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Safe Handling and Use of Biological Research Materials

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New document or new requirements

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 Major requirement change

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Safe Handling and Use of Biological Research Materials

1.0 Introduction

The environment, safety, and health (ES&H) policy of Lawrence Livermore National Laboratory (LLNL) requires operations to be planned and performed safely, with full consideration for the law and for the protection of employees, the public, and the environment. This document in addition to other applicable documents in the *ES&H Manual* constitutes the biosafety manual for LLNL and describes the safety requirements for the conduct of all biological research operations. The requirements address operational hazards, environmental concerns, controls, and the responsibilities of personnel.

One objective of this document and other applicable documents within the *ES&H Manual* is to define LLNL's biological safety program. (The term "biological safety" is used interchangeably with "biosafety.") A biosafety program is a complete program of administrative controls, engineered controls (e.g., containment), and use of personal protective equipment (PPE) for reducing the risk of disease to employees who face potential occupational exposure to infectious agents or biologically derived molecules. The biosafety program is intended to protect employees, coworkers, laboratory support workers, products, and the environment. (For a definition of the terms used in this document, refer to Appendix A.)

All biological research operations conducted at LLNL shall satisfy the requirements in this and other appropriate documents in the *ES&H Manual*. In accordance with Integrated Safety Management (ISM), such work is covered by an Integration Work Sheet (IWS) or safety plan that specifically assesses and references responsibilities, hazards, and the controls necessary to conduct the operation safely. For more information, see Document 2.2, "Managing ES&H for LLNL Work," in the *ES&H Manual*.

1.1 Applicability

This document governs biohazardous operations that are limited to biosafety levels (BSLs) 1, 2 or 3 involving small-scale (i.e., nonproduction, research laboratory-scale) amounts of biological research materials. No work at the BSL 4 level is currently allowed at LLNL. For more information about BSLs, refer to Appendix B.

The requirements of this document apply to:

- All activities (including human-subjects research and environmental restoration) and facilities involving the handling, storage, onsite transportation, or use of biohazardous research materials (biohazardous research materials are defined in Section 2.0).
- All persons (including LLNL employees, subcontract workers, consultants, support and service staff, participating guests, short- and long-term visitors, and summer students) who enter or work in research areas where biological research materials are present.

The requirements of this document do not apply to the following nonresearch services provided by the organizations indicated:

- Health care (Health Services Department).
- Emergency response and cleanup (Fire Department, Protective Force Division).
- Environmental restoration (Environmental Protection Department).
- Sewer and water system maintenance (Plant Engineering).

2.0 Hazards

The hazards associated with working with biological research materials range from personal exposure to accidental environmental releases. Personal exposure may result from the handling of materials such as toxins, infectious agents, or animals. The degree of personal exposure depends on the infectious source or contaminated source, the individual's immune status, and efficiency of the transmission of infection. Personal exposure may have benign results or may cause a disease requiring medical treatment.

Accidental releases into the environment may cause an imbalance in the normal, established microflora in the affected area. Such imbalance may pose a health threat to the general public (particularly persons who are immunologically compromised) and may have an impact on agriculture and sewage treatment facilities, for instance. Because of the serious ramifications of employee exposure and accidental releases into the environment, control measures to prevent such events are required.

The categories of materials, discussed in this section, are as follows:

- Biohazards.
 - Biohazardous materials.
 - Biohazardous agents.

- Human subjects.
- Human blood, body fluids, and tissues.
- Genomic materials.
- Cell cultures of human or animal origin.
- Animals and animal blood, body fluids, and tissues.
- Hazardous materials.

2.1 Biohazards

A biohazard is any biological material or component that presents a risk of illness or injury to humans, plants, or animals. Biohazards can be divided into biohazardous materials and biohazardous agents, which are described in this section.

2.1.1 Biohazardous Materials

Biohazardous materials are not capable of self-replication and are the components of biological agents that present a risk of causing illness or injury to humans, plants, and animals. The types of biohazardous materials are as follows:

Toxins. Toxins (also referred to as biotoxins) are nonliving toxic biochemicals that are naturally produced by many different types of living organisms. Biotoxins are:

- Often more toxic by mass than chemical warfare agents.
- Considered to pose the same level of risk as the microorganisms that produce them.
- Not themselves infectious or contagious. However, a biotoxin-producing organism may be infectious or contagious.

Endotoxins. Endotoxins, which occur in the outer membrane of certain gram-negative bacteria, are not secreted but are released only when the cells are disrupted or destroyed. Endotoxins are complex polysaccharide molecules that elicit an antigenic response, resulting in fever and altered resistance to bacterial infections. Exposure may cause toxic hemorrhagic shock and severe diarrhea.

Clinical and Diagnostic Specimens. A clinical or diagnostic specimen is any human or animal material (e.g., excreta, secreted, blood, components, tissue, and tissue fluids) handled for the purposes of diagnosis, treatment, or research. Such specimens pose a unique hazard because of the material's unknown infectious nature.

2.1.2 Biohazardous Agents

Biohazardous agents (also called pathogenic or etiologic agents) are microbial agents that are capable of self-replication or self-directed replication and have the capacity to produce deleterious effects in other biological organisms (particularly humans). Such agents include (but are not limited to) viruses, prions, chlamydia, bacteria, fungi, yeast, algae, and plants.

Factors to be considered in determining the level of infectiousness of an agent include:

- Agent factors (e.g., virulence, pathogenicity, infectious dose, environmental stability, route of transmission, communicability, operations, quantity, and availability of vaccine or treatment).
- Agent products able to elicit toxicity, physiological activity, or allergenicity.

Classification of Etiologic Agents. The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) have classified biological agents into four risk groups (RGs) by the degree of hazard. A higher RG number indicates a higher level of hazard to a healthy human being.

- RG 1: Agents not associated with disease in healthy human adults.
- RG 2: Agents associated with human diseases that are rarely serious and for which preventive or therapeutic interventions are often available.
- RG 3: Agents associated with serious or lethal human disease for which preventive or therapeutic intervention may be available (i.e., high individual risk but low community risk).
- RG 4: Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic intervention are not available (i.e., high individual risk and high community risk).

Appendix C is a list of biological agents classified into the four RGs in accordance with the CDC and the NIH (see Section 6.3 for related references).

Note that RGs are not the same as biosafety levels (BSLs). RGs are used to classify biological agents themselves, whereas a BSL indicates the level of containment that is required in an operation.

Select Agents and Toxins. The term “Select Agent” refers to a microorganism (i.e., virus, bacterium, fungus, or rickettsia) or toxin that are listed in one of the following:

- 42 CFR 73, “Possession, Use, and Transfer of Select Agents (for Humans).”
- 9 CFR 121, “Possession, Use, and Transfer of Select Agents (for Animals).”
- 7 CFR 331, “Possession, Use, and Transfer of Select Agents (for Plants).”

Select Agents have been identified as potential weapons of mass destruction that can be used in bioterrorism activities. See Appendix D for a detailed list. The term also includes recombinant organisms and isolated genetic material when in a host system in which it can produce an infectious agent or functional toxin.

In addition, the CDC further classifies Select Agents into the following categories according to priority in public health preparedness efforts:

- Category A – Highest-priority agents
- Category B – Second highest-priority agents
- Category C – Third highest-priority agents

For a list of the agents in the above priority categories, see Appendix E.

Classification of Oncogenic Viruses. The National Cancer Institute (NCI) has defined oncogenic (i.e., potentially tumor-causing) viruses as a special class of etiologic agents that have been categorized into three risk levels: low, moderate, and high. For more information, refer to the *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses* (see Section 6.3).

Materials Regulated by the U.S. Department of Agriculture. Use, storage, disposal, and transport of animal and plant pathogens or toxins and soil potentially contaminated with animal or plant pathogens or toxins are regulated by the U.S. Department of Agriculture (USDA). The regulations are enforced by the USDA, as well as by the California Department of Food and Agriculture (CDFA) and county departments of agriculture. A permit is required for the transport, use, handling, or storage of these materials. For a list of USDA-regulated materials, see Appendix F. For more information about USDA-regulated materials, contact the Biosafety Officer or see the following Internet address:

<http://www.aphis.usda.gov/ppq/permits/plantpest/pathogen.html>

For more information about the CDFA, see the following Internet address:

<http://www.cdfa.ca.gov/>

2.2 Human Subjects

Work with human subjects involves the handling of potentially contaminated materials (e.g., blood, bodily fluids, and tissue). For more information regarding the potential hazards of working with such materials, see Section 2.3 of this document, as well as Document 13.2, "Exposure Control Plan: Working Safely with Blood and Bloodborne Pathogens," and Document 13.4, "Research Involving Human Subjects," in the *ES&H Manual* for more information on the topic.

2.3 Human Blood, Body Fluids, and Tissues

In accordance with Occupational Safety and Health Administration (OSHA) regulations, human blood, human body fluids, and unfixed tissues shall be assumed to be contaminated with bloodborne pathogens. Bloodborne pathogens include, but are not limited to, the hepatitis B virus (HBV), hepatitis C virus, and human immunodeficiency virus (HIV). See Document 13.2 for more information.

2.4 Genomic Materials

Recombinant genomic materials include nucleic acids [e.g., deoxyribonucleic acid (DNA) or ribonucleic acid (RNA)] and are defined as either of the following:

- Molecules that are constructed outside living cells by joining natural or synthetic DNA or RNA segments to DNA or RNA molecules that can replicate in a living cell.
- Molecules that result from the replication described above.

Synthetic or recombinant DNA or RNA molecules that are likely to yield a potential harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) are considered as equivalent to their natural DNA counterparts and to be in the same RG as those of the organism from which the DNA originated. Work with genomic materials that are likely to yield a potential harmful polynucleotide or polypeptide requires additional controls or higher containment levels as determined by the ES&H Team industrial hygienist as part of the hazard analysis determination.

Recombinant DNA or RNA experiments may involve microbial, animal, or plant hosts and are defined as any experiments involving:

- Construction and handling of recombinant DNA or RNA molecules.
- Organisms and viruses containing recombinant DNA or RNA molecules.
- Gene therapy.

2.5 Cell Cultures of Human or Animal Origin

Cell cultures of human or animal origin may harbor latent viruses either inadvertently or because of deliberate experimental infections and therefore pose an undetected hazard. These cell cultures include primary and established cell lines of subprimate or normal primate origin. Primary and established (immortal) human or animal cell lines should be assumed to carry adventitious agents and should be classified as RG 2; the Biosafety Officer or the Institutional Biosafety Committee (IBC) may downgrade certain animal cell lines to RG 1 after deliberation.

Under no circumstances should anyone work with cells derived from themselves or from their first-degree relatives, because their immune system may not provide adequate protection against transplantation and growth of the cells.

2.6 Animals

Working with animals poses a unique hazard to the animals themselves and to personnel handling the animals. Special care should be taken to limit exposure to biological agents that are zoonotic (i.e., readily communicable from animals to humans). For more information about the hazards associated with working with vertebrate animals, see Document 13.5, "Vertebrate Animals Used in Research," in the *ES&H Manual*.

2.7 Hazardous Materials

Working with biological materials usually involves working with radioactive and hazardous materials. The hazards of dealing with radioactive and hazardous materials are addressed in other *ES&H Manual* documents [e.g., those in Part 14 (Chemical) and Part 20 (Ionizing Radiation/Nonionizing Radiation)]. The hazards associated with such materials shall be considered during the review and authorization of work with biological research materials.

3.0 Controls

This section describes the three types of controls for work involving biological materials: administrative controls (e.g., protocol and institutional review), engineered controls (e.g., containment), and PPE.

Containment is the safe management of infectious (e.g., biological) or hazardous (e.g., radioactive) materials in a laboratory where such materials are handled or maintained. The purpose of containment is to reduce or eliminate exposure of Laboratory employees, other persons, and the outside environment to potentially infectious or hazardous agents. Containment requirements vary depending on the hazard present. For information about biological containment requirements or biological containment levels, see Appendix B. The terms "biological containment levels" and "biosafety level" are used interchangeably.

