TABLE OF CONTENTS

IMPORTANT TELEPHONE NUMBERS ............................................................................................................ 1

POLICY STATEMENT ...................................................................................................................................... 1

Purpose .......................................................................................................................................................... 1
Policy ........................................................................................................................................................... 1
Responsibilities ................................................................................................................................................ 1

CLASSIFICATION OF POTENTIALLY INFECTIOUS AGENTS ................................................................. 3

Microorganisms ............................................................................................................................................ 3
Genetically Engineered Microorganisms ...................................................................................................... 3
Human Clinical Materials ............................................................................................................................... 5
Biosafety Containment Levels ...................................................................................................................... 5
Animal Facilities ........................................................................................................................................... 5
Clinical Laboratories ..................................................................................................................................... 5

BIOSAFETY CONTAINMENT LEVELS ....................................................................................................... 6

Summary of Recommended Biosafety Levels ............................................................................................... 6

EMERGENCY PROCEDURES ...................................................................................................................... 7

Biological Spills .......................................................................................................................................... 7
General Spill Clean Up Guidelines ................................................................................................................ 7
Specific Spill Clean Up Guidelines ............................................................................................................... 7
Illness or Injury Involving Biological Materials .......................................................................................... 10
Fires Involving Biological Materials ........................................................................................................... 10

DECONTAMINATION AND DISPOSAL .................................................................................................... 10

Chemical Disinfectants ............................................................................................................................... 10
Disinfectants Commonly Used in the Laboratory ......................................................................................... 11
Dilution of Disinfectants ............................................................................................................................... 12
Autoclaving Procedures ............................................................................................................................... 12
Use and Disposal of Sharps .......................................................................................................................... 13
Biological Waste Disposal Procedures ........................................................................................................ 14

BIOSAFETY EQUIPMENT ............................................................................................................................. 16

Biological Safety Cabinets ......................................................................................................................... 16
Operation of Class II Biological Safety Cabinets ....................................................................................... 17
Centrifuge Containment ............................................................................................................................... 17
Protection of Vacuum Lines .......................................................................................................................... 17
IMPORTANT TELEPHONE NUMBERS

Emergency Telephone Numbers

Fire, Police, Rescue, Emergency Medical Service

On-campus 9911
Off-campus 911

Assistance Telephone Numbers

University Medical Center Emergency Center 743-2100
Occupational Medicine Clinic 743-1864
Environmental Health and Safety 742-3876
Biological Safety Officer 742-3876
Biological Waste Pick-up 742-3876
Radiation Safety Officer 742-3876

POLICY STATEMENT

Purpose

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

National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines), May 1999 edition;

Biosafety in Microbiological and Biomedical Laboratories (BMBL), 4th edition.

These documents are available on the publications page of the Environmental Health and Safety (EH&S) web site at www.ehs.ttu.edu. Related documents, such as the University Chemical Hygiene Plan, are also available on the web site.

Policy

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

Responsibilities

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

Environmental Health and Safety (EH&S) shall:

- Prepare this Biosafety Manual, with revisions as necessary;
- Distribute the Manual to each laboratory performing work with biological materials;
- Investigate accidents involving infectious agents;
- Coordinate and track the certification of Biological Safety Cabinets (BSC’s);
- Collect and dispose of biological waste when appropriate;
- Provide or coordinate biosafety training as requested;
- Assist investigators with risk assessment;
- Monitor laboratories for compliance with all elements of the Biosafety Program;
- Assist faculty with submission of registrations to the IBC and maintain registration files.

Principal Investigators (PI) shall:

- Assess the risks of their experiments;
- Ensure the safe operation of their laboratory;
- Train laboratory personnel in safe work practices;
- Comply with this Manual, the University Chemical Hygiene Plan, the University Radiation Safety Manual, and all applicable University OP’s relating to safety and health;
- Comply with all applicable state and federal regulations and guidelines;
- Register the following experiments with the Institutional Biosafety Committee, Human Subjects Committee, and/or Environmental Health and Safety (EH&S), as required:
  - recombinant DNA activities;
  - work with infectious agents; and
  - 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.

The Institutional Biosafety and Hazardous Material Committee (IBC) shall:

- 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;
- 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 the *BMBL*;
- Notify the PI of the results of the IBC’s review;
- 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 Recombinant DNA Activities (ORDA) within 30 days; and
- Follow the guidelines for membership defined by NIH, with the additional requirement of one representative from the Texas Tech University Animal Care and Use Committee.
CLASSIFICATION OF POTENTIALLY INFECTIOUS AGENTS

Federal and state regulations and guidelines govern procedures and facilities involved in protecting laboratory workers, the public, and the environment from laboratory biological hazards. 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 classify pathogenic agents into one of four risk groups according to specific criteria. It is Texas Tech University policy that all laboratories adhere to these NIH/CDC guidelines.

Investigators must register any project involving microorganisms capable of causing infection in humans with the Institutional Biosafety Committee (IBC) and receive its approval before work is begun. Following receipt of the completed registration document by EH&S, the laboratory will be surveyed by the Biological Safety Officer 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. Registration forms are available from EH&S at 742-3876.

Genetically Engineered Organisms

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

Registration Document

Each Principal Investigator is responsible for the preparation and submission of the "Registration Document for Recombinant DNA Experiments" for all recombinant DNA experiments, including those exempt from the NIH Guidelines. The Biosafety Officer is available to assist in completing the form. Send the completed registration document to EH&S to initiate the review process.
Review and Approval of Experiments

The IBC will review the registration documents. Registration forms are available from EH&S.

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

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

- involving the formation of recombinant DNA molecules containing no more than 2/3 of the genome of any eukaryotic virus propagated in tissue culture.
- involving 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.

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 will review the registration document and confirm that the work is classified correctly according to the NIH Guidelines. Exempt experiments are those that:

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

Work with human clinical material is regulated by the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard, 29 CFR, Part 1910.1030. Human blood, unfixed tissue, and certain other body fluids are considered potentially infectious for bloodborne pathogens such as hepatitis B virus (HBV) and human immunodeficiency virus (HIV). All human clinical material should be presumed infectious and handled using Biosafety Level 2 work practices. This concept is called Universal Precautions. Investigators are responsible for notifying EH&S of their use of human clinical materials so training and immunization can be provided as necessary.

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 the summary following and Appendices A-D). Most microbiological work at the Texas Tech University is conducted at BSL1 or BSL2 containment.

Animal Facilities

Four biosafety levels are also described for activities involving infectious disease work with experimental mammals. These four combinations of practices, safety equipment, and facilities are designated Animal Biosafety Levels 1, 2, 3, and 4, (ABSL1, ABSL2, ABSL3, ABSL4), and provide increasing levels of protection to personnel and the environment. These parallel BSL 1-4.

