

Biosafety Manual

For

Tennessee State University

Revised October 2006

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Important Telephone Numbers

Emergency Telephone Numbers:

Campus Police: 963-5171
Fire, Police, Rescue 9-1-1
Biosafety Committee Chair:
Dr. Mohammad Karim
963-5344

Other Important Numbers:

University Health center:
Facilities Management:
Radiation Safety Officer: 963-5344

Useful Websites:

NIH Guidelines:

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

BMBL:

<http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.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/eaipp/>

USDA/APHIS Permit to Import or Transport Livestock Pathogens:

<http://www.aphis.usda.gov/forms/index.html>

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

http://www.aphis.usda.gov/biotechnology/permits_main.shtml

University of Maryland Form for Registration of Biological Materials:

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

Selection, Installation, and Use of Biological Safety Cabinets:

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

Policy Statement

I. Purpose:

The purpose of the manual is to establish the process for compliance with the following documents:

- A. NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines):
- B. Biosafety in Microbiological and Biomedical Laboratories (BMBL)

II. Policy:

Tennessee State University is committed to preserving the health and safety of its students, faculty and staff. The University is also committed to protecting the environment and the community. It is recognized that the use of recombinant DNA or other potentially harmful pathogenic microorganisms is necessary in many research and teaching laboratories at the University. The University requires the compliance with the NIH guidelines and with the recommendations in BMBL to ensure the safe handling of these organisms. 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. Institutional Biosafety Committee (IBC) is to assist the PI with these responsibilities.

A. The University Biosafety Committee 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. Provide or coordinate biosafety training as requested
5. Assist investigators with risk assessment
6. Administer all elements of the Biosafety Program, assist faculty with submission of registrations to the IBC, and maintain registration files
7. 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*;

8. 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*;
9. Notify the PI of the results of the IBC's review and approval;
10. 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 Activities (OBA) within 30 days; and
11. 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.

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, 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
 - d. animal and plant pathogens.

C. The University Health Center (UHC) shall:

1. Provide medical surveillance, 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.

D. Laboratory personnel shall:

1. Comply with safety recommendations for the work being performed; and
2. Report accidents or injuries to the PI.

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) classify pathogenic agents into one of four risk groups according to specific criteria. It is Tennessee State University 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 IBC, the laboratory will be surveyed by the Institutional Biosafety Committee (IBC) 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.

Genetically Engineered Microorganisms

Work with all genetically engineered organisms must comply with the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines-Recent Report April 2002)*. 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 Tennessee State University policy that all laboratories follow these guidelines.

Registration

Each PI is responsible for registering all recombinant DNA experiments with the IBC, including those exempt from the *NIH Guidelines*. The IBC audits all laboratories where BSL2 or BSL3 containment is required. BSL1 laboratories are audited on request of the PI.

Review and Approval of Experiments

The IBC, which oversees recombinant DNA research at Tennessee State University, will review 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 research involving Select Agents.
- Cloning DNA from Risk Group 2, 3, 4, or Select Agents.

- 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 Chair of the IBC or 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.
- 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 Appendix 6](#) for detailed information on handling human clinical material.

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 IBC of their use of human materials. Training and immunization are required by OSHA.

Plant and Animal Pathogens

The IBC requires investigators to register their campus use of plant pathogens. The registration form for animal pathogens is available at the web site: <http://www.tnstate.edu>. Registration of plant pathogens may be completed by forwarding a copy to the Biosafety Office.

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 Biosafety Office immediately if you currently possess or plan to acquire any of the listed agents 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 Biosafety Office, or visit [CDC's Select Agent Program web site](#), which provides links to select agent program information.

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 Tennessee State University is conducted at BSL1 or BSL2 containment. There are no BSL4 laboratories at the university.

Biosafety Level 1

BSL1 is appropriate for undergraduate and secondary educational training and teaching laboratories, and for other facilities in which work is done with well-characterized agents not known to cause disease in healthy adult humans. The laboratory is not necessarily separated from the general traffic patterns in the building. BSL1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing. The following Standard Microbiological Practices apply to all Biosafety Levels. Additional practices recommended for BSL2 are in Appendix 2, and for BSL3 in Appendix 3.