3.1 Administrative Controls

All activities involving biohazards research activities shall be authorized through the IWS process in accordance with ISM. For more information about ISM, see Document 2.1, "Laboratory and ES&H Policies, General Worker Responsibilities, and Integrated Safety Management," in the *ES&H Manual*.

In addition to an IWS, a safety plan is also required when working with:

- Regulated materials, such as
 - Select Agents
 - USDA-regulated materials (e.g., animals, plants, and soil).
 - Bloodborne pathogens.
 - Recombinant DNA or RNA.
- Large-scale quantities (i.e., quantities greater than 10 liters) of any biological research material
- Genomic materials coding for virulence or toxin factors

3.1.1 Institutional Review

Work involving biohazardous materials shall be initially reviewed by the LLNL Biosafety Operations Committee (LBOC) as part of the work authorization process. Additional review may be required depending on the specific hazards involved. The institutional committees' guidelines and forms are discussed below.

LLNL Biosafety Operations Committee. The purpose of LBOC is to facilitate the institutional review of proposed work with biological research materials.

LBOC is comprised of representatives of organizations such as the following:

- The Health Services, Hazards Control, and Environmental Protection Departments.
- The Nonproliferation, Arms Control, and International Security Directorate.
- The Chemistry and Materials Science Directorate.
- The Biology and Biotechnology Research Program Directorate.
- Institutional Biosafety Committee (IBC).

See Section 4.6 for the responsibilities of LBOC.

Institutional Biosafety Committee. The purpose of the IBC is to review LLNL research protocols to ensure that:

- Safe recombinant DNA methods and restrictive use of recombinant materials for human therapy and environmental applications are in accordance with the *NIH Guidelines for Research Involving Recombinant DNA* (see Section 5.2).
- All biohazardous research protocols involving etiologic agents and toxins adhere to the safety requirements specified in the U.S. Department of Health and Human Services' *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, see Section 5.1) and to all applicable regulations pertaining to work with etiologic agents and toxins.

The IBC also functions as a licensee for the Laboratory Registration/Select Agent Transfer Program. For more information, see the following Internet addresses:

<http://ibc.llnl.gov/>

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

See Section 4.7 for the responsibilities of IBC.

Institutional Review Board (IRB). The purpose of the IRB is to review proposed work with human subjects or with tissue or data from human subjects. The IRB ensures that the rights of human subjects are protected and that human subjects give their informed, voluntary consent to participate in the research. For more information, see Documents 13.2 and 13.4. For more information, see the following Internet address:

<http://www.llnl.gov/HumanSubjects/>

Institutional Animal Care and Use Committee (IACUC). The purpose of IACUC is to review proposed experimental work with any vertebrate animal. IACUC coordinates the monitoring of the humane care of animals. For more information regarding IACUC approval, see Document 13.5. Appropriate animal BSLs are assigned according to the hazards involved. All work with live animals is restricted to the Animal Care Facility.

3.1.2 Protocol Review

The protocol review process is a process used by institutional committees, as well as programs to identify the following:

- Health hazards associated with the work.
- Environmental concerns.

- Medical surveillance requirements (see Section 3.1.9).
- Containment requirements.
- Training needs.
- Ethical concerns.
- Regulatory requirements and applicable Work Smart Standards (WSSs) (see Section 5.0).
- Best management practices.

The purpose of review is to determine whether employees are at risk, whether the potential benefits of the research outweigh the risk, and whether adequate provisions or controls have been made to minimize or mitigate the hazards involved. Research involving more than minimal risk may require more frequent review and controls. Research involving the use of human blood or other human bodily fluids shall be handled in accordance with the guidelines and work practices outlined in Document 13.2.

3.1.3 Work Practice Controls

Work with biohazardous agents or materials onsite shall be limited to BSL 1, 2 or 3 containment levels. Appropriate BSL work practices (outlined in Appendix B of this document) are to be followed. Work practices for biological materials include the following:

Universal Precautions. Universal precautions are an approach to infection control in which all human blood and certain human bodily fluids are treated as if known to be contaminated with bloodborne pathogens. Universal precautions should be followed at all times when handling human blood and other bodily fluids. See Appendix G for details.

Hand washing. Frequent hand washing is encouraged. Hands shall be washed with soap after handling potentially infectious materials and before leaving the laboratory.

Decontamination. All surfaces shall be decontaminated before initiating any work and at the end of each workday. The three levels of biological decontamination of microorganisms are:

1. Sanitize – The general reduction of microorganisms by use of general cleaning agents.

2. Disinfect – The destruction of targeted organisms with the use of chemicals or physical agents. For example, table-tops and surfaces can be decontaminated with disinfectants. (See Appendix H for more information about disinfectants.)
3. Sterilize – The complete destruction of all microbes. Sterilization is in general part of the total decontamination process.

Reusable, rigid, leak proof secondary containers used to hold biohazardous waste shall be thoroughly washed and decontaminated each time they are emptied, unless the surfaces of the container have been completely protected from contamination by disposable liners, bags, or other devices removed with the waste. Methods of decontamination are listed in Appendix H and in Document 13.1, “Biological Controls and Operations,” in the *ES&H Manual*. Emergency response procedures are discussed in Document 22.1, “Emergency Management,” in the *ES&H Manual*.

Biohazard Signs. Biohazard signs bearing the biohazard symbol (see Figure 1) shall be placed as a warning sign at the entry of each room where biohazardous materials of RG 2 or higher are handled or stored. The sign shall indicate the biohazard present, the persons responsible for the work, and any restrictions on access to the room. See Document 13.1 for more information about signs and labels.



Figure 1. Biohazard symbol.

In addition, the standard Health Hazard Communication Notice door sign can also be used to indicate that a biohazard is present in a room or area. See Document 10.2, “LLNL Health Hazard Communication Program,” in the *ES&H Manual*.

3.1.4 Medical Waste Management

The LLNL main site is considered a large-quantity generator because 200 pounds or more of medical waste is normally generated within any month of a 12-month period. Medical waste is subject to regulations of the California Department of Health Services, which are enforced by the Alameda County Health Care Services Agency (in the case of the LLNL main site) and by the San Joaquin County Public Health Services (in the case of Site 300). For more information, see Document 36.1, "Hazardous, Radioactive, and Biological Waste Management Requirements," in the *ES&H Manual*.

In general, the following types of medical waste shall be segregated into specific waste streams for proper disposal:

- Syringes and other sharps shall be segregated from solid or liquid biohazardous waste and placed in a properly labeled and approved sharps container. All sharps containers shall be disposed of when 3/4 full. Sharps containers are first autoclaved before final destruction by incineration. (See Section 3.2 for more information about sharps containers.)
- Biohazardous materials that are to be autoclaved shall be placed in properly labeled, leak proof containers containing labeled, autoclavable biohazard bags. Such waste shall be disposed of within 7 days if stored at room temperature or within 90 days if stored at 0°C. All such autoclaves shall have a proper county-issued permit prior to use. Contact your area ES&H Team environmental analyst for more information.
- If biohazardous materials are to be chemically disinfected, contact your area ES&H Team Industrial Hygienist for more information regarding the disinfectant of choice, its contact time, and methods of disposal. Chemically treated artifacts should not be autoclaved because of the vapor hazards of chemical disinfectants. For more information regarding chemical disinfectants, see Appendix H of this document.

Special waste management practices for all BSL 2 laboratories working with Select Agents or RG 3 materials include the following:

- All solid and semi-solid waste is considered biohazardous and is collected into doubled autoclavable red bags held in rigid, leak-proof, puncture-resistant containers with lids.
- When bags are filled, they are gathered at the neck and closed by wrapping with at least a double circumference of autoclave tape, or with a rubber band (as long as there is autoclave tape elsewhere on the bag to serve as a secondary indicator of successful sterilization).

- If the lab contains an autoclave, red bags shall be autoclaved before removal from that lab. Autoclaving conditions shall be appropriate to the nature of the autoclave load, but shall not be less than thirty (30) minutes at 121° C. Each load shall include a centrally placed spore strip or other autoclave validation device. Each autoclave so used shall be tested no less than monthly by a *Geobacillus stearothermophilus* spore vial test and the results posted on an annual summary table near the autoclave.
 - Any failure of an autoclave validation test shall be grounds for immediate cessation of autoclave use and quarantine of any waste being processed at the time of the failure. The Biosafety Officer and ES&H Team industrial hygienist shall be notified immediately. An action plan for retesting, repairing if necessary, and releasing quarantined waste shall be developed and implemented without delay.
- If the lab does NOT contain an autoclave, arrangements shall be made to use an autoclave in another approved laboratory or facility. The same operational and failure requirements shall be employed. Transport of red bags to the autoclave location shall be within a separate rigid, leak-proof, puncture-resistant container with a lid. Transfer to this container shall be made at the entry door to the laboratory.
- All red bags shall be transported after autoclaving to the central autoclave facility in B361 for a second autoclaving or transferred to a contractor licensed by the Department of Transportation and the State of California to transport and dispose of biohazardous and medical waste. On-site transport of red bags shall be within a rigid, leak-proof, puncture-resistant container or cart with a lid.
- Liquid or semi-liquid laboratory waste shall be chemically disinfected by at least 30 minutes exposure to a final concentration of no less than 10% household bleach (0.5% sodium hypochlorite) or other disinfectant approved by the Biosafety Officer. Following disinfection, liquid and semi-liquid laboratory waste can be disposed to the sanitary sewer following secondary disinfection in Waste Retention Tanks.

Special waste management practices for BSL 3 laboratories include the following:

- All solid and semi-solid waste generated in the BSL 3 labs and the BSL 2 anteroom is considered biohazardous and is collected into doubled autoclavable red bags held in rigid, leak-proof, puncture-resistant containers with lids.
- When bags are filled, they are gathered at the neck and closed by wrapping with at least a double circumference of autoclave tape, or with a rubber band

(as long as there is autoclave tape elsewhere on the bag to serve as a secondary indicator of successful sterilization).

- Red bags shall be placed in the autoclave in the anteroom and removed from the autoclave in the mechanical room. Autoclaving conditions shall be appropriate to the nature of the autoclave load but shall not be less than thirty (30) minutes at 121° C. Each load shall include a centrally placed spore strip or other autoclave validation device. Each autoclave so used shall be tested no less than monthly by *Geobacillus stearothermophilus* spore vial test and results posted on an annual summary table near the autoclave.
 - Any failure of an autoclave validation test shall be grounds for immediate cessation of autoclave use and quarantine of any waste being processed at the time of the failure. The Biosafety Officer and ES&H Team industrial hygienist shall be notified immediately. An action plan for retesting, repairing if necessary, and releasing quarantined waste shall be developed and implemented without delay.
- All red bags generated in Building 368 (B368) shall be transported after autoclaving to the central autoclave facility in Building 361 (B361) for a second autoclaving or transferred to a contractor licensed by the Department of Transportation and the State of California to transport and dispose of biohazardous and medical waste. On-site transport of red bags to the B361 autoclave shall be within a rigid, leak-proof, puncture-resistant container or cart with a lid.
- Liquid or semi-liquid laboratory waste shall be chemically disinfected by at least 30 minutes exposure to a final concentration of no less than 10% household bleach (0.5% sodium hypochlorite), or other disinfectant approved by the Biosafety Officer. Following disinfection, liquid and semi-liquid laboratory waste can be disposed to the sanitary sewer following secondary disinfection in Waste Retention Tanks.

3.1.5 Cell Cultures of Human or Animal Origin

Because all human or animal cell lines may harbor adventitious agents or other microbial contaminants, all such cultures are assumed to be in RG 2 and thus should be handled under BSL 2 containment. In rare instances, the BSO or the Institutional Biosafety Committee (IBC) may, after deliberation, downgrade an animal cell line to RG 1. However, it is strongly recommended the basic BSL 2 practices continue to be applied as a means to control outside contamination of the cultures.