Clinical Laboratories

Except in extraordinary circumstances (e.g., suspected hemorrhagic fever), the initial processing of clinical specimens and identification of isolates can be done safely at BSL2, the recommended level for work with bloodborne pathogens such as hepatitis B virus and HIV.
## SUMMARY OF RECOMMENDED BIOSAFETY LEVELS

<table>
<thead>
<tr>
<th>Agents</th>
<th>Practices</th>
<th>Safety Equipment (Primary Barriers)</th>
<th>Facilities (Secondary Barriers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Not known to consistently cause disease in healthy adults</td>
<td>Standard Microbiological Practices</td>
<td>None required</td>
<td>Open bench top sink required</td>
</tr>
<tr>
<td>2 Associated with human disease, hazard = percutaneous injury, ingestion, mucous membrane exposure</td>
<td>BSL-1 practice plus: Limited access Biohazard warning signs &quot;Sharps&quot; precautions Biosafety manual defining any needed waste decontamination or medical surveillance policies</td>
<td>Primary barriers = Class I or II BSC’s or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPE: laboratory coats; gloves; face protection as needed</td>
<td>BSL-1 plus: Autoclave available</td>
</tr>
<tr>
<td>3 Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences</td>
<td>BSL-2 practice plus: Controlled access Decontamination of all waste Decontamination of lab clothing before laundering Baseline serum</td>
<td>Primary barriers = Class I or II BSC’s or other physical containment devices used for all open manipulations of agents; PPE: protective lab clothing; gloves; respirator protection as needed</td>
<td>BSL-2 plus: Physical separation from access corridors Self-closing, double-door access Exhausted air not recirculated Negative airflow into laboratory</td>
</tr>
<tr>
<td>4 Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission</td>
<td>BSL-3 practices plus: Clothing change before entering Shower on exit All material decontaminated on exit from facility</td>
<td>Primary barriers = All procedures conducted in Class III BSC’s or Class I or II BSC’s in combination with full-body, air-supplied, positive pressure personnel suit</td>
<td>BSL-3 plus: Separate building or isolated zone Dedicated supply and exhaust, vacuum, and decon systems Other requirements outlined in the text</td>
</tr>
</tbody>
</table>

## EMERGENCY PROCEDURES

### Biological Spills

A spill kit should be kept in each laboratory where work with microorganisms is conducted. Basic equipment is: diluted disinfectant (such as 10% 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

- Know how to get the HVAC unit servicing the lab space shut down in order to limit the spread of contamination.
- 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.
Allow a 30-minute contact for disinfection.
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

Spill of BSL1 material:
- Wearing gloves and a lab coat, pick up broken glass with forceps and place in sharps container.
- Absorb the spill with paper towels or other absorbent material.
- Discard these contaminated materials into biohazard waste container.
- Wipe the spill area with the appropriate dilution of a disinfectant effective against the organism.
- Autoclave all towels, gloves, and other materials worn or used to clean up the spill.
- Wash hands with soap and water.

Spill of Human Blood:
- Wear gloves and lab coat to clean up spill.
- If broken glass is present, use forceps to pick up and place in sharps container.
- Absorb blood with paper towels and discard in biohazard waste container.
- Wet spill area with disinfectant, allow 30 minute contact time and wipe up.
- Using a detergent solution, clean the spill site of all visible blood.
- Wipe the spill site with paper towels soaked in a disinfectant such as bleach diluted 1:10 (vol/vol).
- Discard all contaminated materials into biohazard waste container.
- Wash hands with soap and water.
- Inform PI and Biosafety Officer of spill.

Spill of BSL2 material:
- Keep other workers out of the area to prevent spreading spilled material.
- Post warning sign, if needed.
- Remove contaminated clothing and put into a biohazard bag for decontamination later.
- Wash hands and exposed skin and inform the PI of the spill. Call the Biological Safety Officer at 742-3876 for assistance, if necessary.
- 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).
- Pick up broken glass with forceps and dispose into sharps container.
- Cover the spill with paper towels and add appropriately diluted disinfectant.
- After at least 30 minutes contact time, pick up the paper towels and re-wipe the spill area with diluted disinfectant.
- Collect all contaminated materials into biohazard waste container and autoclave.
- Wash hands with soap and water.

Spill of a BSL3 material:
- Stop work immediately.
• Evacuate the room. Close the door, and post a warning sign.
• Remove contaminated clothing, turn exposed area inward, and place in a biohazard bag. Wash hands with soap and water.
• Notify the Principal Investigator. Call the Biological Safety Officer at 742-3876 (after hours and weekends call 742-3301) for assistance if necessary.
• Allow 30 minutes for aerosols to disperse before re-entering the laboratory to begin clean up.
• Put on personal protective equipment (HEPA filtered respirator, gown, gloves, and shoe covers) and assemble clean-up materials (disinfectant, autoclavable container or bag, forceps, sharps container, and paper towels).
• 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.
• Pick up broken glass with forceps and discard in sharps container.
• 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.
• Autoclave (or soak in 10% bleach solution) lab coat, gloves, and other protective equipment that was worn for clean up.
• Wash hands thoroughly with soap and water.

Spill in a Biological Safety Cabinet:
• Leave the cabinet turned on.
• Wearing gloves and lab coat, spray or wipe cabinet walls, work surfaces, and equipment with disinfectant such as 70% ethanol. If necessary, flood work surfaces, as well as drain pans and catch basins below the work surface, with disinfectant. Allow 30 minutes contact time.
• 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.
• Surface disinfect all items that may have been spattered before removing them from the cabinet.
• Discard all clean-up materials into biohazard waste container. Wash hands and exposed skin areas with soap and water.
• The Biosafety Officer 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.

Spill of Biological Radioactive Material

Preparation for Clean-up:
• Evacuate the room. Close the door, and post a warning sign.
• Remove contaminated clothing, turn exposed area inward, and place in a biohazard bag.
• Wash all exposed skin with soap or handwashing antiseptic, followed by a three-minute water rinse.
• Inform the PI, Biological Safety Officer and the Radiation Safety Officer 742-3876 of the spill, and monitor all exposed personnel for radiation.
• 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).
• Confirm with the Radiation Safety Office that it is safe to enter the lab.
Clean-up of Biological Radioactive Spill:

- 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 EH&S for advice since the use of respirators requires prior training, fit-testing, and medical approval.
- Pick up any sharp objects with forceps and put in a sharps container labeled according to Radiation Safety guidelines.
- 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 30 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.**
- Wipe surrounding areas where the spill may have splashed with disinfectant.
- 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 RADIATION SAFETY OFFICER.**
- Disinfect contaminated protective clothing prior to disposal as radioactive waste.
- Place contaminated items on absorbent paper and scan for radioactivity. If none is detected, dispose of these items as biohazard waste.
- If radioactive, spray with disinfectant and allow a 30 minute contact time.
- Wrap the items inside the absorbent paper and dispose of as radioactive waste.
- 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 Radiation Safety Officer. If spill area has residual activity, determine if it is fixed or removable and handle it accordingly.
Illness Or Injury Involving Biological Materials

For Severe Injuries:
Call 9911 or 911, as appropriate, 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 the accident to the PI and Environmental Health and Safety.

For Splash to the Eye:
Use an emergency eyewash to 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.
Contact the most convenient local emergency room to obtain care. Report the accident to the PI and
Environmental Health and Safety, 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 most convenient local emergency room to obtain care.
Report the injury to the PI and to the Environmental Health and Safety, and seek additional medical
assistance if necessary.

Fires Involving Biological Materials

- Without placing yourself in danger, put biological materials in a secure location, such as an
  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

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:

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

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.

Hypochlorites (bleach)
User 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.

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

<table>
<thead>
<tr>
<th>Property</th>
<th>Hypochlorites “Household Bleach”</th>
<th>Iodoform “Wescodyne”</th>
<th>Ethyl Alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shelf-life &gt; 1 week</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Corrosive</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Residue</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Inactivation by organic matter</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Skin Irritant</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Respiratory Irritant</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eye Irritant</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Toxic</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
Dilution of Disinfectants

Chlorine compounds (Typical Household Bleach)

<table>
<thead>
<tr>
<th>Dilution in Water</th>
<th>% Available Chlorine</th>
<th>Available Chlorine in mg/l or ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not diluted</td>
<td>5.25</td>
<td>50,000</td>
</tr>
<tr>
<td>1/10</td>
<td>0.5</td>
<td>5,000</td>
</tr>
<tr>
<td>1/100</td>
<td>0.05</td>
<td>500</td>
</tr>
</tbody>
</table>

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%.