Standard Microbiological Practices:

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens are in progress.
2. Persons wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas where there is reasonable likelihood of exposure to potentially infectious materials. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. All procedures are performed carefully to minimize the creation of splashes or aerosols.
6. Work surfaces are decontaminated at least once a day and after any spill of viable material.
7. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leak-proof container and closed for transport from the laboratory. Materials to be decontaminated off-site are packaged in accordance with applicable state and federal regulations before removal from the facility.
8. An insect and rodent control program is in effect.

Biosafety Level 2

BSL2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists, (2) access to the laboratory is limited when work is being conducted, (3) extreme precautions are taken with contaminated sharp items, and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment. With good microbiological techniques, work at BSL2 can be conducted safely on the open bench, provided the potential for producing splashes or aerosols is low. Primary hazards to personnel working with BSL2 agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. BSL2 is appropriate when work is done with any human-derived blood, body fluids, or tissues where the presence of an infectious agent may be unknown. See Appendix 2 for a complete list of BSL2 criteria.

Biosafety Level 3

BSL3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents. Primary hazards to personnel working at BSL3 relate to autoinoculation, ingestion, and exposure to infectious aerosols. See Appendix 3 for a complete list of BSL3 criteria.

Biosafety Level 4

BSL4 is required for work with dangerous and exotic agents which pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease. Agents with a close or identical antigenic relationship to BSL4 agents are handled at this level until sufficient data are obtained either to confirm continued work at this level, or to work with them at a lower level. Members of the laboratory staff have specific and thorough training in handling extremely hazardous infectious agents; and they understand the primary and secondary containment functions of the standard and special practices, the containment equipment, and the laboratory design characteristics. They are supervised by competent scientists who are trained and experienced in working with these agents. Access to the laboratory is strictly controlled by the laboratory director. The facility is either in a separate building or in a controlled area within a building, which is completely isolated from all other areas of the building. A specific facility operations manual is prepared or adopted.

Within work areas of the facility, all activities are confined to Class III biological safety cabinets, or Class II biological safety cabinets used with one-piece positive pressure personnel suits ventilated by a life support system. The BSL4 laboratory has special engineering and design features to prevent microorganisms from being disseminated into the environment.

¹From: Biosafety in Microbiological and Biomedical Laboratories. Centers for Disease Control and Prevention, and National Institutes of Health, 1993.

Biosafety Level	Risk Assessment	Practices and Techniques	Safety Equipment	Examples
BSL1 Basic Laboratory	Individual risk: Low Community risk: Low	Standard Microbiological Practices.	None: primary containment provided by adherence to standard lab practices during open bench operations.	<i>E. coli</i> K12; <i>S. cerevisiae</i> ; short term, long term culture of most non-primate mammalian cells.
BSL2 Basic Laboratory with biosafety cabinets and other physical containment devices as required	Individual risk: Moderate Community risk: Low	Level 1 practices plus: lab coats; autoclaving all biological waste preferred; limited access; biohazard warning signs on doors and equipment.	Partial containment (i.e., Class I or II biosafety cabinets) for procedures which produce aerosols.	<i>E. coli</i> O157; Hepatitis B virus; <i>Salmonella typhimurium</i> ; human blood; <i>Neisseria gonorrhoeae</i> ; culture of lymphoid lines carrying inducible EB; many common human pathogens.
BSL3 Containment Laboratory with special engineering and design features	Individual risk: High Community risk: Low	Level 2 practices plus: special protective clothing; controlled access through entrance room; biological waste must be autoclaved, preferably within the	Partial containment equipment used for <u>all</u> manipulations of infectious materials; directional airflow.	Yellow fever virus; <i>M. tuberculosis</i> ; Industrial scale volumes of HIV.

		laboratory.		
BSL4 Maximum Containment Laboratory	Individual risk: High Community risk: High	Level 3 practices plus: entrance through change room; complete change of clothing from street to laboratory gear; shower at exit; all wastes decontaminated on exit from facility.	Maximum containment equipment (i.e., Class III biosafety cabinet or partial containment in combination with full- body, air-supplied positive-pressure personnel suit) used for all procedures and activities.	Ebola virus; Marburg virus; propagation of herpes virus simiae.