In addition, cell cultures of human origin must be handled under BSL 2 containment and treated as though infectious.

3.1.6 Accidents

In any laboratory operation, the possibility of a spill or other accident exists. A thorough understanding of the potential hazards of the experiment, as well as careful planning, helps minimize personal injury (e.g., needle sticks) and property damage from spills. Carefully following the controls specified in this document and applicable IWSs and safety plans reduces the probability of an accident and mitigates the consequences if an accident occurs.

Needle Sticks. Needle sticks shall be reported as a reportable injury to both the workplace supervisor and the Health Services Department. For more information about injury reporting, see Document 4.5, “Incidents – Notification, Analysis, and Reporting,” in the *ES&H Manual*.

In all laboratories that use biohazardous or potentially biohazardous materials (BSL 2 and BSL 3), the Responsible Individual (RI) shall immediately report any and all real or suspected exposures to the ES&H Team industrial hygienist, the institutional Biosafety Officer and the RI’s immediate supervisor. Actual or suspected exposures to any Select Agent material shall also be reported to the Select Agents Responsible Official (RO). Reporting is critical to both the treatment and follow-up of the exposed individuals and the ultimate confirmation of the etiologic agent by immunologic testing.

Spills. In the event of a large-scale spill, call 911. For more information about spills, see Document 22.1.

In BSL 3 laboratories and BSL 2 laboratories working with Select Agents or RG 3 materials, special spill management practices shall be employed that recognize the elevated risks associated with these agents. In addition to the standard spill control procedures defined in Document 22.1, the following shall be implemented:

- Addition of disinfectant to a biohazardous spill shall be done carefully and only after the spill has been absorbed to avoid splashing or aerosol generation.
- Biohazardous spills shall be disinfected by exposing the spill to at least 10% household bleach (0.5% sodium hypochlorite), or other disinfectant approved by the Biosafety Officer, for no less than 15 minutes.
- Lab coat, gloves, shoe covers and a full-face respirator or PAPR (power, air-purifying respirator) hood equipped with HEPA filters shall be worn during spill management procedures.
- Contaminated sharps included in the spill scenario shall be removed by remote handling and transferred to a biohazard sharps waste container prior to absorption and disinfection of the spill.
- Hands shall be washed thoroughly after any biohazardous spill cleanup.

3.1.7 Procurement

Procurement of any etiologic agent or material specified in Appendices C, E, or F shall be approved by the LLNL Biosafety Officer, who can be contacted through the local ES&H Teams. For more information, see Document 21.1, "Acquisition, Receipt, Transportation, and Tracking of Hazardous Material," in the *ES&H Manual*.

3.1.8 Transfer of Select Agents

Because Select Agents have the potential for causing mass destruction or widespread disease in humans, the CDC has determined that the transfer of these agents from one site to another poses a risk of disease transmission, and is therefore subject to 42 CFR 73.

The CDC requires that, prior to transferring a Select Agent, both the shipping and receiving parties complete the required sections of CDC Form EA-101, "Transfer of Select Agents." The form is available from the Select Agent Responsible Official (RO). Contact the Biology and Biotechnology Research Program (BBRP) office or the ES&H Team 2 industrial hygienist for contact information. The form shall accompany the request or purchase order for obtaining these restricted agents. Copies shall be maintained by both the requesting and transferring facility and forwarded by the RO to a designated central repository at the CDC, for federal and authorized local law enforcement authorities' availability.

Form EA-101 shall be completed and approved by the RO before any Select Agent is transferred from one LLNL location to another, or shipped from LLNL to any other location for any reason. When applicable, this requirement also applies to transfers to and from LLNL-related facilities (e.g., Site 300 or the Production Genome Facility).

3.1.9 Transportation

Biological material shall be transported in a manner that does not pose a threat of injury to personnel or damage to property. Transportation of such materials requires the use of both proper packaging and shipping.

Onsite transportation of biological materials is performed using labeled, leak proof secondary containers. The labeling requirements to be followed are the same as those for the transportation of biological waste. Hazardous materials, substances, and waste (including biological materials but excluding analytical samples) may not be transported in bicycle baskets, laboratory coats, automobiles, or personal vehicles. For more information, see Document 21.2, "Onsite Hazardous Material Packaging and Transportation Safety Manual," in the *ES&H Manual*.

Offsite transportation of articles or substance that are likely to pose a significant risk to health, safety, property, or the environment shall comply with the applicable

regulations of the Department of Transportation, International Air Transport Association, USDA, and CDC. Offsite shipping of hazardous materials (i.e., chemical, biological, and radiological) shall comply with Document 21.1. For specific packaging and shipping procedures, contact either the LLNL Transportation Office or the Materials Management Department.

3.1.10 Medical Surveillance

The types of medical surveillance applicable to employees involved with biological materials may include:

- Pre-employment and periodic screening.
- Vaccination.
- Serum banking.
- Post-exposure medical surveillance.

Staff who work with pathogens may be required to participate in the medical surveillance program. Staff who work with human blood or other human bodily fluids shall participate in the medical surveillance program. Details about participation in the medical surveillance program are determined in the work review process. For additional information, contact the Health Services Department, or see Document 10.1, "Occupational Medical Program," in the *ES&H Manual*.

3.1.11 Training

Employees whose primary job is to handle biological materials should be trained at the time of initial assignment. Individuals who work with bloodborne pathogens, human blood, human tissues, other body fluids, or cell cultures of human origin shall take course HS4400, "Working Safely with Blood and Bloodborne Pathogens"; this training shall be successfully completed within ten days of beginning any work that has the potential for bloodborne pathogens exposure. The annual refresher, HS4400 RW, shall be taken annually thereafter. Investigators and key staff who work with human source material shall also complete HS0035 W, "Human Subject Research Training." Two classes (BR1001 and BR1003) are available through the Biology and Biotechnology Program (BBRP) to familiarize laboratory staff with the basic principles of working with biohazards. Work supervisors should recommend or provide additional training for specific job duties as appropriate. Employees who are new to the field of biological research or who provide direct ES&H support to programs that use biological materials are also recommended to take HS4430, "Biosurety for the Safety Professional" (or the directorate-specified equivalent).

3.1.12 Access Control

The control of access to BSL 3 laboratories and BSL 2 laboratories working with Select Agents or RG 3 materials is important, because the agents handled in these high-level containment laboratories tend to be highly virulent, easily transmitted and have non-existent or poor preventive and therapeutic interventions. As a result, they are attractive to bioterrorists.

Access requirements are unique for each laboratory and may involve any or all of the following:

- Authorization for entry by the relevant Directorate/Department.
- Completion of required training.
- RAP access programming.
- TESA lock access programming.
- Approved access to keys for agent storage freezers.
- Formal inclusion in Select Agent & Toxin Program and FBI clearance.

The specific access requirements and authorization level shall be determined on a case-by-case basis for each laboratory. Access requirements are defined in the LLNL Select Agents and Toxins Security Plan and the specific Security Plans for each facility handling Select Agents.

3.2 Engineered Controls

Engineered controls are used to isolate or remove hazards from the workplace in order to reduce the potential for exposure. Engineered controls, in combination with safe work practices that alter the manner in which tasks are performed, are expected to be the primary means of eliminating or minimizing the risk of occupational exposure. Engineered controls for biological research materials include, but are not limited to, the following:

- **Mechanical aids**

Mouth pipetting is prohibited, and the use of mechanical aids to transfer potentially harmful (i.e., biohazardous) materials is strictly enforced. Personnel shall use such devices for all pipetting.

- **Work Areas**

All work areas shall be decontaminated before and after each use, to reduce the likelihood of personnel exposure and cross-contamination.

- **Dead air boxes**

Dead air boxes are commonly used to reduce the potential of contamination while diluting or transferring stock concentrations of biohazardous materials. Work areas shall be decontaminated before and after each use to reduce the likelihood of cross-contamination.

- **Fume hoods**

Fume hoods are commonly used in the laboratory to draw air away from the work area. Fume hoods shall not be used for long-term storage of materials and equipment. For more information, see Document 12.4, "Work Enclosures and Local Exhaust Systems for Toxic and Radioactive Materials," in the *ES&H Manual*.

- **Negative-air-flow units**

All animals injected with infectious materials or receiving mutagens or carcinogens added to their food or water are to be housed in negative-air-flow animal units. Cages are to be labeled as to the type of hazard present, amount and route of exposure, and the date of administration. Signs placed on each unit shall indicate whether the animals are currently being exposed, were exposed in the past, or have not been exposed.

- **Biological safety cabinets (BSCs)**

BSCs provide sterile laminar airflow onto the work surface, containment of aerosols or droplets, protection of materials, and protection of the user. There are three different classifications of BSCs: Classes 1 through 3. The performance of BSCs shall be verified at the time of installation, whenever they are moved, and at least annually thereafter. BSCs used in conjunction with RG 3 agents or Select Agents shall be recertified at least every six months. For more information regarding BSCs, see Appendix I of this document and Document 13.1.

- **Containment protection for vacuum systems**

The aspiration of either tissue culture media from monolayer cultures or supernatants from centrifuge samples into primary collection flasks is a common laboratory procedure. Protection shall be provided against drawing aerosols of hazardous chemical or biological materials or overflow fluid into the house vacuum system. Protection is provided by the use of an air filter in the line immediately leading into the house vacuum line and an overflow flask for liquids between the collection flask and the air filter. For an example of this assembly, see Appendix J.

- **Sharps containers**

Sharps containers are hard, puncture-proof containers designed to prevent contact with needles and other sharp objects placed inside. Sharps containers

must be labeled with the words SHARPS CONTAINER and should be disposed of after becoming 3/4 full. See Document 36.1 for more information regarding waste disposal protocol.

- **Intrinsically safe needles**

Intrinsically safe hypodermic needles and syringes are designed to sheath the needle after use, help prevent needle sticks, and are to be used for all hypodermic needle applications, except as approved through the IWS process. All hypodermic needles and syringes must be disposed of in a container properly labeled with the words SHARPS CONTAINER.

3.3 Personal Protective Equipment

Identifying and understanding a workplace hazard and then matching the needed PPE to the hazard is the key to selecting effective and appropriate PPE. In most cases, the Hazard Assessment and Control (HAC) form is sufficient documentation for work involving the PPE covered in this document. Examples of PPE used when working with biological materials include the following:

- **Respiratory protection**

Respiratory protection is required in some cases. When a BSC is used, however, respiratory protection is considered unnecessary and is not recommended, unless specified by an ES&H Team industrial hygienist.

- **Laboratory coats**

Laboratory coats minimize skin exposure and protect street clothes from being contaminated. Laboratory coats shall be worn while working in a laboratory but are not to be worn outside of laboratory areas.

- **Gloves**

The use of gloves is required when splashing is anticipated. Glove selection is important when dealing with a variety of chemicals and biological materials.

- **Face protection**

Safety glasses, safety goggles, or a face shield, as appropriate, shall be worn while working with biological research materials in a laboratory. A face shield is required when splashing is anticipated.

For more information regarding the selection and use of PPE, see Document 11.1, "Personal Protective Equipment," in the *ES&H Manual*, or contact the area industrial hygienist.

4.0 Responsibilities

General responsibilities for all workers are described in Document 2.1. Specific responsibilities for key personnel are listed under each title in the following sections.

Each person is expected to make every reasonable effort to protect himself or herself and others from injury or illness. Facility occupants are expected to guide and govern visitors and to assist new or temporary occupants to understand and follow the applicable procedures. Personnel with any doubts regarding the safety of any phase of work shall check with the Responsible Individual.

For responsibilities of the IRB and IACUC, see Documents 13.1, 13.4, and 13.5.

4.1 Workers

Workers shall:

- Participate as appropriate in the LLNL vaccination and medical surveillance programs.
- Complete all required training in a timely manner.
- Perform work safely.
- Report any needle sticks or occupational exposures immediately to the Health Services Department and to the ES&H Team.