**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.

**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 it if it is 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 tear resistant, but can be punctured or burst in the autoclave. Therefore, place bags in a rigid container 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 or in the case of a vial insert a needle, turn the container on its side when possible, and select a container with the lowest sides and widest diameter possible for the autoclave.
Stainless steel containers and pans - Stainless steel is a good 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 closed 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.
- Biohazard autoclave bags 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. Ampoules 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 Biological Safety Officer 742-3876.

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.
• Discard needle and syringe as an intact unit immediately after use into puncture resistant sharps containers.
• Use care and caution when cleaning up after procedures that require the use of syringes and needles.
• Do not overfill sharps containers. Remove from service when 3/4 full, autoclave, and dispose of in accordance with University policy (University Operating Policy 60.10).
• Locate sharps containers in areas in which needles are commonly used. Make containers easily accessible.

In the event of a needle stick injury:

Notify the PI and seek treatment, if needed.

**Biological Waste Disposal Procedures**

**Biological Waste**

All biological waste from BSL1, BSL2, BSL3, and BSL4 laboratories must be decontaminated prior to disposal. Decontamination and disposal are the responsibility of the person/laboratory generating the waste. Collect disposable, solid materials contaminated by an infectious agent, excluding sharps, into an autoclave bag within a sturdy container. When full, these bags are autoclaved, cooled, and then placed in the building's dumpster by laboratory staff. Decontaminate liquids containing a biological agent by the addition of a chemical disinfectant such as sodium hypochlorite (household bleach) or an iodophor, or by autoclaving, then dispose of by pouring down the sink. It is not necessary to autoclave liquids that have been chemically disinfected. However, if 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.

**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.

**Disposal of Blood Products and Body Fluids**

All human blood and other potentially infectious materials should be handled using Universal Precautions. Discard disposable items contaminated with human blood or body fluids (excluding sharps and glassware) into the biowaste boxes that are available from EH&S. 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. For pick-up, call EH&S at 742-3876.

**Disposal of Sharps and Disposable Glassware**

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. Sharps containers may be purchased from the Physical Plant Central Warehouse. When full, autoclave the container and discard in the dumpster. Uncontaminated pasteur pipettes and broken or unbroken glassware are discarded into containers specifically designed for broken glass disposal, or into heavy-duty cardboard boxes that are closeable. When boxes are full, tape closed and place in the building’s dumpster in accordance with University Operating Procedure 60.10. Contaminated pasteur pipettes, and broken or autoclave, then discard into glass disposal boxes as above. Sharps that are contaminated with radioactive materials must be discarded into separate sharps containers labeled with the name of the isotope. Specify isotope content when requesting pick-up by EH&S.

Multi-hazard or Mixed Waste

Avoid generating mixed waste if possible. Keep volume to a minimum. Do not autoclave mixed waste. 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 Radiation Safety Officer at 742-3876 before beginning inactivation procedures. 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 EH&S at 742-3876 for disposal forms and instructions.

Disposal of Animal Tissues, Carcasses and Bedding

Place animal carcasses/tissues into plastic bag. Double-bag when carcass contains zoonotic agents (transmissible from animals to humans). Place bag in freezer until pick-up. Call EH&S at 742-3876 for pick-up. Disposal of animal carcasses/tissues that are contaminated with radioactive materials or hazardous chemicals is through EH&S. Disposal forms and instructions are available by phoning 742-3876.

Disposal Containers

Each laboratory is responsible for purchasing containers for the disposal of biological waste, except when biowaste containers are provided by EH&S. The following types of containers are available:

- Sharps containers may be purchased from local sources, including the Physical Plant Central Warehouse, as well as from laboratory product distributors. They are available in various sizes, and must 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.

- Biohazard Autoclave Bags may be purchased from various laboratory product distributors, such as Vallen Safety Supply, Lab Safety Supply, 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.

- Biowaste containers are provided by EH&S when there is no access to an autoclave. A plastic liner (also provided by EH&S) must be used to prevent contamination of the box.

- Any sturdy, puncture-resistant, closable box may be used for glass disposal or they may be purchased from various laboratory product distributors.
What to do with Filled Waste Containers

Biowaste containers - When full, call EH&S to request a pick-up.

Biohazard autoclave bags, sharps containers, and glass disposal boxes - Close and autoclave, place inside trash bag and place in building dumpster.

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 BSC’s (Class I, II, III) and the horizontal laminar flow cabinet are described below.

The common element to all classes of biological safety cabinets is the high efficiency particulate air (HEPA) filter. This filter removes particulates of 0.3 microns or larger 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. Environmental Health and Safety administers a program for annual certification of all biological safety cabinets at the university. Contact EH&S at 742-3876 to confirm that your cabinet is included in this program.

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.

Class II (Types A, B1, B2, and B3) biological safety cabinets 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).

Class III cabinets (sometimes called Class III glove boxes) were designed for work with infectious agents that require Biosafety Level 4 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 two HEPA filters before it is exhausted to the outdoors. This type of cabinet provides the highest level of product, environmental, and personnel protection.

Horizontal laminar flow "clean air benches" are not BSC’s. 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 biological safety cabinet in research laboratories.

Operation of Class II Biological Safety Cabinets

- Turn on cabinet fan 15 minutes before beginning work.
- Disinfect the cabinet work surface with 70% ethanol or other disinfectant.
- Place supplies in the cabinet. Locate the container for disposal of pipettes inside the cabinet. (Movement of hands in and out of the cabinet to discard pipettes into a container located outside of the cabinet creates turbulence and disrupts the air barrier that maintains sterility inside the cabinet.)
- Work as far to the back (beyond the air split) of the BSC workspace as possible.
- Always use mechanical pipetting aids.
- Avoid using open flames inside BSC’s. 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 BSC’s.
- Do not work in a BSC while a warning light or alarm is signaling.
- Locate liquid waste traps inside the 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 to prevent spilling.
- Wear gloves when there is potential for skin contact with infectious material.
- Keep the work area of the BSC free of unnecessary equipment or supplies.
- Clutter inside the BSC may affect proper airflow and the level of protection provided. Also, keep the front and rear grilles clear.
- When work is completed, remove equipment and supplies from the cabinet. Wipe the work area with 70% ethanol and allow the cabinet to run for 15 minutes.
- Some BSC’s are equipped with ultraviolet (UV) lights. If good procedures are followed, UV lights are not needed. If an UV light is used, due to its limited penetrating ability, surfaces must be dust-free and the UV light tube should be wiped frequently with alcohol to remove dust. UV radiation should not take the place of 70% ethanol for disinfection of the cabinet interior.
- The UV lamp should never be on while an operator is working in the cabinet.
- 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 or higher 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) for importation into the U.S. of:

1) any microorganism that causes disease in humans;
2) biological materials, such as blood and tissues, when known or suspected to contain an infectious agent;
3) live insects, such as mosquitoes, known or suspected of being infected with any disease transmissible to humans; and
4) any animal known or suspected of being infected with any disease transmissible to humans.