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), a package of paper towels, household rubber gloves, autoclave bags, sharps container, and forceps to pick up broken glass

General Spill Cleanup Guidelines

- Wear gloves and lab coat.
- 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 Biosafety Office at 963-5344 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 a 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 Biosafety Office at 963-5344 (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 (PPE) (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 lab coat, spray or wipe cabinet walls, work surfaces, and equipment with disinfectant such as 70% ethanol. If necessary, flood work surface, as well as drain

pans and catch basins below the work surface, with disinfectant. Allow at least 20 minutes contact time.

- 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 Biosafety Office 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 Biological Radioactive 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 you disinfect 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 963-5344 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 handwashing 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 (disinfectant, autoclavable containers, forceps, paper towels, sharps container).
- f. Confirm with the RSO that it is safe to enter the lab.

Clean-up of a Biological Radioactive 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 Biosafety Office.

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 University Health Center (UHC) to obtain care. If UHC is closed, go to the emergency room at the most convenient local emergency hospital.
- Report the accident to the PI and Biosafety Office, 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 963-5291 to obtain care. If UHC is closed, go to the emergency room at a local hospital.
- Report the injury to the PI and to Biosafety Office, 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.

Decontamination and Disposal of Biological Organisms

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 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 wk		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 ppm
Not diluted	5.25	50,000
1/10	0.525	5,000
1/100	0.025	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 in the autoclave. 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 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.

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°C, and 2) heat-resistant spores (*Bacillus stearothermophilis*) that are killed by exposure to 121°C for approximately 15 minutes. Both types of tests should be placed well down in the center of the bag or container of waste, at the point slowest to heat.

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

If you need assistance, please contact the Biosafety Office 963-5344.

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 the sharps containers. Close completely when they are 3/4 full.
- Locate sharps containers in areas in which needles are commonly used. Make containers easily accessible.
- Sharps containers may be purchased from VWR and Fisher Scientific and other scientific supply vendors.

In the event of a needle stick injury:

- Wash thoroughly with soap and water. Notify supervisor and go immediately to Urgent Care Clinic at UHC. If UHC is closed, go to the emergency room at the most convenient local emergency room.

Biological Waste Disposal Procedures

Please read and follow the Waste Disposal Guidelines wall chart.

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. Decontaminated 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 bleach has been used in the tray used to collect 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 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. 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 Biosafety Webpage at <http://www.tnstate.edu/research>. Biosafety Office will contact you and make the necessary action for disposal.

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 Biosafety Office web page at: <http://www.tnstate.edu/research> Alternatively, closed sharps containers may be packaged in incinerator boxes (Section III above). Sharps containers may be purchased from VWR and Fisher Scientific, and other scientific supply vendors.
- B. **Uncontaminated** pasteur pipets 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 pipets, 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 Biosafety Office 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 of as radioactive waste. Seek advice from the RSO at 963-7631 before beginning inactivation procedures.
- D. When discarding waste containing an infectious agent and a hazardous chemical, inactivate the infectious agent first, then dispose of as chemical waste. Seek advice before beginning inactivation procedures. Contact Biosafety Office at 963-7631 for instructions.

VI. Disposal of Animal Tissues, Carcasses and Bedding

- A. Disposal of animal carcasses/tissues is coordinated through the Central Facilities Management.
 - 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 pick-up.
 - 3. Call Facilities Management at 963-5683 for pick-up.
- B. Disposal of animal carcasses/tissues that are contaminated with radioactive materials or biohazardous chemicals is through Biosafety Office.

VII. Disposal Containers

Each laboratory is responsible for purchasing containers for the disposal of biological waste. 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 "Sharps," 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 which are able to withstand autoclaving. They should be placed inside a rigid container with lid while waste is being collected.
- C. Incinerator Boxes must be used. A plastic liner must be used to prevent contamination of the box.
- D. Glass Disposal Boxes may be purchased from Chemistry 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, facilities management
 - B. Biohazard autoclave bags and glass disposal boxes - close and autoclave bags, tape boxes closed; put both in building dumpster.
-

Instructions
Biological, Pathological or Medical Waste Removal Request Form

Biosafety Equipment

Biological Safety Cabinets

The biological safety cabinet (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 particulates of 0.3 microns with an efficiency of 99.97%. However, it does not remove vapors or gases.

The biosafety cabinet 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. Contact Biosafety Office at 963-5344 to confirm that your cabinet is included in this program.