4.2 Facility Points of Contact

Facility Points of Contact for facilities where biological research materials are used or stored shall:

- Know where biological materials are used, produced, stored, or handled in any way in the facility.
- Be familiar with this document and its contents and objectives.

4.3 Responsible Individuals

Responsible Individuals shall:

- Develop IWSs and Safety Plans, as needed.
- Obtain approval from appropriate institutional committees (i.e., IBC, IRB, and IACUC). For more information, contact the chair of the respective committee or the area ES&H Team.

- Implement the controls in this document as applicable to their operations.
- Ensure that workers have applicable information and training regarding health, hazards, and communication before beginning specific tasks involving biological materials.
- Plan activities involving the safe use of biological materials.
- Perform ES&H evaluations in coordination with the ES&H Teams.
- Provide the required PPE to workers who work with biological materials.

4.4 Authorizing Individuals

Authorizing individuals:

- Are appointed by the authorizing organization to fulfill its responsibilities.
- Approve procedures or delegate approval authority.
- Authorize work once all controls have been confirmed to be implemented.
- Shall, before authorizing proposed work with biohazardous research materials, ensure that the ISM process has been followed to incorporate all necessary reviews and approvals.
- Determine which employees are required to participate in the Immunization Program of the Health Services Department.

4.5 Payroll Supervisors

Payroll supervisors shall work with the ES&H Team clinician and Industrial Hygienist to determine those individuals who shall participate in the medical surveillance program.

4.6 LLNL Biosafety Operations Committee

LBOC shall:

- Determine whether a proposed activity requires peer review, institutional review (i.e., through the IBC, IRB, or IACUC), ES&H review, and National Environmental Policy Act (NEPA) review, and inform both the Responsible Individual and the reviewing organizations of the need for review.
- Review the biological research materials, and their hazards, involved in the proposed activity.

- Make initial recommendations for training, medical surveillance, environmental protection, waste disposal, and containment levels for the proposed activity.
- Identify ethical issues associated with the proposed activity.
- Review and recommends proposed changes to the *ES&H Manual* involving biological operations.
- Address biosafety issues as requested by the Council of Biosciences and Biotechnology (CBB).
- Review any new or modified biological research operations and procedures that may impact research activities. Such activities include the intended use of any new equipment or procedures for the handling or processing of biological materials into or from LLNL facilities, or the introduction of new biological materials into LLNL.
- Document the discussions, agreements, and commitments of each meeting in the form of minutes that are retained by the LBOC Chair and distributed to all LBOC members, the CBB Chair, and others as deemed appropriate.

4.7 LLNL Institutional Biosafety Committee

The IBC:

- Shall review and approve all LLNL research activities involving:
 - Recombinant DNA.
 - Artificial gene transfer.
 - Infectious and non-infectious agents (e.g., bacteria, viruses, protozoans, fungi).
 - Human and animal blood, body fluids, and tissues.
 - Cell cultures of human and animal origin.
 - Toxins (natural and synthetic).

Through review, the IBC ensures that such activities and related facilities are in compliance with applicable LLNL policies and external regulations.

- Shall function to fulfill LLNL's obligations under current governmental requirements, including those dealing with recombinant DNA activities, human gene therapy techniques, biohazardous agents, and Select Agent transfer. To this end, the IBC shall assist Responsible Individuals in meeting their responsibilities.
- May at any time suspend an activity involving biohazardous agents or recombinant DNA that is not in compliance with LLNL policies or external

regulations. Upon doing so, the IBC immediately notifies the affected Authorizing Individuals, Responsible Individuals, appropriate LLNL officers, and others as required by LLNL policies and external regulations.

- Shall approve this document as part of the institutional biosafety manual.
- Shall perform the additional duties defined in Section IV B-2 of the *NIH Guidelines*, which are available at the following Internet address:

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

4.8 Health Services Department

The Health Services Department shall:

- Provide a medical surveillance program for employees who work with or who are at risk for occupational exposure to biohazardous materials.
- Provide onsite medical assessment and treatment of significant exposures and (in collaboration with the Hazards Control Department) worksite assessment to reduce the hazard of subsequent exposures.
- Maintain the medical records of employees who work with biological materials, including a record of immunization, surveillance, and post-exposure assessment and treatment.
- Provide advice, medical safety consultation, and prophylactic measures as needed.
- Provide a required member of the IBC.

4.9 Hazards Control Department

The Hazards Control Department shall:

- Assist in the identification of hazards associated with biological materials.
- Assist employees in working safely with biological materials.
- Concur with IWSs for work involving biological research materials.
- Review HAC forms (or equivalent) for operations involving biological materials.
- Determine the need for and frequency of workplace monitoring and inform supervisors, workers, and the Health Services Department of the results.

- Work collaboratively with the Health Services Department in the post-exposure and follow-up programs.
- Provide required training through Hazards Control Department's Safety Education & Training Section (SETS) classes.
- Provide specific training upon request.
- Designate LLNL's biological safety officer as the subject-matter expert on biosafety.
- Works collaboratively with HSD and payroll supervisor to identify individuals requiring medical surveillance.

4.10 Biosafety Officer

The Biosafety Officer's duties include, but are not be limited to, the following:

- Performing periodic inspections to ensure that laboratory standards are rigorously followed.
- Reporting to LLNL and the IBC any significant problems, violations of the *NIH Guidelines*, and any significant research-related accidents or illnesses of which he or she becomes aware, unless he or she determines that a report has already been filed by the Responsible Individual.
- Developing emergency plans for handling accidental spills and personnel contamination and for investigating laboratory accidents.
- Providing advice on general biological laboratory security.
- Providing technical advice to Responsible Individuals and the IBC regarding research safety procedures.
- Represent LLNL in institutional biosafety issues involving external assessments

4.11 Responsible Official

The Responsible Official (RO) and Alternate Responsible Officials (AROs) are appointed by the LLNL Deputy Director for Operations to oversee all Select Agent activities at LLNL.

The RO shall:

- Register all Select Agent activities at LLNL with CDC/APHIS.

- Make all required reports to CDC/APHIS regarding shipments or abnormal events.
- Assist RIs in documenting their activities and Select Agent records.
- Oversee preparation of safety, security and emergency response plans for Select Agent activities.

In the absence of the RO, one of the AROs shall perform these duties.

4.12 Safeguards and Security Department

To meet the regulatory requirements of the Select Agent Program, a *Biological Select Agents and Toxins Security Plan* is available from the Safeguards and Security Department or the Select Agents RO.

4.13 Environmental Protection Department

This section defines the responsibilities of the Environmental Protection Department. Contact the area environmental analyst for information regarding medical waste management, including waste characterization, storage procedures, and preparation for waste treatment or disposal. The area Hazardous Waste Management (HWM) Division field technician can assist in ensuring receipt of proper biohazardous and sharps containers, labels, and waste disposal requisition forms that need to accompany all sharps wastes and biohazardous wastes with a hazardous or radioactive component.

4.13.1 Environmental Analysts

Environmental analysts shall:

- Review the HAC forms (or equivalent) for operations involving the use of biological materials.
- Provide guidance to biohazard handlers on how to implement environmental controls and procedures and on the proper management of hazardous waste contaminated with biohazards to ensure compliance with all applicable federal, state, and local environmental requirements.
- Provide specific training to directorate organizations, as required.

4.13.2 Hazardous Waste Management Technicians

HWM technicians shall:

- Provide specific guidance to biohazard handlers on how to properly segregate, package, and label solid and liquid wastes that are contaminated with biohazardous materials.
- Coordinate the disposal of waste generated in the area.

5.0 Work Standards

5.1 Work Smart Standards

7 CFR, Chapter III, Part 330, "Federal Plant Pest Regulations; General; Plant Pests; Soil; Stone, and Quarry Products; Garbage."

http://www.access.gpo.gov/nara/cfr/waisidx_00/7cfr330_00.html

9 CFR, Chapter I, Part 104, "Permits for Biological Products."

http://www.access.gpo.gov/nara/cfr/waisidx_01/9cfr104_01.html

9 CFR 104, Subchapter E, "Viruses, Serum, Toxins, and Analogous Products, Organisms, and Vectors."

<http://www.washingtonwatchdog.org/documents/cfr/title9/>

9 CFR 121, "Possession, Use, and Transfer of Select Agents (for Animals)."

http://www.access.gpo.gov/nara/cfr/waisidx_03/9cfrv1_03.html

29 CFR 1910.1030, "Bloodborne Pathogens," included in 29 CFR 1910, Subpart Z, Toxic & Hazardous Substances (1910.1000 to 1910.1450 App B).

http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARD&p_id=10051

42 CFR 73, "Possession, Use, and Transfer of Select Agents (for Humans)."

<http://www.cdc.gov/od/sap/docs/42cfr73.pdf>

National Institutes of Health, *NIH Guidelines for Research Involving Recombinant DNA Molecules* (66 FR 1146). Revised January 2001. Available at the following Internet address:

<http://www4.od.nih.gov/oba/rac/frnotices/1-5-01act.htm>

Public Law, 104-32, "The Anti-Terrorism and Effective Death Penalty Act (AEDPA)," 1996.

http://www.llnl.gov/es_and_h/sourcematerial/pl104_32.pdf

Public Law, 107-56, "The Patriot Act of 2001."

<http://www.usdoj.gov/oig/special/03-07/final.pdf>

Public Law 107-188, "Public Health and Security and Bioterrorism Preparedness and Response."

<http://tis.eh.doe.gov/biosafety/library/PL107-188.pdf>

U.S. Dept. of HHS, Public Health Service, *Biosafety in Microbiological and Biomedical Laboratories*, 4th Edition 1999, HHS Publication No. (CDC) 93-8395.

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

5.2 Other Requirements

7 CFR 331 "Possession, Use, and Transfer of Select Agents (for Plants).

<http://www.cdc.gov/od/sap/docs/btarule.pdf>

42 CFR 72, "Interstate Shipment of Etiological Agents."

http://www.access.gpo.gov/nara/cfr/waisidx_01/42cfr72_01.html

National Institutes of Public Health, *NIH Guidelines for Research Involving Recombinant DNA Molecules*. Revised April 2002. Available at the following Internet address:

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

6.0 Resources for More Information

6.1 Contacts

See the ES&H Contact List.

6.2 Lessons Learned

For lessons learned applicable to biological materials, refer to the following Internet address:

http://www-r.llnl.gov/es_and_h/lessons/lessons.shtml

6.3 Other Sources

Hunt, D.L., 1995, "Human Immunodeficiency Virus Type 1 and Other Bloodborne Pathogens," pages 33–66. In Fleming, Richardson, Tulis, Vesley (eds.), *Laboratory Safety: Principles and Practices*, 2nd Edition, American Society of Microbiology.

International Air Transport Association, Dangerous Goods Program Website:

<http://www.iata.org/dangerousgoods/index>

Jahrling, Peter, 1985, "Marburg Virus, Ebola Virus, and the Arenaviruses," pages 796–804. In Lennette, Balows, Hausler, and Shadomy (eds), *Manual of Clinical Microbiology*. 4th Edition, American Society of Microbiology.

National Institutes of Health, NIH Laboratory Safety Monograph, "A Supplement to the NIH Guidelines for Recombinant DNA Research," July 1978.

Swenson, P.D., 1991, "Hepatitis Viruses," pages 959–983. In Balows, Hausler, Herman, Isenberg and Shadomy (eds.), *Manual of Clinical Microbiology*. 5th Edition, American Society of Microbiology.

U.S. Department of Health, Education and Welfare, National Cancer Institute, *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses*, 1974 (DHEW, Government Printing Office, Pub. No. NIH 75-790).