Importation permits are issued only to the importer, who must be located in the U.S. The importation permit, with the proper packaging and labeling, will expedite clearance of the package of infectious materials through the U.S. Public Health Service Division of Quarantine and release by U.S. Customs. Transfers of previously imported material within the U.S. also require a permit. Application for the permit should be made at least 10 working days in advance of the anticipated shipment date. Further information and application forms may be obtained by calling the CDC at (404) 639-3883.
Permits are also required from the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) for importation into the U.S. 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 by calling the USDA/APHIS at (301) 734-4401.

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 from USDA/APHIS (301) 734-4401.

Facility registration and completion of the CDC Form EA-101 are required by the CDC prior to transfer of select infectious agents and toxins (42 CFR Part 72). Select agents are listed in Appendix E. Please contact the Biological Safety Officer if your work includes any of the agents listed in Appendix E.

A validated license is required by the Department of Commerce for export of certain microorganisms and toxins (listed in Appendix F) to all destinations except Canada. Information may be obtained by calling the Department of Commerce, Bureau of Export Administration at (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.
- Clinical 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, liquid-tight if appropriate;
- 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 Organisms

The NIH Guidelines for Experiments Involving Recombinant DNA Molecules (January 1996) 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 Clinical Material

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.
APPENDIX A

Biosafety Level 1

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently 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 neither 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 (responsible person for all work performed in the laboratory) when experiments or work with cultures and specimens are in progress.

2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.

3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use 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 and used 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 splashes or 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, leak-proof container and closed for transport from the laboratory. Materials to be decontaminated outside of the immediate laboratory are packaged in accordance with applicable local, state, and federal regulations before removal from the facility.

9. A biohazard sign can be posted at the entrance to the laboratory whenever infectious agents are present. The sign may include the name of the agent(s) in use and the name and phone number of the investigator.

10. 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 a biological safety cabinet are generally not required for manipulations of agents assigned to Biosafety Level 1.

2. It is recommended that laboratory coats, gowns, or uniforms are 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 is present. Alternatives to powdered latex gloves should be available.

4. Protective eyewear should be worn for conduct of procedures in which splashes of microorganisms or other hazardous materials is anticipated.

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 are 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 to the exterior, they are fitted with fly screens.
APPENDIX B

Biosafety Level 2

Biosafety Level 2 is similar to Biosafety Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs from BSL-1 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, 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. 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 splashes or aerosols.

7. Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern.

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 placed in a durable, leak-proof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the facility are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.

9. 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 have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at increased 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 hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.

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 and support 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. 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-sheathe the needle, needleless systems, and other safety devices are 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, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.

10. Laboratory equipment and work surfaces should be decontaminated with an effective 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 that 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 embryonate 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, face shield or other splatter guard) 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, and 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 hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when 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.

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 are 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 station 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.
APPENDIX C

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 some existing facilities may not have all the facility features recommended for Biosafety Level 3 (i.e., double-door access zone and sealed penetrations). In this circumstance, an acceptable level of safety for the conduct of routine procedures, (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, susceptibility testing, etc.), may be achieved in a Biosafety Level 2 facility, providing: 1) the exhaust air from the laboratory room is discharged to the outdoors, 2) the ventilation to the laboratory is balanced to provide directional airflow into the room, 3) access to the laboratory is restricted when work is in progress, and 4) the recommended Standard Microbiological Practices, Special Practices, and Safety Equipment for Biosafety Level 3 are 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, 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 placed in a durable, leak-proof 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.

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. Persons who are at increased risk of acquiring infection or for whom infection may have serious consequences 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 director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. No minors should be allowed 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 as appropriate and stored for all laboratory and other at-risk personnel. Additional serum specimens may be periodically collected, depending on the agents handled or the function of the laboratory.

7. A biosafety manual specific to the laboratory is prepared or adopted by the laboratory director and biosafety precautions are incorporated into standard operating procedures. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

8. Laboratory and support 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 ensuring 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-sheathe the needle, needleless systems, and other safe devices are 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, and disposed of according to any local, state, or federal regulations.

11. All open 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. Clean up is facilitated by using plastic-backed paper toweling on non-perforated work surfaces within biological safety cabinets.

12. Laboratory equipment and work surfaces should be decontaminated routinely with an effective disinfectant, 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, specimens of body fluids, or wastes 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 are decontaminated before disposal or reuse.

15. Spills and accidents that 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.
16. 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.

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.

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 that are open to unrestricted traffic flow within the building, and access to the laboratory is restricted. Passage through a series of two self-closing doors is the basic requirement for entry into the laboratory from access corridors. Doors are lockable. A clothes change room may be included in the passageway.

2. Each laboratory room contains a sink for handwashing. The sink is hands-free or automatically operated and is located near the room exit door.

3. The interior surfaces of walls, floors, and ceilings of areas where BSL-3 agents are handled are constructed for easy cleaning and decontamination. Seams, if present, must be sealed. Walls, ceilings, and floors should be smooth, impermeable to liquids and resistant to the chemicals and disinfectants normally used in the laboratory. Floors should be monolithic and slip-resistant. Consideration should be given to the use of coved floor coverings. Penetrations in floors, walls, and ceiling surfaces are sealed. Openings such as around ducts and the spaces between doors and frames are 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 in the facility and utilized, preferably within the laboratory (i.e., autoclave, chemical disinfection, incineration, or other approved decontamination method). 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 which draws air into the laboratory from "clean" areas and toward "contaminated" areas. The exhaust air is not recirculated to any other area of the building. Filtration and other treatments of the exhaust air are not required, but may be considered based on site requirements, and specific agent manipulations and use conditions. The outside exhaust must be dispersed away from occupied areas and air intakes, or the exhaust must be HEPA-filtered. Laboratory personnel must verify that the direction of the airflow (into the laboratory) is proper. It is recommended that a visual monitoring device that indicates and confirms directional inward airflow be provided at the laboratory entry. Consideration should be given to installing an HVAC control system to prevent sustained positive pressurization of the laboratory. Audible alarms should be considered to notify personnel of HVAC system failure.

10. HEPA-filtered exhaust air from a Class II biological safety cabinet can be recirculated into the laboratory if the cabinet is tested and certified at least annually. When exhaust air from Class II safety cabinets is to be discharged to the outside through the building exhaust air system, the cabinets must be connected in a manner that avoids any interference with the air balance of the cabinets or the building exhaust system (e.g., an air gap between the cabinet exhaust and the exhaust duct). When Class III biological safety cabinets are used they should be directly connected to the exhaust system. If the Class III cabinets are connected to the supply system, it is done in a manner that prevents positive pressurization of the cabinets.

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. Filters must be replaced as needed. An alternative is to use portable vacuum pumps (also properly protected with traps and filters).