1. **Class I cabinets** 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 to the outside via the building exhaust.
2. **Class II** (Types A1, A2, B1, B2, and B3) BSCs 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 cabinets** (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 pipets. (Movement of hands in and out of the cabinet to discard pipets 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 workspace as possible.

Always use mechanical pipetting aids.

Avoid using open flames inside BSCs. If a flame is necessary, use a burner with a pilot light and place it to the rear of the workspace. Flames disrupt the airflow and contribute to the heat load inside the BSC. Flames have burned holes through HEPA filters and have caused explosions in BSCs.

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 biosafety cabinet and avoid drafts from doors and air conditioning.

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 and above, 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 clinical 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.

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 at <http://www.cdc.gov>

- Permits are also required from the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) for **import or transport** of organisms 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 at <http://www.aphis.usda.gov>
- 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 applications may be obtained at http://www.aphis.usda.gov/brs/regulatory_activities.html.
- Facility registration and completion of the CDC Form EA-101 are required by the CDC and USDS/APHIS prior to transfer of **select agents and toxins** (42 CFR Part 73). Select agents are listed in Appendix 4. Please contact the BSO at 963-7631 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 is a viable microorganism, or its toxin, which causes or may cause disease in humans.
- Diagnostic Specimen is any human or animal material including blood, tissue, and tissue fluids, shipped for the purpose of diagnosis.
- Biological Product is 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;

- An outer rigid shipping container that contains the secondary container and other material.

Genetically Modified Microorganisms

The *NIH Guidelines for Experiments Involving Recombinant DNA Molecules* (April 2002) states that:

- Host organisms should be shipped as etiologic agents, regardless of whether they contain recombinant DNA (rDNA), if they are regulated as human pathogens, animal pathogens, or plant pests.
- Host organisms should be shipped as etiologic agents if they contain 1) rDNA that includes the complete genome of an organism that is a human or animal pathogen or plant pest; 2) rDNA that codes for a toxin involved in eliciting human, animal, or plant disease, and is carried on an expression vector or within the host chromosome; or 3) rDNA from an organism regulated as a human or animal pathogen or a plant pest that has not been adequately characterized.

Human Blood and Tissue

- 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.

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Appendix 1

Biosafety Level 1

Biosafety Level 1 is suitable for work involving well-characterized agents not known to cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or

facility design is not required nor generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

The following standard and special practices, safety equipment and facilities apply to agents assigned to Biosafety Level 1:

A. Standard Microbiological Practices

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens are in progress.
2. Persons wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas where there is reasonable likelihood of exposure to potentially infectious materials. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. All procedures are performed carefully to minimize the creation of splashes or aerosols.
6. Work surfaces are decontaminated at least once a day and after any spill of viable material.
7. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated at off-site from the laboratory are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.
8. An insect and rodent control program is in effect.

B. Special Practices: None

C. Safety Equipment (Primary Barriers)

1. Special containment devices or equipment such as biological safety cabinets are generally not required for manipulation of agents assigned to Biosafety Level 1.
2. It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.
3. Gloves should be worn if the skin on the hands is broken or if a rash exists.
4. Protective eyewear should be worn for anticipated splashes of microorganisms or other hazardous materials to the face.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratories should have doors for access control.
2. Each laboratory contains a sink for handwashing.
3. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
4. Bench tops are impervious to water and resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surface and equipment.
5. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning.

6. If the laboratory has windows that open, they are fitted with fly screens.

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Appendix 2

Biosafety Level 2

Biosafety in Microbiological and Biomedical Laboratories 4th Edition, May 1999 Centers for Disease Control and Prevention and National Institutes of Health

Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists, (2) access to the laboratory is limited when work is being conducted, (3) extreme precautions are taken with contaminated sharp items, and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

The following standard and special practices, safety equipment and facilities apply to agents assigned to Biosafety Level 2:

A. Standard Microbiological Practices

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
2. Persons wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.
4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. All procedures are performed carefully to minimize the creation of splashes or aerosols.
6. Work surfaces are decontaminated at least once a day and after any spill of viable material.
7. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated at off-site from the laboratory are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.
8. An insect and rodent control program is in effect.

B. Special Practices:

1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the

laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.

2. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet specific entry requirements (e.g., immunization) enter the laboratory or animal rooms.
3. A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate information to be posted includes the agent(s) in use, the biosafety level, the required immunizations, the investigator's name and telephone number, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.
4. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).
5. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.
6. Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
7. The laboratory director ensures that laboratory personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural or policy changes.
8. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
 - a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.
 - b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - c. Syringes which re-sheath the needle, needle-less systems, and other safe devices should be used when appropriate.
 - d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.

9. Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.
 10. Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.
 11. Spills and accidents which result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
 12. Animals not involved in the work being performed are not permitted in the lab.
- C. Safety Equipment (Primary Barriers)**
1. Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:
 - a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, 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 if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.
 2. Face protection (goggles, mask, faceshield or other splatter guards) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face, when the microorganisms must be manipulated outside the BSC.
 3. Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.
 4. Gloves are worn when handling infected animals and when hands may contact infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed, or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.
- D. Laboratory Facilities (Secondary Barriers)**
1. Provide lockable doors for facilities that house restricted agents as defined in 42 CFR 72.6.
 2. Consider locating new laboratories away from public areas.
 3. Each laboratory contains a sink for handwashing. Foot, knee, or automatically operated sinks are recommended.

4. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.
5. Bench tops are impervious to water and resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
6. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
7. Install biological safety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Locate biological safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the biological safety cabinets' air flow parameters for containment.
8. An eyewash facility is readily available.
9. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
10. There are no specific ventilation requirements. 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. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

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Appendix 3

Biosafety Level 3

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents.

All procedures involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment. The laboratory has special engineering and design features.

It is recognized, however, that many existing facilities may not have all the facility safeguards recommended for Biosafety Level 3 (e.g. access zone, sealed penetrations, and directional airflow, etc.). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g. diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in Biosafety Level 2 facilities. However, the recommended Standard Microbiological Practices, Special Practices, and Safety Equipment for Biosafety Level 3 must be rigorously followed. The decision to

implement this modification of Biosafety Level 3 recommendations should be made only by the laboratory director.

The following standard and special safety practices, equipment and facilities apply to agents assigned to Biosafety Level 3:

A. Standard Microbiological Practices

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
2. Persons wash their hands after handling infectious materials and animals, after removing gloves, and when they leave the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the laboratory. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.
4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of aerosols.
7. Work surfaces are decontaminated at least once a day and after any spill of viable material.
8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leakproof container and closed for transport from the laboratory. Infectious waste from BSL-3 laboratories should be decontaminated before removal for off-site disposal.
9. An insect and rodent control program is in effect (see Appendix G).

B. Special Practices:

1. Laboratory doors are kept closed when experiments are in progress.
2. The laboratory director controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. For example, persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. Persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.
3. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures, enter the laboratory or animal rooms.
4. When infectious materials or infected animals are present in the laboratory or containment module, a hazard warning sign, incorporating the universal biohazard symbol, is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for immunizations, respirators, or other personal protective measures.

5. Laboratory personnel receive the appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing), and periodic testing as recommended for the agent being handled.
6. Baseline serum samples are collected and stored for all laboratory and other at-risk personnel. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the laboratory.
7. A biosafety manual is prepared or adopted by the laboratory director and precautions are incorporated into standard operating procedures. Personnel are advised of special hazards and are required to read and to follow instructions on practices and procedures.
8. Laboratory personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural changes.
9. The laboratory director is responsible for insuring that, before working with organisms at Biosafety Level 3, all personnel demonstrate proficiency in standard microbiological practices and techniques, and in the practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the laboratory director or other competent scientist proficient in safe microbiological practices and techniques.
10. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
 - a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.
 - b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - c. Syringes which re-sheath the needle, needle-less systems, and other safe devices should be used when appropriate.
 - d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass should be decontaminated before disposal, according to any local, state, or federal regulations.
11. All manipulations involving infectious materials are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.
12. Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials.

- a. Spills of infectious materials are decontaminated, contained and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious material. Spill procedures are developed and posted.
 - b. Contaminated equipment must be decontaminated before removal from the facility for repair or maintenance or packaging for transport in accordance with applicable local, state, or federal regulations.
13. Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.
 14. All potentially contaminated waste materials (e.g., gloves, lab coats, etc.) From laboratories or animal rooms are decontaminated before disposal or reuse.
 15. Spills of infectious materials are decontaminate, contained and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious material.
 16. Spill and accidents which result in overt or potential exposures to infectious materials are immediately reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.
 17. Animals and plants not related to the work being conducted are not permitted in the laboratory.