Appendix A

Acronyms, Terms, and Definitions

Adventitious agent	Microbial agents present in an organism, tissue, culture or cell in which they are not naturally found, are typically unexpected, and are often latent rather than overt.
Biohazardous agent	A substance that is biological in nature and usually capable of self-replication and has the capacity to produce deleterious effects on other biological organisms, particularly humans. Biohazardous agents include, but are not limited to, various viruses, prions, chlamydia, bacteria, fungi, yeast, and algae, as well as plants and animals and their products that contain any of these agents.
Biohazardous material	A biologically derived material, such as a toxin or the component of a biological agent, that presents a risk of causing illness or injury to humans, plants, and animals and that is not capable of self-replication
Biohazard	Any biological material, or a component thereof, that presents a risk of illness or injury to humans, plants, and animals.
Biological safety	A complete program of administrative controls, medical surveillance, vaccination, and containment strategies for reducing the risk of disease to employees facing potential occupational exposure to infectious agents or other biologically derived molecules. Biosafety protects workers, products, coworkers, laboratory support workers, the environment, and the general public. Used interchangeably with "biosafety."
Biological safety cabinet (BSC)	A ventilated cabinet that serves as the primary containment device for operations involving biohazardous materials. There are three classes of biological safety cabinets (i.e., Classes 1 through 3).
Biosafety	See "Biological safety."

Biosafety (containment) level	The level of containment required to perform biohazardous operations safely. Work practices and techniques, safety equipment, and laboratory facilities appropriate for the operations are based on the hazards imposed by the agents used and for laboratory function and activities.
Biosurety	Defined by the Department of Energy (DOE) as an integrated approach to the management and oversight of potentially hazardous biological materials and activities in the fields of recombinant DNA, genetic research, and environmental remediation. Ensures safety, emergency management, security, community relations, and environmental protection.
Blood (human)	Human blood, human blood components, and products made from human blood.
Bloodborne pathogen	A pathogenic microorganism that is present in human blood and can cause disease in humans. Such pathogens include, but are not limited to, HBV and HIV.
BMBL	CDCs Biosafety in Microbiological and Biomedical Laboratories
BSC	See "Biological safety cabinet."
BSL	Biosafety level.
CBB	LLNL Council of Biosciences and Biotechnology.
CDC	Centers for Disease Control and Prevention. An agency of the Department of Health and Human Services.
Containment	A safe method for managing infectious agents in a laboratory environment where they are handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the environment to potentially hazardous agents.
Contaminated	A term indicating the presence or the reasonably anticipated presence of blood or other potentially infectious materials on an item or surface.

Decontamination	The use of physical or chemical means to remove, inactivate, or destroy pathogens on a surface or item to the point where they are no longer capable of transmitting infectious particles and the surface or item is rendered safe for handling, use, or disposal.
Disinfection	The use of antimicrobial agents on inanimate objects to destroy all organisms that could pose a potential hazard to humans or animals or compromise the integrity of an experiment.
DNA	Deoxyribonucleic acid. DNA, the nucleic acid in which the sugar is a deoxyribose, constitutes the primary genetic material of all cellular organisms and DNA viruses. DNA can be single or double stranded.
Engineered control	An engineered device or control that isolates or removes hazards from the workplace.
Etiologic agent	A disease-causing agent.
First-degree relative	A person separated from an individual by only one step in a direct hereditary line of descent or ascent, i.e., a parent, sibling, or child.
Fume hood	A ventilated work enclosure with one open side through which air flows. The height or width of the opening can be adjusted by a sash and/or door(s). Air velocity is highest at the front opening (i.e., face plane). Airflow is distributed inside the hood by means of a plenum at the back with slot-type openings. Unlike a spray booth, where air velocity is essentially constant from front to back, the velocity of air inside the hood decreases rapidly after it has passed through the face plane.
Genomic materials	Material (i.e., DNA or RNA) obtained from the genome of an organism.
HAC	Hazard Assessment and Control (form)
Hand washing facility	A facility providing an adequate supply of running potable water, soap, and single-use towels or hot air drying machines.

Hazardous material	A material that is explosive, flammable, poisonous, an irritant, or otherwise harmful and likely to cause injury or illness.
HBV	Hepatitis B virus.
HEPA	See "High-efficiency particulate air (HEPA) filter."
High-efficiency particulate air (HEPA) filter	A filter capable of trapping and retaining at least 99.97% of all monodispersed particles 0.3 microns in diameter.
HIV	Human immunodeficiency virus.
IACUC	Institutional Animal Care and Use Committee.
Institutional Biosafety Committee (IBC)	A committee for biosafety research that meets the requirements specified in Section IV B-2, "Institutional Biosafety Committee (IBC)," of the <i>NIH Guidelines</i> and that reviews, approves, and oversees projects in accordance with the responsibilities defined in Section IV B-2.
IRB	Institutional Review Board.
Laboratory-scale work	Work with substances in which the containers used for reactions, transfers, and other handling of substances is designed to be easily and safely manipulated by one person. "Laboratory scale" excludes those workplaces whose function is to produce commercial quantities of materials.
LBOC	LLNL Biosafety Operations Committee. An advisory committee that provides guidance to investigators regarding assessments and approvals required for a protocol to be conducted at or under the auspices of LLNL.
Negative-air-flow unit	A unit that draws air into a facility to replace the air removed by exhaust systems or other facility conditions.
NIH	National Institutes of Health (an agency of the Public Health Service).
Oncogenic virus	A virus that causes cancer.
Pathogen	Any agent (usually living) capable of producing disease.

Personal protective equipment (PPE)	Specialized clothing or equipment worn by an employee for protection against a hazard. General work clothes (e.g., uniforms, pants, shirts, or blouses) not intended to function as protection against a hazard are not considered to be PPE.
PPE	See “Personal protective equipment (PPE).”
Prion	Small proteinaceous infectious particles.
Production facility	A facility engaged in industrial-scale, large-volume, or high-concentration production of microorganisms.
Recombinant DNA molecules	Molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell. This definition includes molecules that result from the replication of such molecules.
Regulated biohazardous waste	Liquid or semi-liquid blood or other potentially infectious materials; contaminated items that would release blood or other potentially infectious materials in a liquid or semi-liquid state if compressed; or items that are caked with dried blood or other potentially infectious materials and are capable of releasing these materials during handling (e.g., contaminated sharps, pathological waste, or microbiological wastes containing blood or other potentially infectious materials).
Research laboratory	A laboratory producing or using research laboratory-scale amounts (i.e., amounts greater than 10 liters) of biological materials or chemicals.
Responsible Individual	The individual directly responsible for an operation, activity, or group of activities. The Responsible Individual may be at any level within the organization and is formally identified by the activity’s authorizing individual. In some organizations, this person is called the work supervisor. In most cases the Responsible Individual will be directing the work of others as part of the operation or activity. See Documents 2.1 and 2.2 for more information.
RG	See “Risk group.”

Risk group	A system (developed by the CDC and NIH) for classifying biological agents by the degree of hazard. There are four risk groups: A higher RG number indicates a higher level of hazard.
RNA	Ribonucleic acid. RNA, the nucleic acid in which the sugar is ribose, constitutes the genetic material in the RNA viruses and in all biology is the intermediary between genetic material and the synthesis of protein.
Select Agent	A microorganism (e.g., virus, bacterium, fungus, or rickettsia) or toxin listed in 42 CFR 73, (“Possession, Use, and Transfer of Select Agents and Toxins”) or in 7 CFR 331 and 9 CFR 121 (“Agricultural Bioterrorism Protection Act of 2002;” “Possession, Use and Transfer of Biological Agents and Toxins”) and not subject to the current rules of exemption. A complete list of Select Agents and Toxins may be found in Appendix D.
Select Agents Responsible Official	An individual, registered with the CDC and/or the USDA Select Agents Program, who accepts responsibility, on behalf of the institution, for the management and safe and secure conduct of the institution’s Select Agents Program.
Sharps	Any object that can penetrate the skin, including, but not limited to, needles, scalpels, broken glass, broken capillary tubes, and exposed metal edges (e.g., dental wires).
SOP	Standard operating procedure.
Sterilize	To use a physical or chemical procedure to destroy all microbial life, including highly resistant bacterial endospores.
Universal precautions	An approach to infection control under which all human blood and certain human bodily fluids are treated as if known to be infectious for HIV, HBV, and other <i>bloodborne</i> pathogens (see Appendix G).
Work practice control	A control that reduces the likelihood of exposure by altering the manner in which a task is performed.
Zoonotic	Readily communicable from animals to humans.

Appendix B

Biosafety Levels

This document adopts the classification system of *BMBL* (see Section 5.1) for BSLs. This classification system assigns BSL 1 through 4. The higher the BSL number, the higher the containment level required. Although BSL 4 containment levels are discussed below, work at LLNL is restricted to BSLs 1, 2, and 3. The requirements for BSLs 1, 2 and 3 are summarized in Table B-1.

Biosafety Level 1

BSL 1 requires the use of standard laboratory practices and equipment found in most research and teaching institutions. Such a laboratory provides areas of open bench space, with no special containment equipment, and is generally not well separated from the general traffic of the building. This type of laboratory is suitable for experiments involving RG 1 agents of no known hazard, or minimal potential hazard, to laboratory personnel or the environment.

Biosafety Level 2

BSL 2 requires the use of standard laboratory practices and equipment designed for experiments with agents of moderate hazard to personnel and the environment. BSL 2 differs from BSL 1 mainly in providing areas of containment (e.g., a BSC) to carry out certain laboratory experiments and operations in which aerosols could be created. Examples of experiments requiring BSL 2 containment include:

- Recombinant DNA work involving nonexempt microorganisms (as defined in the *NIH Guidelines*), such as *Salmonella* and *Pseudomonas fluorescens*.
- Work with many bacterial species, including all species and serotypes of *Salmonella*.
- Work with many viral agents, such as measles viruses, rhinoviruses, mumps, and polioviruses.
- Work with low concentrations or small volumes of low- or moderate-risk oncogenic viruses, such as the Epstein-Barr virus or feline leukemia virus.
- Work with tissues or bodily fluids from human or primates.
- Work with viable human tumor cells or tumor cell lines.

Table B-1. Biological research laboratory containment levels of the Centers for Disease Control and National Institutes of Health.

		BSL 1	BSL 2	BSL 3
A	Hazard Levels	Basic/Low	Moderate Risk	Moderate to High Risk
	(Examples)	Undergraduate secondary training	Clinical or diagnostic laboratory	Clinical, diagnostic, production, teaching, or research settings
B	Standard Microbiological Practices			
1	Public access while experiments are in progress	Limited	Limited/ restricted	Limited/ restricted or Controlled
2	Decontamination	Daily	Daily and after spills	Daily and after spills
3	Regulated waste decontamination	Before disposal	Before disposal	Before disposal
4	Pipetting (No mouth pipetting)	Mechanical devices	Mechanical devices	Mechanical devices
5	Eating, drinking, smoking, applying cosmetics, and handling contact lenses	Not permitted	Not permitted	Not permitted
6	Aerosol minimization	Recommended	Recommended	Recommended
7	Food Storage	Outside of work area	Outside of work area	Outside of work area
8	Hand washing	Frequently and before leaving the laboratory	Frequently and before leaving the laboratory	Frequently and before leaving the laboratory
9	Aerosol production	Prohibited in open lab bench	BSC, centrifuge safety cups	BSC, centrifuge safety cups
10	Sharps protocol	Required	Required	Required
11	Lab coats not worn outside the laboratory	Recommended	Required (front-buttoned coats)	Required (wrap-around coats)
12	Face protection	Required	Required	Required
C	Special Practices			
1	Onsite autoclave	Not required in laboratory	Not required, but available onsite	Required onsite
2	Rodent control program	Required	Required	Required
3	Transport of infectious materials for decontamination purposes	In labeled, durable, leak proof container with closeable lid	In labeled, durable, leak proof container with closeable lid	In labeled, durable, leak proof container with closeable lid
4	Animals in work area but not being used in lab experiments	Not permitted	Not permitted	Not permitted

Table B-1. Biological research laboratory containment levels of the Centers for Disease Control and National Institutes of Health. (cont'd)

