks and appropriately applying these recommendations. The recommended biosafety level represents those conditions under which the agent can ordinarily be safely handled. Special characteristics of the agents used, the training and experience of personnel, procedures being conducted and the nature or function of the laboratory may further influence the director in applying these recommendations.

Summary of Recommended Biosafety Levels

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

ANIMAL FACILITIES

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

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

CLINICAL LABORATORIES

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

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

BSL-2 recommendations and OSHA requirements focus on the prevention of percutaneous and mucous membrane exposures to clinical material. Primary barriers such as BSCs (Class I or II) should be used when performing procedures that might cause splashing, spraying, or splattering of droplets. Biological safety cabinets also should be used for the initial processing of clinical specimens when the nature of the test requested or other information suggests the likely presence of an agent readily transmissible by infectious aerosols (e.g., *M. tuberculosis*), or when the use of a BSC (Class II) is indicated to protect the integrity of the specimen. The segregation of clinical laboratory functions and limited or restricted access to such areas is the responsibility of the laboratory director. It is also the director's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented.

From: *Biosafety in Microbiological and Biomedical Laboratories*. Centers for Disease Control and Prevention, and National Institutes of Health, 2007.

EMERGENCY PROCEDURES

BIOLOGICAL SPILLS

A spill kit should be kept in each laboratory where work with microorganisms is conducted. Basic equipment is: concentrated disinfectant (such as chlorine bleach), absorbent material, household rubber gloves, autoclave bags, sharps container, and forceps to pick up broken glass.

GENERAL SPILL CLEANUP GUIDELINES

- ✓ Wear gloves and labcoat.
- ✓ Use forceps to pick up broken glass and discard into sharps container.
- ✓ Cover spilled material with paper towels.
- ✓ Add diluted disinfectant in sufficient quantity to ensure effective microbial inactivation.
- ✓ Dispose of towels in biohazard waste container.
- ✓ Wipe spill area with diluted disinfectant.
- ✓ Wash hands with soap and water when finished.

SPECIFIC SPILL CLEANUP GUIDELINES

1. Spill of BSL1 material

- a. Wearing gloves and a lab coat, pick up broken glass with forceps and place in sharps container.
- b. Absorb the spill with paper towels or other absorbent material.
- c. Discard these contaminated materials into biohazard waste container.
- d. Wipe the spill area with the appropriate dilution of a disinfectant effective against the organism.
- e. Autoclave all towels, gloves, and other materials worn or used to clean up the spill.
- f. Wash hands with soap and water.

2. Spill of Human Blood

- a. Wear gloves and lab coat to clean up spill.
- b. If broken glass is present, use forceps to pick up and place in sharps container.
- c. Absorb blood with paper towels and discard in biohazard waste container.
- d. Using a detergent solution, clean the spill site of all visible blood.
- e. Wipe the spill site with paper towels soaked in a disinfectant such as bleach diluted 1:10 (vol/vol).
- f. Discard all contaminated materials into biohazard waste container.
- g. Wash hands with soap and water.

3. **Spill of BSL2 material**

- a. Keep other workers out of the area to prevent spreading spilled material. Post warning sign, if needed.
- b. Remove contaminated clothing and put into a biohazard bag for decontamination later.
- c. Wash hands and exposed skin and inform the PI of the spill. Call the BSO at (40)5-3975 for assistance, if necessary.
- d. Put on protective clothing (lab coat, gloves, and if needed, face protection and shoe covers) and assemble clean-up materials (disinfectant, autoclavable container or bag, forceps, sharps container, and paper towels).
- e. Pick up broken glass with forceps and dispose into sharps container.
- f. Cover the spill with paper towels and add appropriately diluted disinfectant.
- g. After at least 20 minutes contact time, pick up the paper towels and re-wipe the spill area with diluted disinfectant.
- h. Collect all contaminated materials into biohazard waste container and autoclave.
- i. Wash hands with soap and water.

4. **Spill of BSL3 material**

- a. Stop work immediately.
- b. Avoid inhaling airborne material while quickly leaving the room. Notify others to leave. Close door, and post with warning sign.
- c. Remove contaminated clothing, turn exposed area inward, and place in a biohazard bag. Wash hands with soap and water.
- d. Notify the PI. Call the BSO at (40)5-3975 (after hours and weekends call 911) for assistance if necessary.
- e. Allow 30 minutes for aerosols to disperse before re-entering the laboratory to begin clean-up.
- f. Put on personal protective equipment (HEPA filtered respirator, gown, gloves, and shoe covers) and assemble clean-up materials (disinfectant, autoclavable container or bag, forceps, sharps container, and paper towels).
- g. Contain the spill with absorbent paper towels or disposable pads. Carefully add 10% chlorine bleach to the spill; avoid creating aerosols when pouring the disinfectant. Leave the room and allow 30 minutes for the bleach to inactivate the material.
- h. Pick up broken glass with forceps and discard in sharps container.
- i. Clean up liquid with paper towels and collect all contaminated materials into biohazard bag or container. Remove all spilled materials and decontaminate the area again with an appropriate disinfectant.
- j. Autoclave (or soak in 10% bleach solution) lab coat, gloves, and other protective equipment that was worn for clean up.
- k. Wash hands thoroughly with soap and water.

5. Spill in a Biological Safety Cabinet

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

6. Spill of Radioactive Biological Material

A spill involving both radioactive and biological materials requires emergency procedures that are different from the procedures used for either material alone. As a general rule, disinfect the microorganism using a chemical disinfectant, then dispose of all clean-up materials in a separate bag/container labeled to indicate that the radioisotope is mixed with a chemically disinfected microorganism. **Do not use bleach solutions as a disinfectant on materials that contain iodinated compounds because radioactive iodine gas may be released.** Be sure to use procedures to protect yourself from the radionuclide while disinfecting the biological material. Before any clean-up, consider the type of radionuclide, the characteristics of the microorganism, and the volume of the spill. Contact the Radiation Safety Officer (RSO) at (31)4-8336 for specific radioisotope clean-up procedures.