13. An eyewash station 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 D

Biosafety Level 4

Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease. Agents with a close or identical antigenic relationship to Biosafety Level 4 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 Biosafety Level 4 laboratory has special engineering and design features to prevent microorganisms from being disseminated into the environment. The following standard and special safety practices equipment, and facilities apply to agents assigned to Biosafety Level 4:

A. Standard Microbiological Practices

1. Access to the laboratory is limited by the laboratory director when experiments are in progress.

2. Policies for safe handling of sharps are instituted.

3. All procedures are performed carefully to minimize the creation of aerosols.

4. Work surfaces are decontaminated at least once a day and after any spill of viable material.

5. All waste is decontaminated before disposal by an approved method such as autoclaving.

6. An insect and rodent control program is in effect.

B. Special Practices

1. Only persons whose presence in the facility or individual laboratory rooms is required for program or support purposes are authorized to enter. Persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. Therefore, persons who may be at increased risk of acquiring infection or for whom infection may be unusually hazardous, such as children or pregnant women, are not allowed in the laboratory or animal rooms. The laboratory supervisor (individual directly responsible for day to day operations of the lab) has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. Access to the facility is limited by means of secure, locked doors; accessibility is managed by the laboratory director, biohazard control officer, or other person responsible for the physical security of the facility. Before entering, persons are advised of the potential biohazards and instructed as to appropriate
safeguards for ensuring their safety. Authorized persons comply with the instructions and all other applicable entry and exit procedures. A logbook, signed by all personnel, indicates the date and time of each entry and exit. Practical and effective protocols for emergency situations are established.

2. When infectious materials or infected animals are present in the laboratory or animal rooms, hazard warning signs, incorporating the universal biohazard symbol, are posted on all access doors. The sign identifies the agent, lists the name of the laboratory director or other responsible person(s), and indicates any special requirements for entering the area (e.g., the need for immunizations or respirators).

3. The laboratory director is responsible for ensuring that, before working with organisms at Biosafety Level 4, all personnel demonstrate a high proficiency in standard microbiological practices and techniques, and in the special 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 these unique safe microbiological practices and techniques.

4. Laboratory personnel receive available immunizations for the agents handled or potentially present in the laboratory.

5. Baseline serum samples for all laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be periodically collected, depending on the agents handled or the function of the laboratory. The decision to establish a serologic surveillance program takes into account the availability of methods for the assessment of antibody to the agent(s) of concern. The program provides for the testing of serum samples at each collection interval and the communication of results to the participants.

6. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

7. Laboratory and support 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.

8. Personnel enter and leave the laboratory only through the clothing change and shower rooms. They take a decontaminating shower each time they leave the laboratory. Personnel use the airlocks to enter or leave the laboratory only in an emergency.

9. Personal clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing, including undergarments, pants or jumpsuits, shoes, and gloves, is provided and used by all personnel entering the laboratory. When leaving the laboratory and before proceeding into the shower area, personnel remove their laboratory clothing in the inner change room. Soiled clothing is autoclaved before laundering.

10. Supplies and materials needed in the facility are brought in by way of the double-doored autoclave, fumigation chamber, or airlock, which is appropriately decontaminated between each use. After securing the outer doors, personnel within the facility retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors are secured after materials are brought into the facility.
11. 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 are restricted in the laboratory for use only when there is no alternative, such as for 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 that re-sheath the needle, needleless systems, and other safety devices are 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 must be decontaminated before disposal, according to any local, state, or federal regulations.

12. Biological materials to be removed from the Class III cabinet or from the Biosafety Level 4 laboratory in a viable or intact state are transferred to a non-breakable, sealed primary container and then enclosed in a non-breakable, sealed secondary container. This is removed from the facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose.

13. No materials, except biological materials that are to remain in a viable or intact state, are removed from the Biosafety Level 4 laboratory unless they have been autoclaved or decontaminated before they leave the laboratory. Equipment or material that might be damaged by high temperatures or steam may be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

14. Laboratory equipment is decontaminated routinely after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials. Equipment is decontaminated before it is sent for repair or maintenance.

15. Spills of infectious materials are contained and cleaned up by appropriate professional staff or others properly trained and equipped to work with concentrated infectious material. A spill procedure is developed and posted within the laboratory.

16. A system is established for reporting laboratory accidents and exposures and employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses. Written records are prepared and maintained. An essential adjunct to such a reporting-surveillance system is the availability of a facility for the quarantine, isolation, and medical care of personnel with potential or known laboratory-associated illnesses.
17. Materials not related to the experiment being conducted (e.g., plants, animals, and clothing) are not permitted in the facility.

C. **Safety Equipment** (Primary Barriers)

All procedures within the facility are conducted in the Class III biological safety cabinet or in Class II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life support system.

D. **Laboratory Facility (Secondary Barriers)**

There are two models for Biosafety Level 4 laboratories: (A) the Cabinet Laboratory where all handling of the agent is performed in a Class III Biological Safety Cabinet, and (B) the Suit Laboratory where personnel wear a protective suit. Biosafety Level-4 laboratories may be based on either model or a combination of both models in the same facility. If a combination is used, each type must meet all the requirements identified for that type.

(A) **Cabinet Laboratory**

1. The Biosafety Level 4 facility consists of either a separate building or a clearly demarcated and isolated zone within a building. The rooms in the facility are arranged to ensure passage through a minimum of two doors prior to entering the room(s) containing the Class III biological safety cabinet (cabinet room). Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the cabinet room. A double-door autoclave, dunk tank, fumigation chamber, or ventilated anteroom for decontamination is provided at the containment barrier for passage of those materials, supplies, or equipment that are not brought into the cabinet room through the change room.

2. Daily inspections of all containment parameters (e.g., directional airflow) and life support systems are completed before laboratory work is initiated to ensure that the laboratory is operating according to its operating parameters.

3. Walls, floors, and ceilings of the cabinet room and inner change room are constructed to form a sealed internal shell which facilitates fumigation and is resistant to entry and exit of animals and insects. Floors are integrally sealed and coved. The internal surfaces of this shell are resistant to liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Openings around doors into the cabinet room and inner change room are minimized and are capable of being sealed to facilitate decontamination. Any drains in the cabinet room floor are connected directly to the liquid waste decontamination system. Sewer vents and other service lines contain HEPA filters and protection against vermin.

4. Bench tops have seamless or sealed surfaces which are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.

5. Laboratory furniture is of simple open construction, capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning and decontamination. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
6. A hands-free or automatically operated handwashing sink is provided near the door of the cabinet room(s) and the outer and inner change rooms.

7. If there is a central vacuum system, it does not serve areas outside the cabinet room. In-line HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services to the cabinet room are protected by devices that prevent backflow.

8. If water fountains are provided, they are automatically or foot-operated and are located in the facility corridors outside the laboratory. The water service to the fountain is isolated from the distribution system supplying water to the laboratory areas and is equipped with a backflow preventer.

9. Access doors to the laboratory are self-closing and lockable.

10. Any windows are breakage-resistant and sealed.

11. Double-door autoclaves are provided for decontaminating materials passing out of both the Class III biological safety cabinet(s) and the cabinet room(s). Autoclaves that open outside of the containment barrier must be sealed to the wall of the containment barrier. The autoclave doors are automatically controlled so that the outside door can only be opened after the autoclave "sterilization" cycle has been completed.

12. Pass-through dunk tanks, fumigation chambers, or equivalent decontamination methods are provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from both the Class III biological safety cabinet(s) and the cabinet room(s).

13. Liquid effluents from the dirty-side inner change room (including toilets) and cabinet room sinks, floor drains (if used), autoclave chambers, and other sources within the cabinet room are decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer. Effluents from showers and clean-side toilets may be discharged to the sanitary sewer without treatment. The process used for decontamination of liquid wastes must be validated physically and biologically.

14. A dedicated non-recirculating ventilation system is provided. The supply and exhaust components of the system are balanced to ensure directional airflow from the area of least hazard to the area(s) of greatest potential hazard. The differential pressure/directional airflow between adjacent areas is monitored and alarmed to indicate any system malfunction. An appropriate visual pressure monitoring device that indicates and confirms the pressure differential of the cabinet room is provided and located at the entry to the clean change room. The airflow in the supply and exhaust components is monitored and the HVAC control system is designed to prevent sustained positive pressurization of the laboratory. The Class III cabinet should be directly connected to the exhaust system. If the Class III cabinet is connected to the supply system, it is done in a manner that prevents positive pressurization of the cabinet.