		BSL 1	BSL 2	BSL 3
D	Safety Equipment			
1	Biological safety cabinet (BSC) or other containment systems	Recommended	Class II BSC required for use of aerosol-generating equipment or high concentrations of agents, for animal inoculation, or for harvesting of infected animal tissue or eggs	Class II BSC required for use of aerosol-generating equipment or high concentrations of agents, for animal inoculation, or for harvesting of infected animal tissue or eggs
2	BSC testing	When new, after moving, annually thereafter	When new, after moving, annually thereafter	When new, after moving, annually thereafter
3	Personal protective equipment			
	a. Face protection	Recommended	Required whenever splashes are anticipated	Required whenever splashes are anticipated
	b. Gloving	Recommended. Required for workers with broken skin	Required when handling infectious materials. Double gloving suggested	Required when handling infectious materials. Double gloving suggested
E	Laboratory facilities			
1	Ventilation	Not required	Negative pressure	Negative pressure with audible alarm system. No air recirculation to other parts of the building
2	Open windows	Permitted, but fly screens required	Permitted, but fly screens required	Prohibited. Closed and sealed windows required
3	Doors	Required	Lockable door required for certain agents	Double doors
4	Posted warning sign	Recommended with agent and RI identified	Required with agent and RI identified	Required with agent and RI identified
5	Lab separated from general public	No, but access control required	Yes. Access control also required	Yes. Access control also required

Table B-1. Biological research laboratory containment levels of the Centers for Disease Control and National Institutes of Health. (cont'd)

		BSL 1	BSL 2	BSL 3
6	Hand washing facility	Required	Required	Required
7	Shower facilities	Not required	Not required	Recommended
8	Chemical shower	Not required	Not required	Recommended
9	HEPA vacuum lines	Recommended	Recommended	Required
F	Technical training	Required	Required	Required
G	Medical surveillance	Recommended	Required when appropriate	Required when appropriate

Reference: U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories*, 4th edition, (U.S. Government Printing Office, Washington, DC, 1999, DHHS Pub. No. 93-8395).

Operations that involve large volumes or high concentrations of certain RG 2 agents, such as the hepatitis viruses or Epstein-Barr virus, should be carried out at BSL 3. See *BMBL* for guidance.

Biosafety Level 2 Safe Work Practices for Laboratory Staff

Safe work practices for laboratory staff include, but are not limited to, the following:

- Follow the universal precautions (see Appendix G) at all times.
- Hands shall be washed frequently, and shall always be washed before beginning operations, after glove removal, after any spill management procedure, and before leaving the laboratory.
- Protective eyewear and face shield shall be worn for procedures that commonly result in the generation of droplets or the splashing of blood or other bodily fluids.
- Laboratory coats shall be worn during all laboratory procedures. Additional protection (e.g., gowns and aprons) should be worn during procedures in which the splashing of blood or other bodily fluids can be reasonably anticipated.
- Gloves shall be worn during all procedures that involve the handling of items containing or contaminated with blood and in areas where items (e.g., benches) could be contaminated with potentially infectious materials.
- A glove that is torn shall be removed and replaced promptly.
- Gloves shall be changed and hands washed after completion of specimen processing.

- All specimens of blood and bodily fluids shall be put in a well-constructed container with a secure lid to prevent leaking during transport.
- Care shall be taken when collecting each specimen to avoid contaminating the outside of the container or any form (or other paperwork) accompanying the specimen.
- For routine procedures, such as histological and pathological studies or microbiological culturing, a BSC is highly recommended.
- BSCs (or equivalent protective ventilation controls) shall always be used for procedures that have a high potential for generating aerosols.
- Mechanical pipetting devices shall be used for manipulating all liquids in the laboratory. Mouth pipetting is prohibited.
- Needles and syringes should be used only when no alternatives exist. Employees should pay attention to their hands whenever handling needles and syringes.
- Laboratory work surfaces shall be decontaminated with an appropriate chemical germicide after a spill of blood or other bodily fluids and when work activities are completed.
- Equipment contaminated with blood or other potentially infectious materials *shall* (and equipment contaminated with any other biological material *should*) be cleaned with a mild bleach solution (i.e., 1:10 to 1:100 dilution of household bleach) or with an appropriate chemical germicide immediately after completion of laboratory procedures. Contaminated equipment should never be stored without the appropriate biohazard label. An acceptable alternative is autoclaving.

[The cleaning of chemically or radiologically contaminated equipment is covered in other *ES&H Manual* documents [e.g., those in Part 14 (Chemical) and Part 20 (Ionizing Radiation/Nonionizing Radiation).]

- All laboratory staff shall wash their hands after completing laboratory activities and shall remove protective clothing before leaving the laboratory.
- Any personal clothing that is contaminated with blood or other bodily fluids during collection procedures shall be removed immediately (or as soon as possible) to prevent further contamination and separated from other clothing until properly laundered.

In accordance with *BMBL* and the *NIH Guidelines for Research Involving Recombinant DNA Molecules*, certain work with RG 3 agents in BSL 2 containment facilities may be allowed after approval of the program, the IBC, and the ES&H Team has been obtained. Such work requires additional controls.

BSL 2 laboratories working with Select Agents or Risk Group 3 materials

Certain Select Agents and RG 3 agents may be handled in BSL 2 facilities when the agents are known or suspected to be present in very low quantity. Such a condition is often encountered in diagnostic samples and diluted materials.

Entry procedures for these laboratories shall include the following:

- All authorized entrants shall have received all relevant clearances and authorizations, and completed all required training.
- Immediately upon entry to the laboratory, don lab coat or gown (with at least center and neck ties fastened), sleeve covers (if used), eye protection and gloves.

Exit procedures for these laboratories shall include the following:

- Immediately prior to exiting laboratory, remove lab coat or gown, safety glasses, gloves and sleeve covers (if used).
- Perform hygienic hand wash before exiting the anteroom.

Work practices for these laboratories shall include all standard BSL 1 and BSL 2 work practices, plus the following:

- Lab coat or gown and eye protection shall be worn at all times when in the laboratory.
- Gloves shall be worn during all operations that involve the potential for direct contact with agents.
- Hands shall be washed frequently, and always before beginning operations, after glove removal, after any spill management procedure, and before leaving the laboratory.
- Operators shall be especially aware of any procedures that may generate an aerosol, so that all precautions may be taken to control potential exposure to the aerosol. Some examples of these precautions are (1) deliberate pointing of a potential jet orifice into the biosafety cabinet working volume; (2) use of double containment devices; and (3) implementation of additional shielding prior to start of procedure step.
- The use of needles and other sharps, or of glass containers or devices, shall be minimized and eliminated where possible. If hypodermic syringe needles, surgical scalpels or other sharps must be used, they shall incorporate engineered sharps injury protection (ESIP) features whenever possible.
- Containers that are routinely reused shall be inspected before each use for cracks, chips, and other stress marks and discarded without use when such indicators of potential breakage are noted.

- All centrifuge carriers (rotors, buckets, etc.) shall be inspected before each use for corrosion, cracked or broken O-rings, or any other evidence of potential breakage or leakage. Carriers shall be retired or repaired before use when these types of indicators are noted.
- If any evidence of centrifuge rotor, bucket or tube failure during operation (e.g., unusual noise, sudden imbalance) with a biohazardous or potentially biohazardous material is noted, the centrifuge shall remain sealed until the ES&H Team industrial hygienist and the Biosafety Officer have approved the proposed recovery operation.
- An appropriate disinfectant shall be on hand for spill management at all times.
- All biohazardous spills shall be reported to the Laboratory RO, the ES&H Team industrial hygienist and the Biosafety Officer immediately.
- All work with biohazardous materials should be done using the smallest volume and most dilute preparation possible.
- No infectious or toxic material shall be handled outside the biosafety cabinet work volume.
- Authorized maintenance and service personnel entering the laboratory must be escorted at all times and follow the entry and exit protocols in effect at the time of entry.

Biosafety Level 3 (BSL 3)

At LLNL, "BSL 3" refers to a containment level consisting of a combination of primary and secondary barriers and Standard and Special Practices as defined in the *BMBL*. This containment level is typically used to work with RG 3 agents which are known or suspected to be present in high titers or large volumes. Such conditions are often encountered in purified cultures and many typical research or manufacturing preparations. All BSL 3 laboratories at LLNL are located in B368.

Entry procedures for all of B368 shall include the following:

- All authorized entrants must have received all relevant clearances and authorizations and completed all required training.
- In the change room, don eye protection, shoe covers, hair and beard covers, gloves and standard lab coat or scrubs.
- Prior to BSL 3 lab entry, don the following in the anteroom (BSL 2):
 - closed-front disposable gown (with at least center and neck ties fastened).
 - sleeve covers (if used).

- second pair of gloves.
- If working only in BSL 2 anteroom, PPA/PPE donned in change room is adequate.

Exit procedures for all BSL 3 laboratories shall include the following:

- Immediately prior to exiting BSL 3 laboratory, remove and discard disposable gown, sleeve covers (if used), and second pair of gloves.
- Immediately prior to exiting the anteroom, remove and discard hair, beard and shoe covers and gloves, and perform hygienic hand wash.
- In the change room, remove scrubs and deposit in bag for laundering.

Work practices for BSL 3 shall include all BSL 1 and BSL 2 work practices plus the following:

- Ultracentrifuges and super speed centrifuges shall be equipped with HEPA filters.
- A requirement that at least two operators shall be present in the BSL 3 laboratory may be imposed by the Institutional Biosafety Committee (IBC) whenever warranted by conditions or activities.
- With the exception of appropriate items of clothing, personal effects shall not be taken into the laboratory. Notes taken in the laboratory must be either electronically transferred, faxed out of the lab, or autoclaved.

Biosafety Level 4

No work classified at the BSL 4 level is authorized at LLNL. Such work would involve dangerous and exotic agents that present a high risk of life-threatening disease. Examples of such agents are the Lassa fever virus, Kyasanur Forest disease virus, Marburg virus, wild strains of yellow fever virus, recombinant DNA agents that carry genes for the biosynthesis of molecules highly toxic for vertebrates, and recombinant DNA agents that carry genes for drug resistance in microorganisms not known to acquire resistance naturally when acquisition could compromise the use of the drug to control disease. Stringent laboratory practices are required, and maximum containment equipment and facilities are required.

Appendix C

Classification of Human Etiologic Agents

This appendix (excerpted from Appendix B of the *NIH Guidelines for Research Involving Recombinant DNA Molecules*, January 2001) includes biological agents known to infect humans, as well as selected animal agents that may pose theoretical risks if inoculated into humans. Included are lists of representative genera and species known to be pathogenic; mutated, recombined, and nonpathogenic species and strains are not considered. Noninfectious life cycle stages of parasites are excluded.