Preparation For Clean-up

- a. Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave. Close door, and post with warning sign.
- b. Remove contaminated clothing, turn exposed area inward, and place in a biohazard bag.
- c. Wash all exposed skin with soap or hand washing antiseptic, followed by a three minute water rinse.
- d. Inform the PI and the RSO at (40)5-3985 of the spill, and monitor all exposed personnel for radiation.
- e. Allow aerosols to disperse for at least 30 minutes before reentering the laboratory. Assemble clean-up materials (diluted disinfectant, autoclaveable containers, forceps, paper towels, sharps container).
- f. Confirm with the RSO that it is safe to enter the lab.

Clean-up of Radioactive Biological Spill

- a. Put on protective clothing (lab coat, surgical mask, gloves, and shoe covers). Depending on the nature of the spill, it may be advisable to wear a HEPA filtered respirator instead of a surgical mask. In setting up your spill plan, contact DES for advice since the use of many types of respirators requires prior training, fit-testing, and medical approval.
- b. Pick up any sharp objects with forceps and put in sharps container labeled according to Radiation Safety guidelines.
- c. Cover the area with paper towels, and carefully pour diluted disinfectant around and into the spill. Avoid enlarging the contaminated area. Use additional disinfectant as it becomes diluted by the spill. Allow at least 20 minutes contact time.
DO NOT USE BLEACH SOLUTIONS ON IODINATED MATERIALS: RADIOIODINE GAS MAY BE RELEASED. INSTEAD, USE AN ALTERNATIVE DISINFECTANT SUCH AS AN IODOPHOR.
- d. Wipe surrounding areas where the spill may have splashed with disinfectant.
- e. Absorb the disinfectant and spill materials with additional paper towels, and place into an approved radioactive waste container. Keep separate from other radioactive waste.
DO NOT AUTOCLAVE CONTAMINATED WASTE UNLESS APPROVED BY THE RSO.
- f. Disinfect contaminated protective clothing prior to disposal as radioactive waste.
 - (I) Place contaminated item(s) on absorbent paper and scan for radioactivity. **If none is detected, dispose of these items as biohazard waste.**
 - (ii) If radioactive, spray with disinfectant and allow a 20 minute contact time.
 - (iii) Wrap the item(s) inside the absorbent paper and dispose of as radioactive waste.
- g. Wash hands and exposed skin areas with soap and water, and monitor personnel and spill area for residual radioactive contamination. If skin contamination is detected, repeat decontamination procedures under the direction of the RSO. If spill area has residual activity, determine if it is fixed or removable and handle it accordingly.

INJURY INVOLVING BIOLOGICAL MATERIALS

For Severe Injuries

- ✓ Call 911 for assistance and transportation to the nearest emergency room.
- ✓ Accompany the injured person to the medical facility and provide information to personnel about the accident/exposure.
- ✓ Report accident to the PI and DES.

For Splash To The Eye

- ✓ Immediately flush the eye with a gentle stream of clean, temperate water for 15 minutes. Hold the eyelid open. Be careful not to wash the contaminant into the other eye. Use an emergency eyewash if one is accessible.
- ✓ Contact the UHC at (31)4-8162 to obtain care. If UHC is closed, go to the emergency room at Washington Adventist Hospital (7600 Carroll Ave., Takoma Park, MD) or to the most convenient local emergency room.
- ✓ Report the accident to the PI and DES, and seek additional medical assistance if necessary.

For Contamination To The Body

- ✓ Immediately remove contaminated clothing and drench skin with water. Wash with soap and water, and flush the area for 15 minutes.
- ✓ Contact the UHC at (31)4-8162 to obtain care. If UHC is closed, go to the emergency room at Washington Adventist Hospital (7600 Carroll Ave., Takoma Park, MD) or to the most convenient local emergency room.
- ✓ Report the injury to the PI and to DES, and seek additional medical assistance if necessary.

FIRES INVOLVING BIOLOGICAL MATERIALS

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

Any individual who receives an exposure or potential exposure will be given a medical consultation and advised of available treatments.

DECONTAMINATION AND DISPOSAL

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

CHEMICAL DISINFECTANTS

Chemical disinfectants are used to render a contaminated material safe for further handling, whether it is a material to be disposed of as waste, or a laboratory bench on which a spill has occurred. It is important to choose a disinfectant that has been proven effective against the organism being used. Chemical disinfectants are registered by the EPA under the following categories:

1. Sterilizer or Sterilant - will destroy all microorganisms including bacterial and fungal spores on inanimate surfaces.
2. Disinfectant - will destroy or irreversibly inactivate specific viruses, bacteria, and pathogenic fungi, but not bacterial spores.
3. Hospital Disinfectant - agent shown to be effective against *S. aureus*, *S. choleraesuis* and *P. aeruginosa*. It may be effective against *M. tuberculosis*, pathogenic fungi or specifically named viruses.
4. Antiseptic - agent formulated to be used on skin or tissue - not a disinfectant.

DISINFECTANTS COMMONLY USED IN THE LABORATORY

1. Iodophors

- ✓ Recommended dilution is 75 ppm, or approximately 4.5 ml/liter water.
- ✓ Effective against vegetative bacteria, fungi, and viruses.
- ✓ Effectiveness reduced by organic matter (but not as much as with hypochlorites).
- ✓ Stable in storage if kept cool and tightly covered.
- ✓ Built-in color indicator; if solution is brown or yellow, it is still active.
- ✓ Relatively harmless to humans.