15. The supply air to and exhaust air from the cabinet room, inner change room, and anteroom pass through HEPA filter(s). The air is discharged away from occupied spaces and air intakes. The HEPA filter(s) are located as near as practicable to the source in order to minimize the length of potentially contaminated ductwork. All HEPA filters need to be tested and certified annually. The HEPA filter housings are designed to allow for in situ decontamination of the filter prior to removal, or removal of the filter in a sealed, gas-tight primary container for subsequent decontamination and/or destruction by

37
incineration. The design of the HEPA filter housing should facilitate validation of the filter installation. The use of pre-certified HEPA filters can be an advantage. The service life of the exhaust HEPA filters can be extended through adequate prefiltration of the supply air.

16. The Biosafety Level 4 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 annually against these procedures as modified by operational experience.

17. Appropriate communication systems are provided between the laboratory and the outside (e.g., voice, fax, computer).

(B) Suit Laboratory

1. The Biosafety Level 4 facility consists of either a separate building or a clearly demarcated and isolated zone within a building. The rooms in the facility are arranged to ensure passage through the changing and decontamination areas prior to entering the room(s) where work is done with BSL-4 agents (suit area). Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the suit area. A specially designed suit area is maintained in the facility to provide personnel protection equivalent to that provided by Class III biological safety cabinets. Personnel who enter this area wear a one-piece positive pressure suit that is ventilated by a life-support system protected by HEPA filtration. The life support system includes redundant breathing air compressors, alarms and emergency backup breathing air tanks. Entry to this area is through an airlock fitted with airtight doors. A chemical shower is provided to decontaminate the surface of the suit before the worker leaves the area. An automatically starting emergency power source is provided at a minimum for the exhaust system, life support systems, alarms, lighting, entry and exit controls, and BSC’s. The air pressure within the suit is positive to the surrounding laboratory. The air pressure within the suit area is lower than that of any adjacent area. Emergency lighting and communication systems are provided. All penetrations into the internal shell of the suit area, chemical shower, and airlocks, are sealed.

2. A daily inspection of all containment parameters (e.g., directional airflow, chemical showers) and life support systems is completed before laboratory work is initiated to ensure that the laboratory is operating according to its operating parameters.

3. A double-doored autoclave is provided at the containment barrier for decontaminating waste materials to be removed from the suit area. The autoclave door, which opens to the area external to the suit area, is sealed to the outer wall of the suit area and is automatically controlled so that the outside door can be opened only after the autoclave "sterilization" cycle. A dunk tank, fumigation chamber, or ventilated airlock for decontamination is provided for passage of materials, supplies, or equipment that are not brought into the suit area through the change room. These devices can be also used for the safe removal of materials, supplies, or equipment from the laboratory that cannot be decontaminated in the autoclave.

4. Walls, floors, and ceilings of the suit area are constructed to form a sealed internal shell, which facilitates fumigation and is animal and insect prohibitive. The internal surfaces of this shell are resistant to liquids and chemicals, facilitating cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Any drains in the floor of the suit area contain traps filled with a chemical disinfectant of demonstrated efficacy against the target agent, and they are connected directly to the liquid waste decontamination system. Sewer vents and other service lines contain HEPA filters.
5. Internal facility appurtenances in the suit area, such as light fixtures, air ducts, and utility pipes, are arranged to minimize the horizontal surface area.

6. Bench tops have seamless surfaces which are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.

7. Laboratory furniture is of simple open construction capable of supporting anticipated loading and uses. Non-porous materials are preferable. Spaces between benches, cabinets, and equipment are accessible for cleaning and decontamination. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.

8. A hands-free or automatically operated handwashing sink is provided in the suit area(s); handwashing sinks in the outer and inner change rooms should be considered based on the risk assessment.

9. If there is a central vacuum system, it does not serve areas outside the suit area. In-line HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services to the suit area are protected by devices that prevent backflow.

10. Access doors to the laboratory are self-closing and lockable. Inner and outer doors to the chemical shower and inner and outer doors to airlocks are interlocked to prevent both doors from being opened simultaneously.

11. Any windows are breakage-resistant and are sealed.

12. Liquid effluents from sinks, floor drains (if used), autoclave chambers and other sources within the containment barrier are decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer. Effluents from showers and toilets may be discharged to the sanitary sewer without treatment. The process used for decontamination of liquid wastes must be validated physically and biologically.

13. A dedicated non-recirculating ventilation system is provided. The supply and exhaust components of the system are balanced to ensure directional airflow from the area of least hazard to the area(s) of greatest potential hazard. Redundant supply fans are recommended. Redundant exhaust fans are required. The differential pressure/directional airflow between adjacent areas is monitored and alarmed to indicate malfunction of the system. An appropriate visual pressure monitoring device that indicates and confirms the pressure differential of the suit area must be provided and located at the entry to the clean change room. The airflow in the supply and exhaust components is monitored and an HVAC control system is installed to prevent positive pressurization of the laboratory.

14. The supply air to the suit area, decontamination shower, and decontamination airlock is protected by passage through a HEPA filter. The general room exhaust air from the suit area, decontamination shower and decontamination airlock is treated by a passage through two HEPA filters in series prior to discharge to the outside. The air is discharged away from occupied spaces and air intakes. The HEPA filters are located as near as practicable to the source in order to minimize the length of potentially contaminated ductwork. All HEPA filters need to be tested and certified annually. The HEPA filter housings are designed to allow for in situ decontamination of the filter prior to removal. Alternatively,
the filter can be removed in a sealed, gas-tight primary container for subsequent decontamination and/or destruction by incineration. The design of the HEPA filter housing should facilitate validation of the filter installation. The use of pre-certified HEPA filters can be an advantage. The service life of the exhaust HEPA filters can be extended through adequate prefiltration of the supply air.

15. The positioning of the supply and exhaust points should be such that dead air space in the suit room is minimized.

16. The treated exhaust air from Class II biological safety cabinets, located in a facility where workers wear a positive pressure suit, may be discharged into the room environment or to the outside through the facility air exhaust system. If the treated exhaust is discharged to the outside through the facility exhaust system, it is connected to this system in a manner that avoids any interference with the air balance of the cabinets or the facility exhaust system.

17. The Biosafety Level 4 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 annually against these procedures as modified by operational experience.

18. Appropriate communication systems should be provided between the laboratory and the outside.
APPENDIX E

Additional Requirements for Facilities Transferring or Receiving Select Agents
Department of Health and Human Services

Appendix A to 42 CFR Part 72--SELECT AGENTS

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

Viruses
- Crimean-Congo hemorrhagic fever virus
- Eastern Equine Encephalitis virus
- Ebola viruses
- Equine Morbillivirus
- Lassa fever virus
- Cercopithecine herpesvirus 1 (Herpes B virus)
- Monkeypox virus
- Marburg virus
- Rift Valley fever virus
- 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) and Variola minor virus (Alastrim).
- Venezuelan Equine Encephalitis virus
- Viruses causing hantavirus pulmonary syndrome
- Yellow fever virus

Exemptions: Vaccine strains of viral agents (Junin Virus strain candid #1, Rift Valley fever virus strain MP-12, Venezuelan Equine encephalitis virus strain TC-83, Yellow fever virus strain 17-C) are exempt.