RISK GROUP (RG) 1 AGENTS
Bacillus subtilis or Bacillus licheniformis (Asporogenic)
Bacillus subtilis or Bacillus licheniformis host-vector systems, exceptions
Escherichia coli-K12
Host-vector systems, exceptions
Adeno-associated virus (AAV) Types 1 through 4, recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus.
Those agents not listed in RG 2, 3, and 4 are not automatically or implicitly classified in RG 1; a risk assessment shall be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.
RG 2 AGENTS
Bacterial Agents including Chlamydia
Acinetobacter baumannii (formerly Acinetobacter calcoaceticus)
Actinobacillus
Actinomyces pyogenes (formerly Corynebacterium pyogenes)
Aeromonas hydrophila
Amycolata autotrophica
Archanobacterium haemolyticum (formerly Corynebacterium haemolyticum)
Arizona hinshawii (all serotypes)
Bacillus anthracis
Bartonella henselae, B. quintana, B. vinsonii
Bordetella including B pertussis
Borrelia recurrentis
B. burgdorferi
Burkholderia (formerly Pseudomonas species), except those listed in this Appendix as RG 3
Campylobacter coli
Campylobacter fetus

RG 2 AGENTS (cont'd)
Bacterial Agents including Chlamydia
Campylobacter jejuni
Chlamydia psittaci
Chlamydia trachomatis
Chlamydia pneumoniae
Clostridium botulinum
Clostridium chauvoei
Clostridium haemolyticum
Clostridium histolyticum
Clostridium novyi
Clostridium septicum
Clostridium tetani
Corynebacterium diphtheriae
Corynebacterium pseudotuberculosis
Corynebacterium renale
Dermatophilus congolensis
Edwardsiella tarda
Erysipelothrix rhusiopathiae
Escherichia coli (all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen), including E. coli O157:H7
Haemophilus ducreyi
Haemophilus influenzae
Helicobacter pylori
Klebsiella – all species except K. oxytoca (RG 1)
Legionella including L. pneumophila
Leptospira interrogans (all serotypes)
Listeria
Moraxella
Mycobacterium (except those listed in this Appendix as RG 3, including M. avium complex)
M. asiaticum
M. bovis (BCG vaccine strain)
M. chelonae
M. fortuitum
M. kansasii
M. leprae
M. malmoense

RG 2 AGENTS (cont'd)
Bacterial Agents including Chlamydia
M. marinum
M. paratuberculosis
M. scrofulaceum
M. simia
M. szulgai
M. ulcerans
M. xenopi
Mycoplasma, except M. mycoides and M. agalactiae, which are restricted animal pathogens
Neisseria gonorrhoeae
Neisseria meningitidis
Nocardia asteroides
Nocardia brasiliensis
Nocardia otitidiscaviarum
Nocardia transvalensis
Rhodococcus equi
Salmonella, including:
S. arizonae
S. cholerasuis
S. enteritidis
S. gallinarum-pullorum
S. meleagridis
S. paratyphi-A, B, C
S. typhi
S. typhimurium
Shigella, including:
S. boydii
S. dysenteriae-type 1
S. flexneri
S. sonnei
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus pneumoniae
Streptococcus pyogenes
Treponema carateum

RG 2 AGENTS (cont'd)
Bacterial Agents including Chlamydia
Treponema pallidum
Vibrio cholerae
Vibrio parahemolyticus
Vibrio vulnificus
Yersinia enterocolitica
Fungal Agents – RG 2
Blastomyces dermatitidis
Cladosporium bantianum
C. (Xylohypha) trichoides
Cryptococcus neoformans
Dactylaria galopava (ochroconis gallopavum)
Epidermophyton
Exophiala (Wangiella) dermatitidis
Fonsecaea pedrosoi
Microsporum
Paracoccidioides braziliensis
Penicillium marneffeii
Sporothrix schenck
Trichophyton
Parasitic Agents – RG 2
Ancylostoma human hookworms, including A. duodenale A. ceylanicum
Ascaris, including Ascaris lumbricoides suum
Babesia, including: B. divergens B. microti
Brugia filaria worms, including: B. malayi B. timori
Coccidia
Cryptosporidium, including C. parvum

RG 2 AGENTS (cont'd)
Parasitic Agents – RG 2
Cysticercus cellulosae (hydatid cyst, larva of T. solium)
Echinococcus including: E. granulosus E. multilocularis E. vogeli
Endamoeba histolytica
Enterobius
Fasciola including: F. gigantica F. hepatica
Giardia, including G. lamblia
Heterophyes
Hymenolepis including: H. diminuta H. nana
Isospora
Leishmania, including: L. braziliensis L. donovani L. ethiopia L. major L. mexicana L. peruviana L. tropica
Loa loa filaria worms
Microsporidium
Naegleria fowleri
Necator human hookworms, including N. americanus
Onchocerca filaria worms, including O. volvulus
Plasmodium, including simian species
P. cynomologi
P. falciparium
P. malariae
P. ovale
P. vivax

RG 2 AGENTS (cont'd)
Parasitic Agents – RG 2
Sarcocystis, including <i>S. sui hominis</i>
Schistosoma, including: <ul style="list-style-type: none"> <i>S. haematobium</i> <i>S. intercalatum</i> <i>S. japonicum</i> <i>S. mansoni</i> <i>S. mekongi</i>
Strongyloides, including <i>S. stercoralis</i>
Taenia solium
Toxocara, including <i>T. canis</i>
Toxoplasma, including <i>T. gondii</i>
Trichinella spiralis
Trypanosoma, including: <ul style="list-style-type: none"> <i>T. brucei bruce</i> <i>T. brucei gambiense</i> <i>T. brucei rhodesiense</i> <i>T. cruzi</i>
Wuchereria bancrofti filaria worms
RG 2 VIRUSES
Adenoviruses (human, all types)
Alphaviruses (Togaviruses) – Group A Arboviruses <ul style="list-style-type: none"> – Eastern equine encephalomyelitis virus – Venezuelan equine encephalomyelitis vaccine strain TC-83 – Western equine encephalomyelitis virus
Arenaviruses <ul style="list-style-type: none"> – Lymphocytic choriomeningitis virus (non-neurotropic strains) – Tacaribe virus complex – Other viruses
Bunyaviruses <ul style="list-style-type: none"> – Bunyamwera virus – Rift Valley fever virus vaccine strain MP-12 – Other viruses (See Ref.)
Caliciviruses
Coronaviruses

RG 2 VIRUSES (cont'd)
Flaviviruses (Togaviruses) – Group B Arboviruses <ul style="list-style-type: none"> – Dengue virus serotypes 1, 2, 3, and 4 – Yellow fever virus vaccine strain 17D – Other viruses (See Ref.)
Hepatitis A, B, C, D, and E viruses
Herpesviruses [except Herpesvirus simiae (Monkey B virus), which is in RG4] <ul style="list-style-type: none"> – Cytomegalovirus – Epstein-Barr virus – Herpes simplex types 1 and 2 – Herpes zoster – Human herpesvirus types 6 and 7
Orthomyxoviruses <ul style="list-style-type: none"> – Influenza viruses types A, B, and C – Other tick-borne orthomyxoviruses (See Ref.)
Papovaviruses <ul style="list-style-type: none"> – All human papilloma viruses
Paramyxoviruses <ul style="list-style-type: none"> – Newcastle disease virus – Measles virus – Mumps virus – Parainfluenza viruses types 1, 2, 3, and 4 – Respiratory syncytial virus
Parvoviruses <ul style="list-style-type: none"> – Human parvovirus (B19)
Picornaviruses <ul style="list-style-type: none"> – Coxsackie viruses types A and B – Echoviruses (all types) – Polioviruses (all types, wild and attenuated) – Rhinoviruses (all types)
Poxviruses – all types except Monkey-pox virus (see RG 3) Viruses and Prions and restricted poxviruses, including: Alastrim Smallpox Whitepox
Reoviruses – all types except coltivirus, human rotavirus, and orbivirus (Colorado tick virus)
Rhaboviruses, including: Rabies virus (all strains) Vesicular stomatitis virus (laboratory-adapted strains, including VSV-Indian, San Juan, and Glasgow)

RG 2 VIRUSES (cont'd)
Togaviruses (see Alphaviruses and Flaviviruses)
– Rubivirus (rubella)
RG 3
Bacterial Agents, including Rickettsia
Bartonella
Brucella, including:
B. abortus
B. canis
B. suis
Burkholderia (Pseudomonas) mallei
B. pseudomallei
– Coxiella burnetii
– Francisella tularensis
– Mycobacterium bovis (except BCG strain) RG 2
M. tuberculosis
Pasteurella multocida type B (“buffalo” and other virulent strains)
Rickettsia akari
R. australis
R. canada
R. conorii
R. prowazekii
R. rickettsii
R. siberica
R. tsutsugamushi
R. typhi (R. mooseri)
Yersinia pestis
Fungal Agents – RG 3
Coccidioides immitis (sporulating cultures and contaminated soil)
Histoplasma capsulatum
Histoplasma capsulatum var. duboisii
Parasitic Agents – RG 3
None

RG 3 (cont'd)
Viruses and Prions – RG 3
Alphaviruses (Togaviruses) – Group A Arboviruses
<ul style="list-style-type: none"> – Semliki Forest viruses – St. Louis encephalitis virus
<ul style="list-style-type: none"> – Venezuelan equine encephalomyelitis virus (except the vaccine strain TC-83) – Other viruses
Arenaviruses
<ul style="list-style-type: none"> – Flexal – Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)
Bunyaviruses
<ul style="list-style-type: none"> – Hantaviruses
Hantaan virus
<ul style="list-style-type: none"> – Rift Valley fever virus
Flaviviruses (togaviruses) Group B arboviruses
<ul style="list-style-type: none"> – Japanese encephalitis virus – Yellow fever virus – Other viruses
Poxviruses
Monkeypox virus
Prions
<ul style="list-style-type: none"> – Transmissible spongiform encephalopathies (TSE) agents (Creutzfeldt-Jacob disease and kuru agents)
Retroviruses
<ul style="list-style-type: none"> – Human immunodeficiency virus (HIV) types 1 and 2 – Human T cell lymphotropic virus (HTLV) types 1 and 2
Simian immunodeficiency virus (SIV)
Rhabdoviruses
<ul style="list-style-type: none"> – Vesicular stomatitis virus
RG 4 AGENTS
Bacterial Agents – RG 4
None
Fungal Agents – RG 4
None
Parasitic Agents – RG 4
None

RG 4 AGENTS (cont'd)
Viral Agents – RG 4
<p>Arenaviruses</p> <ul style="list-style-type: none"> – Guaranito virus – Lassa virus – Junin virus – Machupo virus – Sabia
<p>Bunyaviruses (Nairovirus)</p> <ul style="list-style-type: none"> – Crimean-Congo hemorrhagic fever virus
<p>Filoviruses</p> <ul style="list-style-type: none"> – Ebola virus – Marburg virus
<p>Flaviruses (Togaviruses) – Group B arboviruses</p> <p>Tick-borne encephalitis virus complex, including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses</p>
<p>Herpesviruses (alpha)</p> <ul style="list-style-type: none"> – Herpesvirus simiae (Herpes B or Monkey B virus)
<p>Paramyxoviruses</p> <ul style="list-style-type: none"> – Equine morbillivirus – Hemorrhagic fever agents and viruses as yet undefined
Animal Viral Etiologic Agents in Common Use
<p>The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents are associated with disease in healthy adult humans, but are commonly used in laboratory experimental work. A containment level appropriate for RG 1 human agents is recommended for their use. For agents that are infectious to human cells (e.g., amphotropic and xenotropic strains of murine leukemia virus), a containment level appropriate for RG 2 human agents is recommended.</p>
<p>Baculoviruses</p>
<p>Herpesviruses</p> <ul style="list-style-type: none"> – Herpesvirus atele
<p>Herpesvirus saimiri</p> <ul style="list-style-type: none"> – Marek's disease virus – Murine cytomegalovirus
<p>Papovaviruses</p> <ul style="list-style-type: none"> – Bovine papilloma virus – Polyoma virus – Shope papilloma virus – Simian virus 40 (SV40)

Animal Viral Etiologic Agents in Common Use (cont'd)**Retroviruses**

- Avian leukosis virus
- Avian sarcoma virus
- Bovine leukemia virus
- Feline leukemia virus
- Feline sarcoma virus
- Gibbon leukemia virus
- Mason-Pfizer monkey virus
- Mouse mammary tumor virus
- Murine leukemia virus
- Murine sarcoma virus
- Rat leukemia virus

Murine Retroviral Vectors

Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered under BSL 1 containment.