2. Hypochlorites (bleach)

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

3. Alcohols (ethanol, isopropanol)

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

Important Characteristics of Disinfectants

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

DILUTION OF DISINFECTANTS

1. Chlorine compounds (Household Bleach)

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

Bleach solutions decompose at room temperature and should be made fresh daily. However, if stored in tightly closed brown bottles, bleach solutions retain activity for 30 days. The use concentration is dependent on the organic load of the material to be decontaminated. Use a 1% solution to disinfect clean surfaces, and 10% solution to disinfect surfaces contaminated with a heavy organic load. To disinfect liquid biological waste before disposal, add concentrated bleach to a final concentration of 1%.

2. Iodophor

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

3. Alcohols

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

AUTOCLAVING PROCEDURES

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

Container Selection

- ✓ **Polypropylene bags.** Commonly called biohazard or autoclave bags, these bags are able to withstand autoclaving and are tear resistant, but can be punctured or burst during autoclaving. Therefore, **place bags in a rigid container such as a polypropylene or stainless steel pan during autoclaving.** Bags are available in a variety of sizes, and some are printed with an indicator that changes color when processed.
- ✓ Polypropylene bags are impermeable to steam, and for this reason should not be twisted and taped shut, but gathered loosely at the top and secured with a large rubber band or autoclave tape. This will create an opening through which steam can penetrate.
- ✓ **Polypropylene containers and pans.** Polypropylene is a plastic capable of withstanding autoclaving, but resistant to heat transfer. Therefore, materials contained in a polypropylene pan will take longer to autoclave than the same materials in a stainless steel pan. To decrease the time required to sterilize material in these containers,
 - remove the lid (if applicable).
 - turn the container on its side when possible.
 - select a container with the lowest sides and widest diameter possible for the autoclave.
- ✓ **Stainless steel containers and pans.** Stainless steel is an efficient conductor of heat and is less likely to increase sterilizing time, though is more expensive than polypropylene.

Preparation and Loading of Materials

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

Cycle Selection

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

Time Selection

- ✓ Take into account the size of the articles to be autoclaved. A 2-liter flask containing 1 liter of liquid takes longer to sterilize than four 500 ml flasks each containing 250 ml of liquid.
- ✓ Material with a high insulating capacity (animal bedding, high-sided polyethylene containers) increases the time needed for the load to reach sterilizing temperatures.
- ✓ Bags of biological waste should be autoclaved for 50 minutes to assure decontamination.

Removing the Load

- ✓ Check that the chamber pressure is zero.
- ✓ Wear lab coat, eye protection, heat insulating gloves, and closed-toe shoes.
- ✓ Stand behind door when opening it.
- ✓ Slowly open door only a crack. Beware of rush of steam.
- ✓ After the slow exhaust cycle, open autoclave door and allow liquids to cool for 20 minutes before removing.
- ✓ All autoclaved bags of waste must be put into plain opaque household trash bags before being put into the dumpster.

Monitoring

Autoclaves used to decontaminate laboratory waste should be tested periodically to assure effectiveness. Two types of tests are used: 1) a chemical indicator that fuses when the temperature reaches 121^o C, and 2) heat-resistant spores (*Bacillus stearothermophilis*) that are killed by exposure to 121^o C for approximately 15 minutes. Both types of tests should be placed well down in the center of the bag or container of waste, at the point slowest to heat.

The chemical test should be used first to determine that the temperature in the center of the container reaches 121^o C. Ampules of heat-resistant spores should be used in subsequent test runs to determine the length of time necessary to achieve sterilization.

If you need assistance, please contact the BSO at (40)5-6513.

AUTOCLAVE SAFETY

CAUTION - AUTOCLAVES MAY CAUSE SERIOUS BURNS

TO PREVENT INJURY:

- Loosen screw caps on bottles and tubes of liquids before autoclaving.
- Check that chamber pressure has returned to zero before opening door.
- Wear eye and face protection.
- Stand behind door when opening it.
- Slowly open door only a crack. Beware rush of steam.
- Keep face away from door as it opens. Escaping steam may burn face.
- Wait 5 minutes after opening door before removing liquids.
- Liquids removed too soon may boil up and out of container, burning operator.

USE AND DISPOSAL OF SHARPS

To prevent needle stick injuries:

- ✓ Avoid using needles whenever possible.
- ✓ Do not bend, break, or otherwise manipulate needles by hand.
- ✓ Do not recap needles by hand. Do not remove needles from syringes by hand.
- ✓ Immediately after use, discard needle and syringe (whether contaminated or not) into puncture resistant sharps containers.
- ✓ Never discard sharps into regular trash.
- ✓ Never discard sharps into bags of biological waste.
- ✓ Use care and caution when cleaning up after procedures that require the use of syringes and needles.
- ✓ Do not overfill sharps containers. Close completely when they are 3/4 full and request pickup online at <http://www.des.umd.edu>

- ✓ Locate sharps containers in areas in which needles are commonly used. Make containers easily accessible.
- ✓ Sharps containers may be purchased from the Chemistry Stores (405-1838) on campus, as well as from laboratory supply distributors such as VWR and Fisher Scientific.

In the event of a needle stick injury:

- ✓ Wash thoroughly with soap and water. Notify supervisor and go immediately to Urgent Care Clinic at the UHC. If the UHC is closed, go to the emergency room at Washington Adventist Hospital (7600 Carroll Ave., Takoma Park, MD) or to the most convenient local emergency room.

BIOLOGICAL WASTE DISPOSAL PROCEDURES

Please read and follow the Waste Disposal Guidelines wall chart. Copies may be obtained by calling (40)5-3960.

I. Biological Waste

- A. All biological waste from BSL1, BSL2, and BSL3 laboratories must be decontaminated prior to disposal.
- B. Decontamination and disposal are the responsibility of the person/laboratory generating the waste.
 - 1. Collect disposable, solid materials contaminated by an infectious agent, **excluding sharps, or broken or unbroken glass**, into an autoclave bag within a sturdy container. When full, these bags are autoclaved, cooled, and then placed in the building's dumpster.
 - 2. Decontaminate liquids containing a biological agent by the addition of a chemical disinfectant such as sodium hypochlorite (household bleach) or an iodophor, **or** by autoclaving, then dispose of by pouring down the sink. It is not necessary to autoclave liquids that have been chemically disinfected. However, if a bleach solution has been used in the collection tray for labware that will later be autoclaved, sodium thiosulfate must be added to the bleach to prevent the release of chlorine gas during autoclaving.