C. Safety Equipment (Primary Barriers)

1. Protective laboratory clothing such as solid-front or wrap-around gowns, scrub suits, or coveralls are worn by workers when in the laboratory. Protective clothing is not worn outside the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when overtly contaminated.
2. Gloves must be worn when handling infectious materials, infected animals, and when handling contaminated equipment.
3. Frequent changing of gloves accompanied by hand washing is recommended. Disposable gloves are not reused.
4. All manipulations of infectious materials, necropsy of infected animals, harvesting of tissues or fluids from infected animals or embryonate eggs, etc., are conducted in a Class II or Class III biological safety cabinet (see Appendix A).
5. When a procedure or process cannot be conducted within a biological safety cabinet, then appropriate combinations of personal protective equipment (e.g., respirators, face shields) and physical containment devices (e.g., centrifuge safety cups or sealed rotors) are used.
6. Respiratory and face protection are used when in rooms containing infected animals.

D. Laboratory Facilities (Secondary Barriers)

1. The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of self-closing doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. A clothes change room (shower optional) may be included in the passageway.
2. Each laboratory contains a sink for handwashing. The sink is hands-free or automatically operated and is located near the laboratory exit door.
3. The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontamination.
4. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and those chemicals used to decontaminate the work surfaces and equipment.

5. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
6. All windows in the laboratory are closed and sealed.
7. A method for decontaminating all laboratory wastes is available, preferably within the laboratory (i.e., autoclave, chemical disinfection, incineration, or other approved decontamination system). Consideration should be given to means of decontaminating equipment. If waste is transported out of the laboratory, it should be properly sealed and not transported in public corridors.
8. Biological safety cabinets are required and are located away from doors, from room supply louvers, and from heavily-traveled laboratory areas.
9. A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air from "clean" areas into the laboratory toward "contaminated" areas. The exhaust air is not recirculated to any other area of the building, and is discharged to the outside with filtration and other treatment optional. The outside exhaust must be dispersed away from occupied areas and air intakes. Laboratory personnel must verify that the direction of the airflow (into the laboratory) is proper.
10. The High Efficiency Particulate Air (HEPA)-filtered exhaust air from Class II or Class III biological safety cabinets is discharged directly to the outside or through the building exhaust system. If the HEPA-filtered exhaust air from Class II or III biological safety cabinets is to be discharged to the outside through the building exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection) that avoids any interference with the air balance of the cabinets or building exhaust system. Exhaust air from Class II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every twelve months.
11. Continuous flow centrifuges or other equipment that may produce aerosols are contained in devices that exhaust air through HEPA filters before discharge into the laboratory. These HEPA systems are tested at least annually. Alternatively, the exhaust from such equipment may be vented to the outside if it is dispersed away from occupied areas and air intakes.
12. Vacuum lines are protected with liquid disinfectant traps and HEPA filters, or their equivalent, which are routinely maintained and replaced as needed.
13. An eyewash facility is readily available inside the laboratory.
14. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
15. The Biosafety Level 3 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified, at least annually, against these procedures as modified by operational experience.
16. Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment, the site conditions, or other applicable federal, state, or local regulations.

Appendix 4

Additional Requirements for Facilities Transferring or Receiving Select Agents
Department of Health and Human Services
42 CFR Part 72
RIN 0905-AE70

Appendix A to Part 72--Select Agents

Agents that require facility registration and completion of CDC Form EA-101 prior to transferring or receiving.