Bacteria
- Bacillus anthracis
- Brucella abortus, B. melitensis, B. suis
- Burkholderia (Pseudomonas) pseudomallei
- Clostridium botulinum
- Francisella tularensis
- Yersinia pestis

Exemptions: vaccine strains as described in Title 9 CFR, Part 78.1 are exempt.

Rickettsiae
- Coxiella burnetii
- Rickettsia prowazekii
- Rickettsia rickettsia
Fungi
- Coccidioides immitis

Toxins
- Abrin
- Aflatoxins
- Botulinum neurotoxins
- Clostridium perfringens epsilon toxin
- Conotoxins
- Diacetoxyscirpenol
- Ricin
- Saxitoxin
- Shigatoxin
- Staphylococcal enterotoxins
- Tetrodotoxin
- T-2 toxin

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

Genetically modified microorganisms or genetic elements from organisms on Appendix A, shown to produce or encode for a factor associated with a disease.

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

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. 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 F

Export Administration Regulations
Department of Commerce

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

List of Items Controlled

a. Viruses, as follows:
   ♦ African swine fever virus;
   ♦ Avian influenza virus;
   ♦ Bluetongue virus;
   ♦ Chikungunya virus;
   ♦ Congo-Crimean haemorrhagic fever virus;
   ♦ Dengue fever virus;
   ♦ Eastern equine encephalitis virus;
   ♦ Ebola virus;
   ♦ Foot and mouth disease virus;
   ♦ Goat pox virus;
   ♦ Hantaan virus;
   ♦ Porcine herpes virus (Aujeszky's disease);
   ♦ Hog cholera virus (syn. Swine fever virus)
   ♦ Japanese encephalitis virus;
   ♦ Junin virus;
   ♦ Lassa fever virus;
   ♦ Lymphocytic choriomeningitis virus;
   ♦ Lyssa virus;
   ♦ Machupo virus;
   ♦ Marburg virus;
   ♦ Monkey pox virus;
   ♦ Newcastle disease virus;
   ♦ Pestes des petits ruminants virus;
   ♦ Porcine enterovirus type 9 (syn. Swine vesicular disease virus);
   ♦ Rift Valley fever virus;
   ♦ Rinderpest virus;
   ♦ Sheep pox virus;
   ♦ Teschen disease virus;
   ♦ Tick-borne encephalitis virus (Russian Spring-Summer encephalitis virus);
   ♦ Variola virus;
   ♦ Venezuelan equine encephalitis virus;
   ♦ Vesicular stomatitis virus;
   ♦ Western equine encephalitis virus;
   ♦ White pox; or
   ♦ Yellow fever virus
b. Rickettsiae, as follows:
   ♦ Coxiella burnetii;
   ♦ Rickettsia quintana;
   ♦ Rickettsia prowasecki; or
   ♦ Rickettsia rickettsii.

c. Bacteria, as follows:
   ♦ Bacillus anthracis;
   ♦ Brucella abortus;
   ♦ Brucella melitensis;
   ♦ Brucella suis;
   ♦ Chlamydia psittaci;
   ♦ Clostridium botulinum;
   ♦ Francisella tularensis;
   ♦ Mycoplasma mycoides;
   ♦ Pseudomonas mallei;
   ♦ Pseudomonas pseudomallei;
   ♦ Pseudomonas solanacerum;
   ♦ Salmonella typhi;
   ♦ Shigella dysenteriae;
   ♦ Vibrio cholerae;
   ♦ Xanthomonas albilineas;
   ♦ Xanthomonas campestris pv citri;
   ♦ Xanthomonas campestris pv oryzae; or
   ♦ Yersinia pestis.

d. Fungi, as follows:
   ♦ Colletotrichum coffeanum var. virulans;
   ♦ Cochliobolus miyabeanaus (Helminthosporium oryzae);
   ♦ Microcyclus ulei (syn. Dothidella ulei)
   ♦ Puccinia glumarum;
   ♦ Puccinia graminis (syn. Puccinia graminis f. sp. tritici);
   ♦ Puccinia striiformis (syn. Puccinia glumarum); or
   ♦ Pyricularia grisea/ Pyricularia oryzae.