II. Reusable Labware

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

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

III. Disposal of Blood Products and Body Fluids

- A. All human blood and other potentially infectious materials (OPIM) should be handled using Universal Precautions.
- B. Discard disposable items contaminated with human blood or body fluids (**excluding sharps and glassware**) into the incinerator boxes that are available from DES. Do not overfill boxes or use without the plastic liners provided with them. These boxes may be used for temporary storage and accumulation of waste. When full, close and seal the plastic liner and box.
- C. Biological waste pickup request forms may be filled out and submitted electronically from the DES web site at <http://www.des.umd.edu>. DES will collect and dispose of all incinerator boxes.

IV. Disposal of Sharps and Disposable Glassware

- A. Discard all needles, needle and syringe units, scalpels, and razor blades, **whether contaminated or not**, directly into rigid, red, labeled sharps containers. Do not recap, bend, remove or clip needles. Sharps containers should not be overfilled. To request pickup of sharps containers, fill out and submit a biological waste pickup request form from the DES web site at <http://www.des.umd.edu>. Alternatively, closed sharps containers may be packaged in incinerator boxes (Section III above). Sharps containers may be purchased from Chemistry Stores.
- B. **Uncontaminated** Pasteur pipettes and broken or unbroken glassware are discarded into containers specifically designed for broken glass disposal, or into heavy-duty cardboard boxes that are closeable. When boxes are full, tape closed and place in the building's dumpster.
- C. **Contaminated** Pasteur pipettes and broken or unbroken glassware may be treated in one of two ways:
 - 1. Discarded into approved sharps containers, as in Section A above, or
 - 2. Decontaminated by autoclaving or chemical disinfection, then discarded into glass disposal boxes as in Section B above.
- D. Sharps that are contaminated with radioactive materials or hazardous chemicals should be discarded into separate sharps containers labeled with the name of the isotope or chemical. Contact DES (5-3960) for disposal information.

V. Multi-hazard or Mixed Waste

- A. Avoid generating mixed waste if possible. Keep volume to minimum.
- B. Do not autoclave mixed waste.
- C. When discarding waste containing an infectious agent and radioactive material, inactivate the infectious agent first, then dispose as radioactive waste. Seek advice from the RSO at (40)5-3985 before beginning inactivation procedures.
- D. When discarding waste containing an infectious agent and a hazardous chemical, inactivate the infectious agent first, then dispose as chemical waste. Seek advice before beginning inactivation procedures. Contact DES at (40)5-3960 for instructions.

VI. Disposal of Animal Tissues, Carcasses and Bedding

- A. Disposal of animal carcasses/tissues is coordinated through the Central Animal Resource Facility (CARF).
 - 1. Place animal carcasses/tissues into plastic bag. Double-bag when carcass contains zoonotic agent (transmissible from animals to humans).
 - 2. Place bag in freezer until pickup.
 - 3. Call CARF at (40)5-4921 for pickup.
- B. Disposal of animal carcasses/tissues that are contaminated with radioactive materials or hazardous chemicals is through DES. Disposal instructions are available by phoning (40)5-3960.

VII. Disposal Containers

Each laboratory is responsible for purchasing containers for the disposal of biological waste, EXCEPT incinerator boxes (with liners), which will be provided by DES. The following types of containers are available:

- A. Sharps containers may be purchased from local sources (including Chemistry Stores) as well as from laboratory product distributors. They are available in various sizes, and should be puncture resistant, red, labeled as "s/wharps," and have a tightly closing lid. Do not purchase "needle-cutter" devices, which may produce aerosols when used.
- B. Biohazard Autoclave Bags may be purchased from various laboratory product distributors, such as Fisher Scientific, VWR, and Baxter. Be sure to select polypropylene bags that are able to withstand autoclaving. They should be placed inside a rigid container with lid while waste is being collected.
- C. Incinerator Boxes are provided by DES. A plastic liner (also provided by DES) must be used to prevent contamination of the box.

- D. Glass Disposal Boxes may be purchased from General Stores and various laboratory product distributors. Alternatively, heavy-duty, closeable cardboard boxes may be used for disposal of broken glass.

VIII. What to do with Filled Waste Containers

- A. Sharps containers and incinerator boxes - To request pickup, fill out and submit a Biological Waste Pickup Removal Request form from the DES web site at <http://www.des.umd.edu>.
- B. Biohazard autoclave bags and glass disposal boxes - close and autoclave bags, tape glass disposal boxes closed; put both in building dumpster.

BIOSAFETY EQUIPMENT

BIOLOGICAL SAFETY CABINETS (BSCs)

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

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

The BSC requires regular maintenance and certification by a professional technician to assure that it protects you, your experiments, and the environment. Each cabinet should be certified when it is installed, each time it is moved or repaired, and at least annually. DES administers a program for annual certification of all BSCs at the University at no cost to the user. Contact DES at (40)5-6513 to confirm that your cabinet is included in this program.