HHS Select Agents and Toxings

- Crimean-Congo haemorrhagic fever virus
- *Coccidioides posadasii*
- Ebola viruses
- Cercopithecine herpesvirus 1 (Herpes B virus)
- Lassa fever virus
- Marburg virus
- Monkeypox virus
- *Rickettsia prowazekii*
- *Rickettsia rickettsii*
- South American haemorrhagic fever viruses
 - Junin
 - Machupo
 - Sabia
 - Flexal
 - Guanarito
- Tick-borne encephalitis complex (flavi) viruses
 - Central European tick-borne encephalitis
 - Far Eastern tick-borne encephalitis
 - Russian spring and summer encephalitis
 - Kyasanur forest disease
 - Omsk hemorrhagic fever
- Variola major virus (Smallpox virus)
- Variola minor virus (Alastrim)
- *Yersinia pestis*
- Abrin (100 mg)
- Conotoxins (100 mg)
- Diacetoxyscirpenol (1000 mg)
- Ricin (100 mg)
- Saxitoxin (100 mg)
- Shiga-like ribosome inactivating proteins (100 mg)
- Tetrodotoxi (100 mg)

HHS Select Agents/ USDA High Consequence Livestock Pathogens (Overlap Agents)

- *Bacillus anthracis*
- *Brucella abortus*
- *Brucella melitensis*
- *Brucella suis*
- *Burkholderia mallei* (formerly *Pseudomonas mallei*)
- *Burkholderia pseudomallei* (formerly *Pseudomonas pseudomallei*)
- Botulinum neurotoxin producing species of *Clostridium*
- *Coccidioides immitis*
- *Coxiella burnetii*
- Eastern equine encephalitis virus
- Hendra virus
- *Francisella tularensis*
- Nipah Virus
- Rift Valley fever virus
- Venezuelan equine encephalitis virus
- Botulinum neurotoxin (0.5 mg)
- *Clostridium perfringens* epsilon toxin (100 mg)
- Shigatoxin (100 mg)
- Staphylococcal enterotoxin (5.0 mg)
- T-2 toxin (1000 mg)

USDA High Consequence Livestock Pathogens and Toxins

- Akabane virus
- African swine fever virus
- African horse sickness virus
- Avian influenza virus (highly pathogenic)
- Blue tongue virus (Exotic)
- Bovine spongiform encephalopathy agent
- Camel pox virus
- Classical swine fever virus
- *Cowdria ruminantium* (Heartwater)
- Foot and mouth disease virus
- Goat pox virus
- Lumpy skin disease virus
- Japanese encephalitis virus
- Malignant catarrhal fever virus (Exotic)
- Menangle virus
- *Mycoplasma capricolum*/ M.F38/*M. mycoides capri*
- *Mycoplasma mycoides mycoides*
- Newcastle disease virus (velogenic)
- Peste Des Petits Ruminants virus
- Rinderpest virus
- Sheep pox virus
- Swine vesicular disease virus
- Vesicular stomatitis virus (Exotic)

Listed Plant Pathogens

- *Liberobacter africanus*
- *Liberobacter asiaticus*
- *Peronosclerospora philippinensis*
- *Ralstonia solanacearum* race 3, biovar 2
- *Schlerophthora rayssiae* var *zeae*
- *Synchytrium endobioticum*
- *Xanthomonas oryzae*
- *Xylella fastidiosa* (citrus variegated chlorosis strain)

Exemptions: Toxins for medical use, inactivated for use as vaccines, or toxin preparations for biomedical research use at an LD₅₀ for vertebrates of more than 100 nanograms per kilogram body weight are exempt. National standard toxins required for biologic potency testing as described in 9 CFR Part 113 are exempt.

Recombinant organisms/molecules

1. Genetically modified microorganisms or genetic elements from organisms on Appendix A, shown to produce or encode for a factor associated with a disease.
2. Genetically modified microorganisms or genetic elements that contain nucleic acid sequences coding for any of the toxins listed in this Appendix, or their toxic subunits.

Other restrictions

The deliberate transfer of a drug resistance trait to microorganisms listed in this Appendix that are not known to acquire the trait naturally is prohibited by NIH "Guidelines for Research Involving Recombinant DNA Molecules," if such acquisition could compromise the use of the drug to control these disease agents in humans or veterinary medicine.

Additional Exemptions

1. Products subject to regulation under the Federal Insecticide Fungicide and Rodenticide Act (7 U.S.C. & 136 et seq.) and the Toxic Substances Control Act (15 U.S.C. & 2601 et seq.) are exempt.
2. Additional exemptions for otherwise covered strains will be considered when CDC reviews and updates the list of select agents in this Appendix. Individuals seeking an exemption should submit a request to CDC that specifies the agent or strain to be exempted and explains why such an exemption should be granted. Future exemptions will be published in the Federal Register for review and comment prior to inclusion in this Appendix.