e. Genetically modified microorganisms, as follows:
   ♦ Genetically modified micro-organisms or genetic elements that contain nucleic acid sequences
     associated with pathogenicity and are derived from organisms identified in this ECCN;
   ♦ Genetically modified micro-organisms or genetic elements that contain nucleic acid sequences
     associated with pathogenicity derived from plant pathogens identified in this ECCN; or
   ♦ Microorganisms genetically modified to produce any of the toxins listed in paragraph f of this
     ECCN.
f. Toxins
   ♦ Botulinum toxins;
   ♦ Clostridium perfringens toxins;
   ♦ Conotoxin;
   ♦ Microcystin (cyanginosin);
   ♦ Ricin;
   ♦ Saxitoxin;
   ♦ Shiga toxin;
   ♦ Staphylococcus aureus toxins:
     ♦ Tetrodotoxin;
     ♦ Verotoxin; or
   ♦ Aflatoxins.
<table>
<thead>
<tr>
<th>A</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSL1</td>
<td>6</td>
</tr>
<tr>
<td>ABSL2</td>
<td>6</td>
</tr>
<tr>
<td>ABSL3</td>
<td>6</td>
</tr>
<tr>
<td>ABSL4</td>
<td>6</td>
</tr>
<tr>
<td>aerosols</td>
<td>8, 10, 11, 20, 22, 26, 28, 30, 32, 36, 38</td>
</tr>
<tr>
<td>agents</td>
<td>38, 46</td>
</tr>
<tr>
<td>alcohols</td>
<td>14, 16</td>
</tr>
<tr>
<td>Animal Care and Use Committee</td>
<td>3</td>
</tr>
<tr>
<td>animal facilities</td>
<td>6</td>
</tr>
<tr>
<td>animal pathogens</td>
<td>6, 24</td>
</tr>
<tr>
<td>annual certification</td>
<td>20</td>
</tr>
<tr>
<td>antimicrobial</td>
<td>13</td>
</tr>
<tr>
<td>antiseptic</td>
<td>13</td>
</tr>
<tr>
<td>autoclave</td>
<td>9, 10, 16, 36, 39, 41, 42, 43, 44</td>
</tr>
<tr>
<td>autoclave bags</td>
<td>9, 16, 17, 20</td>
</tr>
<tr>
<td>autoclaving procedures</td>
<td>16</td>
</tr>
<tr>
<td>B</td>
<td>46, 49</td>
</tr>
<tr>
<td>bacteria</td>
<td>46, 49</td>
</tr>
<tr>
<td>biohazard bag</td>
<td>10, 11</td>
</tr>
<tr>
<td>biological agent</td>
<td>18</td>
</tr>
<tr>
<td>biological safety cabinet</td>
<td>2, 10, 20, 27, 30, 35, 41</td>
</tr>
<tr>
<td>Biological Safety Officer</td>
<td>1, 4, 10, 11, 17, 23</td>
</tr>
<tr>
<td>biological spills</td>
<td>9</td>
</tr>
<tr>
<td>biological waste</td>
<td>1, 2, 16, 17, 18, 20</td>
</tr>
<tr>
<td>biological waste disposal procedures</td>
<td>18</td>
</tr>
<tr>
<td>biosafety cabinet</td>
<td>20, 22</td>
</tr>
<tr>
<td>Biosafety Containment Levels</td>
<td>6</td>
</tr>
<tr>
<td>biosafety equipment</td>
<td>20</td>
</tr>
<tr>
<td>Biosafety in Microbiological and Biomedical Laboratories</td>
<td>1, 3, 4, 6</td>
</tr>
<tr>
<td>Biosafety Level 1</td>
<td>4, 26, 27, 28</td>
</tr>
<tr>
<td>Biosafety Level 2</td>
<td>6, 28, 32</td>
</tr>
<tr>
<td>Biosafety Level 3</td>
<td>32, 33, 37</td>
</tr>
<tr>
<td>Biosafety Level 4</td>
<td>21, 38, 39, 40, 41, 43, 45</td>
</tr>
<tr>
<td>biowaste boxes</td>
<td>19, 20</td>
</tr>
<tr>
<td>bleach</td>
<td>9, 10, 11, 14, 16, 18</td>
</tr>
<tr>
<td>Bloodborne Pathogens</td>
<td>6, 24</td>
</tr>
<tr>
<td>BMBL</td>
<td>1, 3, 4, 6</td>
</tr>
<tr>
<td>body fluids</td>
<td>2, 6, 19, 30, 34</td>
</tr>
<tr>
<td>BSC</td>
<td>2, 3, 5, 8, 20, 21, 22, 30, 43</td>
</tr>
<tr>
<td>BSC’s</td>
<td>21</td>
</tr>
<tr>
<td>BSL1</td>
<td>4, 6, 9, 18</td>
</tr>
<tr>
<td>BSL2</td>
<td>4, 6, 7, 9, 18, 22</td>
</tr>
<tr>
<td>BSL3</td>
<td>4, 10, 18</td>
</tr>
<tr>
<td>BSL4</td>
<td>4, 18</td>
</tr>
<tr>
<td>C</td>
<td>27, 31</td>
</tr>
<tr>
<td>carpets and rugs</td>
<td>27, 31</td>
</tr>
<tr>
<td>CDC</td>
<td>4, 6, 23, 46, 47</td>
</tr>
<tr>
<td>Centers for Disease Control and Prevention</td>
<td>4, 23</td>
</tr>
<tr>
<td>centrifuge</td>
<td>18, 22, 30, 35</td>
</tr>
<tr>
<td>centrifuge containment</td>
<td>22</td>
</tr>
<tr>
<td>characteristics of disinfectants</td>
<td>14</td>
</tr>
<tr>
<td>chemical disinfectants</td>
<td>13</td>
</tr>
<tr>
<td>Chemical Hygiene Plan</td>
<td>1, 2</td>
</tr>
<tr>
<td>chemical waste</td>
<td>19</td>
</tr>
<tr>
<td>chlorine compounds</td>
<td>16</td>
</tr>
<tr>
<td>chloroplasts</td>
<td>5</td>
</tr>
<tr>
<td>Class I</td>
<td>8, 20, 21</td>
</tr>
<tr>
<td>Class II</td>
<td>21, 30, 35, 36, 38, 41, 45</td>
</tr>
<tr>
<td>Class III</td>
<td>8, 21, 35, 36, 38, 40, 41, 42, 43</td>
</tr>
<tr>
<td>classification of potentially infectious agents</td>
<td>4</td>
</tr>
<tr>
<td>clean air benches</td>
<td>21</td>
</tr>
<tr>
<td>clinical laboratories</td>
<td>6</td>
</tr>
<tr>
<td>clinical specimens</td>
<td>7, 23</td>
</tr>
<tr>
<td>collection flask</td>
<td>22</td>
</tr>
<tr>
<td>contaminated clothing</td>
<td>10, 11, 13</td>
</tr>
<tr>
<td>D</td>
<td>24</td>
</tr>
<tr>
<td>decontamination</td>
<td>8, 10, 11, 12, 13, 16, 17, 26, 28, 29, 33, 34, 35, 36, 37, 40, 41, 42, 43, 44, 45</td>
</tr>
<tr>
<td>decontamination and disposal</td>
<td>13</td>
</tr>
<tr>
<td>Department of Transportation</td>
<td>24</td>
</tr>
<tr>
<td>dilution of disinfectants</td>
<td>16</td>
</tr>
<tr>
<td>disinfectant</td>
<td>9, 10, 11, 13, 14, 16, 18, 21, 30, 34, 36, 40, 44</td>
</tr>
<tr>
<td>disinfectants commonly used in the laboratory</td>
<td>14</td>
</tr>
<tr>
<td>disinfection</td>
<td>13, 16, 22, 36</td>
</tr>
<tr>
<td>disposal</td>
<td>12, 16, 18, 26, 28, 29, 30, 33, 38, 40</td>
</tr>
<tr>
<td>disposal containers</td>
<td>19</td>
</tr>
<tr>
<td>disposal of animal tissues, carcasses and bedding</td>
<td>19</td>
</tr>
<tr>
<td>disposal of blood products and body fluids</td>
<td>18</td>
</tr>
<tr>
<td>disposal of sharps and disposable glassware</td>
<td>19</td>
</tr>
<tr>
<td>DOT</td>
<td>24</td>
</tr>
<tr>
<td>double-bag</td>
<td>19</td>
</tr>
<tr>
<td>E</td>
<td>2, 4, 5, 6, 11, 19, 20</td>
</tr>
<tr>
<td>EH&amp;S</td>
<td>2, 4, 5, 6, 11, 19, 20</td>
</tr>
<tr>
<td>emergency eyewash</td>
<td>13</td>
</tr>
<tr>
<td>emergency procedures</td>
<td>9</td>
</tr>
<tr>
<td>Environmental Health and Safety</td>
<td>1, 2, 13, 20</td>
</tr>
<tr>
<td>ethanol</td>
<td>10, 14, 21, 22</td>
</tr>
<tr>
<td>etiologic agents</td>
<td>24, 29</td>
</tr>
<tr>
<td>eukaryotic</td>
<td>5, 6</td>
</tr>
<tr>
<td>export administration regulations</td>
<td>48</td>
</tr>
<tr>
<td>F</td>
<td>20</td>
</tr>
<tr>
<td>filled waste containers</td>
<td>20</td>
</tr>
<tr>
<td>fires involving biological materials</td>
<td>13</td>
</tr>
<tr>
<td>fungi</td>
<td>14, 46, 49</td>
</tr>
<tr>
<td>G</td>
<td>9</td>
</tr>
<tr>
<td>general spill cleanup guidelines</td>
<td>9</td>
</tr>
<tr>
<td>genetically engineered microorganisms</td>
<td>4</td>
</tr>
<tr>
<td>genetically engineered organisms</td>
<td>4, 23</td>
</tr>
<tr>
<td>genetically modified microorganisms</td>
<td>24</td>
</tr>
<tr>
<td>genome</td>
<td>5, 6, 24</td>
</tr>
<tr>
<td>H</td>
<td>19</td>
</tr>
<tr>
<td>hazardous chemical</td>
<td>19</td>
</tr>
<tr>
<td>HBV</td>
<td>6</td>
</tr>
<tr>
<td>helper virus</td>
<td>5</td>
</tr>
<tr>
<td>hemorrhagic fever</td>
<td>7, 46</td>
</tr>
<tr>
<td>HEPA</td>
<td>10, 11, 20, 21, 36, 37, 41, 42, 43, 44, 45</td>
</tr>
</tbody>
</table>