- 1. Class I BSCs** protect personnel and the environment, but not research materials. They provide an inward flow of unfiltered air, similar to a chemical fume hood, which protects the worker from the material in the cabinet. The environment is protected by HEPA filtration of the exhaust air before it is discharged into the laboratory or ducted outside via the building exhaust.
- 2. Class II BSCs** (Types A1, A2, B1, B2) provide personnel, environment, and product protection. Air is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward laminar flow of HEPA-filtered air within the cabinet provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air passes through the exhaust HEPA filter, it is contaminant-free (environmental protection), and may be recirculated back into the laboratory (Type A) or ducted out of the building (Type B).
- 3. Class III BSCs** (sometimes called Class III glove boxes) were designed for work with infectious agents that require BSL4 containment, and provide maximum protection to the environment and the worker. The cabinet is gas-tight with a non-opening view window, and has rubber gloves attached to ports in the cabinet that allow for manipulation of materials in the cabinet. Air is filtered through one HEPA filter as it enters the cabinet, and through 2 HEPA filters before it is exhausted to the outdoors. This type of cabinet provides the highest level of product, environmental, and personnel protection.
- 4. Horizontal laminar flow "clean air benches"** are not BSCs. They discharge HEPA-filtered air across the work surface and toward the user, providing only product protection. They can be used for certain clean activities, such as dust-free assembly of sterile equipment or electronic devices. However, they should never be used when handling cell culture materials or potentially infectious materials, or as a substitute for a BSC in research laboratories.

OPERATION OF CLASS II BSCs

- 1) Turn on cabinet fan 15 minutes before beginning work.
- 2) Disinfect the cabinet work surface with 70% ethanol or other disinfectant.
- 3) Place supplies in the cabinet. Locate container inside the cabinet for disposal of pipettes. (Movement of hands in and out of the cabinet to discard pipettes into a container located outside of the cabinet creates turbulence and disrupts the air barrier that maintains sterility inside the cabinet.)

Work as far to the back (beyond the air split) of the BSC work space as possible.

Always use mechanical pipetting aids.

Do not work in a BSC while a warning light or alarm is signaling.

- 4) Locate liquid waste traps inside cabinet and use a hydrophobic filter to protect the vacuum line. If traps must be located on the floor, place them in a secondary container (such as a cardboard box) to prevent spilling.
- 5) Wear gloves when there is potential for skin contact with infectious material.
- 6) Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC may affect proper air flow and the level of protection provided. Also, keep the front and rear grilles clear.
- 7) When work is completed, remove equipment and supplies from the cabinet. Wipe the work area with 70% ethanol and allow cabinet to run for 15 minutes.
- 8) Some BSCs are equipped with ultraviolet (UV) lights. However, if good procedures are followed, UV lights are not needed. If one is used, due to the limited penetrating ability of UV light the tube should be wiped with alcohol every two weeks, while turned off, to remove dust. UV radiation should not take the place of 70% ethanol for disinfection of the cabinet interior.
- 9) The UV lamp should never be on while an operator is working in the cabinet.
- 10) Minimize traffic around the BSC and avoid drafts from doors and air conditioning.
- 11) Do not put your head inside the BSC. This compromises the sterility of the environment and, more importantly, could expose you to infectious pathogens.
- 12) Do not tamper with the BSC or interfere with its designed function. It was engineered to operate optimally with no obstructions around the sash or grilles.

- 13) Open flames are not required in the near microbe-free environment of a biological safety cabinet. On an open bench, flaming the neck of a culture vessel will create an upward air current which prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates turbulence which disrupts the pattern of HEPA-filtered air supplied to the work surface. Therefore, the **use of open flames and gas burners is strongly discouraged in biosafety cabinets**. When deemed absolutely necessary, touch-plate microburners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized. The burner must be turned off when work is completed. Small electric "furnaces" are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable sterile loops can also be used.

CENTRIFUGE CONTAINMENT

- ✓ Examine centrifuge tubes and bottles for cracks or stress marks before using them.
- ✓ Never overfill centrifuge tubes since leakage may occur when tubes are filled to capacity. Fill centrifuge tubes no more than 3/4 full.
- ✓ Centrifuge safety buckets and sealed rotors protect against release of aerosols.

PROTECTION OF VACUUM LINES

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

Collection and Overflow Flasks

- ✓ Collection tubes should extend at least 2 inches below the sidearm of the flask.
- ✓ Locate the collection flask inside the biosafety cabinet instead of on the floor, so the liquid level can be seen easily and the flask emptied before it overflows. The second flask (overflow) may be located outside the cabinet.
- ✓ If a glass flask is used at floor level, place it in a sturdy cardboard box or plastic container to prevent breakage by accidental kicking.
- ✓ In BSL2 and BSL3 laboratories, the use of Nalgene flasks is recommended to reduce the risk of breakage.

Vacuum Line Filter

A hydrophobic filter will prevent fluid and aerosol contamination of central vacuum systems or vacuum pumps. The filter will also prevent microorganisms from being exhausted by a vacuum pump into the environment. Hydrophobic filters such as the Gelman Vacushield are available from several scientific supply companies (Fisher Scientific, catalog #09-730-211, and VWR, catalog #55095-006).

SHIPMENT OF BIOLOGICAL MATERIALS

GENERAL INFORMATION

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

In general, **first** determine whether the material you wish to ship requires a permit and begin the application process, if required. **Second**, decide on a carrier, and learn the packaging and labeling requirements of that carrier. **Third**, contact the BSO to find out if training is required.

PERMITS

- ✓ Permits are required from the Centers for Disease Control and Prevention (CDC) to **import or transport** 1) any microorganism that causes disease in humans; 2) biological materials, such as blood and tissues, when known or suspected to contain an infectious agent; 3) live insects, such as mosquitoes, known or suspected of being infected with any disease transmissible to humans; and 4) any animal known or suspected of being infected with any disease transmissible to humans. Importation permits are issued only to the importer, who must be located in the U.S. The importation permit, with the proper packaging and labeling, will expedite clearance of the package of infectious materials through the U.S. Public Health Service Division of Quarantine and release by U.S. Customs. Transfers of previously imported material within the U.S. also require a permit. Application for the permit should be made at least 10 working days in advance of the anticipated shipment date. Further information and application forms may be obtained by calling the CDC at (404) 639-3235, or through the CDC web site at <http://www.cdc.gov/od/ohs/biosfty/imprrtper.htm>
- ✓ Permits are required from the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) for **importation or domestic transport of agents** infectious to livestock; and of biological reagents containing animal, particularly livestock, material (this includes tissue culture media containing growth stimulants of bovine origin such as calf serum). Further information and application forms may be obtained by calling the USDA/APHIS at (301) 734-4401, or through the APHIS web site at <http://www.aphis.usda.gov/forms/index.html>
- ✓ Permits are also required from the USDA/APHIS for **interstate movement, importation, or release into the environment (i.e., field tests)** of genetically engineered organisms that are **plant pests**, or that contain portions (plasmids, DNA fragments, etc.) of **plant pests**. Application should be made at least 120 days in advance of the anticipated release or shipment date. Information and application forms may be obtained by calling the USDA/APHIS at (301) 734-4401, or through the APHIS web site at <http://www.aphis.usda.gov/brs/regulatory-activities.html>