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.

List of Items Controlled

- a. Viruses, as follows:
 - a.1. African swine fever virus;
 - a.2. Avian influenza virus;
 - a.3. Bluetongue virus;
 - a.4. Chikungunya virus;
 - a.5. Congo-Crimean haemorrhagic fever virus;
 - a.6. Dengue fever virus;
 - a.7. Eastern equine encephalitis virus;
 - a.8. Ebola virus;
 - a.9. Foot and mouth disease virus;
 - a.10. Goat pox virus;
 - a.11. Hantaan virus;
 - a.12. Herpes virus (Aujeszky's disease);
 - a.13. Hog cholera virus (syn. Swine fever virus)
 - a.14. Japanese encephalitis virus;
 - a.15. Junin virus;
 - a.16. Lassa fever virus;
 - a.17. Lymphocytic choriomeningitis virus;
 - a.18. Machupo virus;
 - a.19. Marburg virus;
 - a.20. Monkey pox virus;
 - a.21. Newcastle disease virus;
 - a.22. Pestes des petits ruminants virus;
 - a.23. Porcine enterovirus type 9 (syn. Swine vesicular disease virus);
 - a.24. Rift Valley fever virus;
 - a.25. Rinderpest virus;
 - a.26. Sheep pox virus;
 - a.27. Teschen disease virus;
 - a.28. Tick-borne encephalitis virus (Russian Spring-Summer encephalitis virus);
 - a.29. Variola virus;
 - a.30. Venezuelan equine encephalitis virus;
 - a.31. Vesicular stomatitis virus;
 - a.32. Western equine encephalitis virus;
 - a.33. White pox; or
 - a.34. Yellow fever virus.

- b. Rickettsiae, as follows:
 - b.1. *Coxiella burnetii*;
 - b.2. *Rickettsia quintana*;
 - b.3. *Rickettsia prowasecki*; 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. *Chlamydia psittaci*;
 - c.6. *Clostridium botulinum*;
 - c.7. *Francisella tularensis*;
 - c.8. *Mycoplasma mycoides*;
 - c.9. *Pseudomonas mallei*;
 - c.10. *Pseudomonas pseudomallei*;
 - c.11. *Pseudomonas solanaceum*;
 - c.12. *Salmonella typhi*;
 - c.13. *Shigella dysenteriae*;
 - c.14. *Vibrio cholerae*;
 - c.15. *Xanthomonas albilineas*;
 - c.16. *Xanthomonas campestris* pv *citri*;
 - c.17. *Xanthomonas campestris* pv *oryzae*; or
 - c.18. *Yersinia pestis*.

- d. Fungi, as follows:
 - d.1. *Colletotrichum coffeanum* var. *virulans*;
 - d.2. *Cochliobolus miyabeanus* (*Helminthosporium oryzae*);
 - d.3. *Helminthosporium maydis*;
 - d.4. *Helminthosporium oryzae*;
 - d.5. *Microcyclus ulei* (syn. *Dothidella ulei*)
 - d.6. *Puccinia glumarum*;
 - d.7. *Puccinia graminis* (syn. *Puccinia graminis* f. sp. *tritici*);
 - d.8. *Puccinia striiformis* (syn. *Puccinia glumarum*);
 - d.9. *Pyricularia grisea*/ *Pyricularia oryzae*; or
 - d.10. *Ustilago maydis*.

- e. Genetically modified microorganisms, as follows:
 - e.1. Genetically modified micro-organisms or genetic elements that contain nucleic acid sequences associated with pathogenicity and are derived from organisms identified in this ECCN;
 - e.2. Genetically modified micro-organisms or genetic elements that contain nucleic acid sequences associated with pathogenicity derived from plant pathogens identified in this ECCN; or
 - e.3. Micro-organisms genetically modified to produce any of the toxins listed in paragraph f of this ECCN.

- f. Toxins, as follows:
 - f.1. Botulinum toxins;
 - f.2. Clostridium perfringens toxins;
 - f.3. Conotoxin;
 - f.4. Microcystin (cyanogenosin);
 - f.5. Ricin;
 - f.6. Saxitoxin;
 - f.7. Shiga toxin;
 - f.8. Staphylococcus aureus toxins;
 - f.9. Tetrodotoxin; or
 - f.10. Verotoxin.