- ✓ Facility registration and completion of the CDC/USDA Form 2 are required by the CDC prior to transfer of **select agents and toxins** (42 CFR Part 73). Select agents are listed in Appendix 4, and a copy of the regulation is available at <http://www.des.umd.edu>. Contact the BSO at (40)5-3975 if your work includes any of the agents listed in Appendix 4.
- ✓ A validated license is required by the Department of Commerce for **export** of certain microorganisms and toxins (listed in Appendix 5) to all destinations except Canada. Information may be obtained by calling (202) 482-0896.

PACKAGING

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

- ✓ Infectious Substance: a viable microorganism, or its toxin, which causes or may cause disease in humans. DOT requires shippers of infectious substances to attend training every 3 years.
- ✓ Diagnostic Specimen: any human or animal material including blood, tissue, and tissue fluids, shipped for the purpose of diagnosis.
- ✓ Biological Product: a product for human or veterinary use, such as vaccines and investigational new drugs.

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

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

GENETICALLY MODIFIED MICROORGANISMS

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

- ✓ GMOs of Category A agents must be shipped as Category A.
- ✓ GMOs of Category B agents must be shipped as Category B.
- ✓ Non-infectious GMOs are exempt from the shipping regulations.

HUMAN CLINICAL MATERIALS

- ✓ The OSHA Bloodborne Pathogens Standard requires that all packages containing human blood and other potentially infectious materials be labeled with the universal biohazard symbol or color-coded. Various carriers may have additional requirements.

ON-CAMPUS TRANSPORT BETWEEN LABORATORIES OR BUILDINGS

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

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

NOTE: Shipment of chemicals, including formaldehyde or samples fixed in formalin, are not covered in training provided by DES. For shipment of chemicals or non-biological materials, contact Doug Waterman at the UM Physical Distribution Center (301 405 5852).

APPENDIX 1

Biosafety Level 1 (BSL1)

Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, February 2007
Centers for Disease Control and Prevention and National Institutes of Health

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

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

Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
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. Plasticware 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. 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. Agent information should be posted in accordance with the institutional policy.
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 child-bearing 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.

Special Practices

None required.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. Special containment devices or equipment, such as 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. 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, integrity has been 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.

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 windows that open to the exterior should be fitted with screens.

APPENDIX 2

Biosafety Level 2 (BSL-2)

Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, February 2007
Centers for Disease Control and Prevention and National Institutes of Health

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:

- laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures;
- access to the laboratory is restricted when work is being conducted; and
- all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

The following standard and special practices, safety equipment, and facility requirements apply to BSL-2:

Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
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. 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. Posted information must include: the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required. See Appendix G.
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 child-bearing 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.

Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

2. Laboratory personnel must be provided medical evaluation and offered appropriate immunizations for agents handled or potentially present in the laboratory.
3. Each institution must establish policies and procedures describing the 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 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 safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, evaluation, 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.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. Properly maintained BSCs (preferably Class II), 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, 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, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. 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, integrity has been 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 should be used in rooms containing infected animals as determined by the risk assessment.

Laboratory Facilities (Secondary Barriers)

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.

2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.

3. 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 High Efficiency Particulate Air (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.
9. There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.
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).

APPENDIX 3

Biosafety Level 3 (BSL-3)

Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, February 2007
Centers for Disease Control and Prevention and National Institutes of Health

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 inhalation route 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, other physical containment devices, or by personnel wearing appropriate personal protective equipment.

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:

Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
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. Plasticware 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. 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. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
 10. An effective integrated pest management program is required. See Appendix G.
 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 child-bearing 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.

Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

2. Laboratory personnel must be provided medical evaluation and offered appropriate immunizations for agents handled or potentially present in the laboratory.
3. Each institution must establish policies and procedures describing the 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.
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 safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, evaluation, 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.

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. Protective laboratory clothing with a solid-front such as tie-back or wraparound gowns, scrub suits, or coveralls are worn by workers when in the laboratory. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated with appropriate disinfectant before being laundered. Clothing is changed when contaminated.

3. Eye and face protection (goggles, mask, face shield or other splatter 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 should:
 - a. Change gloves when contaminated, integrity has been 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.

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. Access to the laboratory is restricted to entry by a series of 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 of the biological agents in use.

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 air flow. A visual monitoring device which confirms directional air flow 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.
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 a direct (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, incineration, or other validated decontamination method).
12. Equipment that may produce infectious aerosols must be contained in 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 means of 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. 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.
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.

APPENDIX 4: HHS and USDA Select Agents & Toxins

HHS Non-Overlap Select Agents and Toxins

- Abrin
- Botulinum neurotoxin
- Botulinum neurotoxin-producing species of *Clostridium*
- Cercopithecine herpesvirus (Herpes B virus)
- *Clostridium perfringens* epsilon toxin
- *Coccidioides posadasii*/ *Coccidioides immitis*
- Conotoxins
- Crimean-Congo haemorrhagic fever virus
- Diacetoxyscirpenol
- Eastern Equine Encephalitis virus
- Ebola virus
- Lassa fever virus
- Marburg virus
- Monkeypox virus
- Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
- Ricin
- *Rickettsia prowazekii*
- *Rickettsia rickettsii*
- Saxitoxin
- Shiga-like ribosome inactivating proteins
- South American Haemorrhagic Fever viruses
 - Flexal
 - Guanarito
 - Junin
 - Machupo
 - Sabia
- Staphylococcal enterotoxins
- T-2 toxin
- Tetrodotoxin
- Tick-borne encephalitis complex (flavi) viruses
 - Central European tick-borne encephalitis
 - Far Eastern tick-borne encephalitis
 - Kyasanur forest disease
 - Omsk hemorrhagic fever

- Russian spring and summer encephalitis
- Variola major virus (Smallpox virus)
- Variola minor virus (Alastrim)
- *Yersinia pestis*

Overlap Select Agents and Toxins

- *Bacillus anthracis*
- *Brucella abortus*
- *Brucella melitensis*
- *Brucella suis*
- *Burkholderia mallei* (formerly *Pseudomonas mallei*)
- *Burkholderia pseudomallei* (formerly *Pseudomonas pseudomallei*)
- Hendra virus
- Nipah Virus
- Rift Valley fever virus
- Venezuelan Equine Encephalitis virus

USDA Select Agents and Toxins

- African horse sickness virus
- African swine fever virus
- Akabane virus
- Avian influenza virus (highly pathogenic)
- Bluetongue virus (Exotic)
- Bovine spongiform encephalopathy agent
- Camel pox virus
- Classical swine fever virus
- *Ehrlichia ruminantium* (Heartwater)
- Foot-and-mouth disease virus
- Goat pox virus
- Japanese encephalitis virus
- Lumpy skin disease virus
- Malignant catarrhal fever virus (Alcelaphine herpesvirus type 1)
- Menangle virus
- *Mycoplasma capricolum* subspecies *capripneumoniae*(contagious caprine pleuropneumonia)
- *Mycoplasma mycoides* subspecies *mycoides* small colony (*MmmSC*) (contagious bovine pleuropneumonia)
- Peste des petits ruminants virus
- Rinderpest virus
- Sheep pox virus

- Swine vesicular disease virus
- Vesicular stomatitis virus (exotic): Indiana subtypes VSV-IN2, VSV-IN3
- Virulent Newcastle disease virus¹

USDA Plant Pathogens

- *Peronosclerospora philippinensis* (*Peronosclerospora sacchari*)
- *Phoma glycinicola* (formerly *Pyrenochaeta glycines*)
- *Ralstonia solanacearum* race 3, biovar 2
- *Sclerophthora rayssiae* var *zeae*
- *Synchytrium endobioticum*
- *Xanthomonas oryzae*
- *Xylella fastidiosa*(citrus variegated chlorosis strain)

¹ A virulent Newcastle disease virus (avian paramyxovirus serotype 1) has intracerebral pathogenicity index in day-old chicks (*Gallus gallus*) of 0.7 or greater or has an amino acid sequence at the fusion (F) protein cleavage site that is consistent with virulent strains of Newcastle disease virus. A failure to detect a cleavage site that is consistent with virulent strains does not confirm the absence of a virulent virus.

APPENDIX 5

Export Administration Regulations

Department of Commerce

15 CFR Parts 730-799

Microorganisms and toxins that require a validated license for export to all destinations except Canada.

a. Viruses, as follows:

- a.1. Chikungunya virus;
- a.2. Congo-Crimean haemorrhagic fever virus (a.k.a. Crimean-Congo haemorrhagic fever virus);
- a.3. Dengue fever virus;
- a.4. Eastern equine encephalitis virus;
- a.5. Ebola virus;
- a.6. Hantaan virus;
- a.7. Japanese encephalitis virus;
- a.8. Junin virus;
- a.9. Lassa fever virus
- a.10. Lymphocytic choriomeningitis virus;
- a.11. Machupo virus;
- a.12. Marburg virus;
- a.13. Monkey pox virus;
- a.14. Rift Valley fever virus;
- a.15. Tick-borne encephalitis virus (Russian Spring-Summer encephalitis virus);
- a.16. Variola virus;
- a.17. Venezuelan equine encephalitis virus;
- a.18. Western equine encephalitis virus;
- a.19. White pox;
- a.20. Yellow fever virus;
- a.21. Kyasanur Forest virus;

- a.22. Louping ill virus;
- a.23. Murray Valley encephalitis virus;
- a.24. Omsk haemorrhagic fever virus;
- a.25. Oropouche virus;
- a.26. Powassan virus;
- a.27. Rocio virus;
- a.28. St. Louis encephalitis virus;
- a.29. Hendra virus (Equine morbillivirus);
- a.30. South American haemorrhagic fever (Sabia, Flexal, Guanarito);
- a.31. Pulmonary and renal syndrome- haemorrhagic fever viruses (Seoul, Dobrava, Puumala, Sin Nombre); or
- a.32. Nipah virus.

b. Rickettsiae, as follows:

- b.1. Bartonella quintana (Rochalimea quintana, Rickettsia quintana);
- b.2. Coxiella burnetii;
- b.3. Rickettsia prowasecki (a.k.a. Rickettsia prowazekii); or
- b.4. Rickettsia rickettsii.

c. Bacteria, as follows:

- c.1. Bacillus anthracis;
- c.2. Brucella abortus;
- c.3. Brucella melitensis;
- c.4. Brucella suis;
- c.5. Burkholderia mallei (Pseudomonas mallei);
- c.6. Burkholderia pseudomallei (Pseudomonas pseudomallei);
- c.7. Chlamydia psittaci;
- c.8. Clostridium botulinum;
- c.9. Francisella tularensis;
- c.10. Salmonella typhi;
- c.11. Shigella dysenteriae;

- c.12. *Vibrio cholerae*;
- c.13. *Yersinia pestis*;
- c.14. *Clostridium perfringens*, epsilon toxin producing types; or
- c.15. Enterohaemorrhagic *Escherichia coli*, serotype O157 and other verotoxin producing serotypes.

d. "Toxins", as follows, and "subunits" thereof:

- d.1. Botulinum toxins;
- d.2. *Clostridium perfringens* toxins;
- d.3. Conotoxin;
- d.4. Microcystin (Cyanginosin);
- d.5. Ricin;
- d.6. Saxitoxin;
- d.7. Shiga toxin;
- d.8. *Staphylococcus aureus* toxins;
- d.9. Tetrodotoxin;
- d.10. Verotoxin and other Shiga-like ribosome inactivating proteins;
- d.11. Aflatoxins;
- d.12. Abrin;
- d.13. Cholera toxin;
- d.14. Diacetoxyscirpenol toxin;
- d.15. T-2 toxin;
- d.16. HT-2 toxin;
- d.17. Modeccin toxin;
- d.18. Volkensin toxin; or
- d.19. *Viscum Album* Lectin 1 (Viscumin).

e. "Fungi", as follows:

- e.1. *Coccidioides immitis*; or
- e.2. *Coccidioides posadasii*.

Animal pathogens, as follows:

a. Viruses, as follows:

- a.1. African swine fever virus;
- a.2. Avian influenza virus that are:
 - a.2.a. Defined as having high pathogenicity, as follows:
 - a.2.a.1. Type A viruses with an IVPI (intravenous pathogenicity index) in 6 week old chickens of greater than 1.2; or
 - a.2.a.2. Type A viruses H5 or H7 subtype for which nucleotide sequencing has demonstrated multiple basic amino acids at the cleavage site of haemagglutinin;
- a.3. Bluetongue virus;
- a.4. Foot and mouth disease virus;
- a.5. Goat pox virus;
- a.6. Porcine herpes virus (Aujeszky's disease);
- a.7. Swine fever virus (Hog cholera virus);
- a.8. Lyssa virus;
- a.9. Newcastle disease virus;
- a.10. Peste des petits ruminants virus;
- a.11. Porcine enterovirus type 9 (swine vesicular disease virus);
- a.12. Rinderpest virus;
- a.13. Sheep pox virus;
- a.14. Teschen disease virus;
- a.15. Vesicular stomatitis virus;
- a.16. Lumpy skin disease virus;
- a.17. African horse sickness virus.

b. Bacteria, as follows:

- b.1 Mycoplasma mycoides, as follows:
 - b.1.a. Mycoplasma mycoides subspecies mycoides SC (small colony) (a.k.a. contagious bovine pleuropneumonia);
 - b.1.b. Mycoplasma capricolum subspecies capripneumoniae ("strain F38").

Genetic elements and genetically- modified organisms, as follows:

a. Genetic elements, as follows:

- a.1. Genetic elements that contain nucleic acid sequences associated with the pathogenicity of microorganisms controlled by 1C351.a to .c, 1C352, 1C354, or 1C360;
- a.2. Genetic elements that contain nucleic acid sequences coding for any of the "toxins" controlled by 1C351.d or "sub-units of toxins" thereof.

b. Genetically modified organisms, as follows:

- b.1. Genetically modified organisms that contain nucleic acid sequences associated with the pathogenicity of microorganisms controlled by 1C351.a to .c, 1C352, 1C354, or 1C360;
- b.2. Genetically modified organisms that contain nucleic acid sequences coding for any of the "toxins" controlled by 1C351.d or "sub-units of toxins" thereof.

Technical Note: 1. "Genetic elements" include, inter alia, chromosomes, genomes, plasmids, transposons, and vectors, whether genetically modified or unmodified.

2. This ECCN does not control nucleic acid sequences associated with the pathogenicity of enterohaemorrhagic Escherichia coli, serotype O157 and other verotoxin producing strains, except those nucleic acid sequences that contain coding for the verotoxin or its sub-units.

3. "Nucleic acid sequences associated with the pathogenicity of any of the microorganisms controlled by 1C351.a to .c, 1C352, 1C354, or 1C360" means any sequence specific to the relevant controlled microorganism that:

- a. In itself or through its transcribed or translated products represents a significant hazard to human, animal or plant health; or
- b. Is known to enhance the ability of a microorganism controlled by 1C351.a to .c, 1C352, 1C354, or 1C360, or any other organism into which it may be inserted or otherwise integrated, to cause serious harm to human, animal or plant health.

Plant pathogens, as follows:

a. Bacteria, as follows:

- a.1. *Xanthomonas albilineans*;
- a.2. *Xanthomonas campestris* pv. *Citri* including strains referred to as *Xanthomonas campestris* pv. *citri* types A,B,C,D,E or otherwise classified as *Xanthomonas citri*, *Xanthomonas campestris* pv. *aurantifolia* or *Xanthomonas campestris* pv. *citrumelo*;
- a.3. *Xanthomonas oryzae* pv. *oryzae* (syn. *Pseudomonas campestris* pv. *oryzae*);
- a.4. *Clavibacter michiganensis* subspecies *sepedonicus* (syn. *Corynebacterium michiganensis* subspecies *sepedonicum* or *Corynebacterium sepedonicum*);
- a.5. *Ralstonia solanacearum* Races 2 and 3 (syn. *Pseudomonas solanacearum* Races 2 and 3 or *Burkholderia solanacearum* Races 2 and 3);

b. Fungi, as follows:

- b.1. *Colletotrichum coffeanum* var. *virulans* (*Colletotrichum kahawae*);
- b.2. *Cochliobolus miyabeanus* (*Helminthosporium oryzae*);
- b.3. *Microcyclus ulei* (syn. *Dothidella ulei*);
- b.4. *Puccinia graminis* (syn. *Puccinia graminis* f. sp. *tritici*);
- b.5. *Puccinia striiformis* (syn. *Puccinia glumarum*);
- b.6. *Magnaporthe grisea* (*pyricularia grisea/pyricularia oryzae*);

c. Viruses, as follows:

- c.1. Potato Andean latent tymovirus;
- c.2. Potato spindle tuber viroid.