Appendix D

APHIS Plant Pathogens, HHS Select Infectious Agents, and USDA High Consequence Livestock Pathogens or Toxins

Viruses

1. African horse sickness virus ^β
2. African swine fever virus ^β
3. Akabane virus ^β
4. Avian influenza virus (highly5. pathogenic) ^β
5. Blue tongue virus (exotic) ^β
6. Camel pox virus ^β
7. Cercopithecine herpes virus (Herpes B virus) ^ψ
8. Classical swine fever virus ^β
9. Crimean-Congo haemorrhagic fever virus ^ψ
10. Eastern equine encephalitis virus ^x
11. Ebola viruses ^ψ
12. Foot and mouth disease virus ^β
13. Goat pox virus ^β
14. Japanese encephalitis virus ^β
15. Lassa fever virus ^ψ
16. Lumpy skin disease virus ^β
17. Malignant catarrhal fever ^β
18. Marburg virus ^ψ
19. Menangle virus ^β
20. Monkeypox virus ^ψ
21. Newcastle disease virus (exotic) ^β
22. Nipah and Hendra complex viruses ^x
23. Peste des petits ruminants ^β
24. Plum pox potyvirus ^α
25. Rift Valley fever virus ^x
26. Rinderpest virus ^β
27. Sheep pox ^β
28. South American haemorrhagic fever viruses [(Junin, Machupo, Sabia, Flexal, Guanarito)] ^ψ
29. Swine vesicular disease virus ^β
30. 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)] ^ψ

31. Variola major virus (Smallpox virus) and Variola minor (Alastrim) ^ψ

32. Venezuelan equine encephalitis virus ^x

33. Vesicular stomatitis virus (exotic) ^β

Prion

1. Bovine spongiform encephalopathy agent ^β

Toxins

1. Abrin ^ψ
2. Botulinum neurotoxins ^x
3. *Clostridium perfringens* epsilon toxin ^x
4. Conotoxins ^ψ
5. Diacetoxyscirpenol ^ψ
6. Ricin ^ψ
7. Saxitoxin ^ψ
8. Shigatoxin and Shiga-like ribosome inactivating proteins ^x
9. Staphylococcal enterotoxins ^x
10. Tetrodotoxin ^ψ
11. T-2 toxin ^x

Bacteria

1. *Bacillus anthracis* ^x
2. Botulinum neurotoxin producing strains of *Clostridium* ^x
3. *Brucella abortus* ^x
4. *Brucella melitensis* ^x
5. *Brucella suis* ^x
6. *Burkholderia mallei* ^x
7. *Burkholderia pseudomallei* ^x
8. *Coxiella burnetii* ^x
9. *Cowdria Ruminantium* (Heartwater) ^β
10. *Francisella tularensis* ^x
11. *Liberobacter africanus*, *Liberobacter asiaticus* ^α

12. *Mycoplasma capricolu*/M. F38/*M. mycoides capri* (contagious caprine pleuropneumonia agent)^β
13. *Mycoplasma mycoides mycoides* (contagious bovine pleuropneumonia agent)^β
14. *Ralstonia solanacearum* Race 3^α
15. *Rickettsia prowazekii* ^ψ
16. *Rickettsia rickettsii* ^ψ
17. *Xanthomonas oryzae* pv. *oryzicola* ^α
18. *Xylella fastidiosa* (citrus variegated chlorosis strain)^α
19. *Yersinia pestis* ^ψ

Fungi

1. *Coccidioides immitis* ^x
2. *Coccidioides posadasii* ^ψ
3. *Peronosclerospora philippinensis* ^α
4. *Phakopsora pachyrhizi* ^α
5. *Sclerophthora rayssiae var zea* ^α
6. *Synchytrium endobioticum* ^α

Exemptions

The following agents or toxins are exempt if the aggregate amount under the control of a principal investigator does not, at any time, exceed:

- 0.5 mg of Botulinum neurotoxins
- 5 mg of *Staphylococcal* enterotoxins
- 100 mg of abrin, *Clostridium perfringens* epsilon toxin, conotoxin, ricin, saxitoxin, shigatoxin, shiga-like ribosome inactivating protein, and tetrodotoxin
- 1,000 mg of diacetoxyscirpenol and T-2 toxin

The following agents or toxins are also exempt:

- Any agent or toxin that is in its naturally occurring environment provided it has not been intentionally introduced, cultivated, collected, or otherwise extracted from its natural source.

- Non-viable select agent organisms or nonfunctional toxins.
- The vaccine strains of Junin virus (Candid #1), Rift Valley fever virus (MP-12), Venezuelan Equine encephalitis virus vaccine strain TC-83.

The medical use of toxins for patient treatment is exempt.

Genetic Elements, Recombinant Nucleic Acids, and Recombinant Organisms

1. Select agent viral nucleic acids (synthetic or naturally derived, contiguous or fragmented, in host chromosomes or in expression vectors) that can encode infectious and/or replication competent forms of any of the select agent viruses.
2. Nucleic acids (synthetic or naturally derived) that encode for the functional form(s) of any of the listed toxins if the nucleic acids: a) are in a vector or host chromosome; b) can be expressed *in vivo* or *in vitro*; or c) are in a vector or host chromosome and can be expressed *in vivo* or *in vitro*.
3. Listed viruses, bacteria, fungi, and toxins that have been genetically modified.

Other Restrictions

1. Experiments utilizing recombinant DNA that involve the deliberate transfer of a drug resistance trait to the listed agents that are not known to acquire the trait naturally, if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture.
2. Experiments involving the deliberate formation of recombinant DNA containing genes for the biosynthesis of listed toxins lethal for vertebrates at an LD50 < 100 ng/kg body weight.

Appendix E

Priority Categories Used for Select Agents by the Centers for Disease Control and Prevention

This appendix lists the agents in each of the three categories (A, B, and C) that the CDC uses to classify the Select Agents (see Appendix D), as well as the agents in each category. The categories indicate priority in public health preparedness efforts, with Category A having the highest priority.

Category A – Highest-Priority Agents

Agents in this category:

- Can be easily disseminated or transmitted from person to person.
- Can cause high mortality, with potential for major public health impact.
- Could cause public health panic and social disruption.
- Require special action for public preparedness.

List of Category A agents:

Arenaviruses (Lassa and Junin)

Bacillus anthracis

Clostridium botulinum toxin (botulinum)

Filoviruses (Ebola and Marburg hemorrhagic fever)

Francisella tularensis (tularemia)

Variola major (i.e., smallpox) virus

Yersinia pestis

Category B – Second Highest-Priority Agents

Agents in this category:

- Are moderately easy to disseminate.
- Cause moderate morbidity.
- Require specific enhancements of CDC's diagnostic capacity and enhanced disease surveillance.

List of Category B agents:

Alphaviruses (Venezuelan, eastern, and western equine encephalomyelitis viruses)

Brucella species (Brucellosis)

Burkholderia mallei (Glanders)

Coxiella burnetti (Q-fever)

Epsilon toxin of *Clostridium perfringens*

Ricin toxin (from the castor bean)

Staphylococcus enterotoxin B (SEB)

A subset list including, but not limited to, food or water-borne agents (i.e., *Salmonella* species, *Shigella dysenteriae*, *E. coli* O157:H7, *Vibrio cholerae*, and *Cryptosporidium parvum*)

Category C – Third Highest-Priority Agents

This category contains emerging pathogens that could be engineered for mass dissemination because of their availability, ease of production, high mortality, and major health impact.

List of Category C agents:

Hantaviruses

Multi-drug resistant tuberculosis

Nipah virus

Tickborne hemorrhagic fever viruses

Yellow fever virus

Appendix F

USDA-Regulated Materials

This appendix lists some of the materials regulated by the USDA. Approval from the USDA is required for their use, handling, transport, or storage. This list is not exhaustive; for more information, refer to the following Internet address:

<http://www.aphis.usda.gov/>

F.1 Animal Pathogens

This is a list of animal pathogens of particularly high consequence to livestock.

African horse sickness virus	Lumpy skin disease virus
African swine fever	Malignant catarrhal fever virus
Akabane virus	Menangle virus
Avian influenza virus (highly pathogenic)	<i>Mycoplasma capricolum</i> , mycoplasma strain F-38, <i>M. mycoides capri</i> (contagious caprine pleuropneumonia agent)
Blue tongue virus (exotic)	<i>Mycoplasma mycoides mycoides</i> (contagious bovine pleuropneumonia agent)
Bovine spongiform encephalopathy agent	Newcastle disease virus (exotic)
Camel pox virus	Peste des petits ruminants virus
Classical swine fever	Rinderpest virus
<i>Cowdria ruminantium</i> (heartwater)	Sheep pox virus
Foot and mouth disease virus	Swine vesicular disease virus
Goat pox virus	Vesicular stomatitis virus
Japanese encephalitis virus	

F.2 Plant Pathogens

The following is a list of plant pathogens regulated by the State of California and was taken from the following USDA website:

<http://www.aphis.usda.gov/ppq/permits/plantpest/pathogen.html>

F.2.1 Bacteria (by Scientific Name)

Agrobacterium radiobacter, *Agrobacterium rubi*, *Agrobacterium tumefaciens*, *Agrobacterium vitis*, *Burkholderia andropogonis*, *Burkholderia caryophylli*, *Burkholderia cepacia*, *Burkholderia cichorii*, *Burkholderia corrugata*, *Burkholderia gladioli* pv. *gladioli*, *Clavibacter michiganensis* subsp. *insidiosus*, *Clavibacter michiganensis* subsp. *michiganensis*, *Clavibacter michiganensis* subsp. *sepedonicus*, *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, *Erwinia amylovora*, *Erwinia carotovora* subsp. *atroseptica*, *Erwinia carotovora* subsp. *carotovora*, *Erwinia chrysanthemi*, *Erwinia chrysanthemi* pv. *chrysanthemi*, *Erwinia chrysanthemi* pv. *dieffenbachiae*, *Erwinia chrysanthemi* pv. *zetae*, *Erwinia tracheiphila*, *Pantoea stewartii* subsp. *stewartii*, *Pseudomonas syringae* pv. *apii*, *Pseudomonas syringae* pv. *atrofaciens*, *Pseudomonas syringae* pv. *coronafaciens*, *Pseudomonas syringae* pv. *glycinea*, *Pseudomonas syringae* pv. *lachrymans*, *Pseudomonas syringae* pv. *mori*, *Pseudomonas syringae* pv. *papulans*, *Pseudomonas syringae* pv. *phaseolicola*, *Pseudomonas syringae* pv. *psi*, *Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *tabaci*, *Pseudomonas syringae* pv. *tomato1*, *Ralstonia solanacearum2*, *Rhodococcus fascians*, *Spiroplasma citri*, *Streptomyces scabies*, *Xanthomonas campestris* pv. *armoraciae*, *Xanthomonas campestris* pv. *campestris*, *Xanthomonas campestris* pv. *carotae*, *Xanthomonas campestris* pv. *cucurbitae*, *Xanthomonas campestris* pv. *hederae*, *Xanthomonas campestris* pv. *juglandis*, *Xanthomonas campestris* pv. *papavericola*, *Xanthomonas campestris* pv. *pelargonii*, *Xanthomonas campestris* pv. *pruni*, *Xanthomonas campestris* pv. *raphani*, *Xanthomonas campestris* pv. *vitians*, *Xanthomonas campestris* pv. *zinniae*, *Xanthomonas fragariae*, *Xanthomonas phaseoli* pv. *alfalfae*, *Xanthomonas phaseoli* pv. *begoniae*, *Xanthomonas phaseoli* pv. *glycines*, *Xanthomonas phaseoli* pv. *phaseoli*, *Xanthomonas translucens* pv. *translucens*, *Xanthomonas vesicatoria*.

F.2.2 Fungi (by Scientific Name)

CHYTRIDIOMYCETES

Physoderma maydis

OOMYCETES

Albugo candida, *Peronospora sojae*, *Peronospora trifoliorum*, *Peronospora viticola*, *Phytophthora cactorum*, *Phytophthora capsici*, *Phytophthora cinnamomi*, *Phytophthora citricola*, *Phytophthora fragariae*, *Phytophthora infestans*, *Phytophthora megasperma*, *Phytophthora megasperma* f.sp. *medicaginis*, *Phytophthora rubi* s.sp. *fragariae*, *Phytophthora sojae*, *Plasmodiophora brassicae*, *Pythium aphanidermatum*, *Pythium arrhenomanes*, *Pythium graminicola*, *Pythium irregulare*, *Pythium ultimum*, *Sclerophthora macrospora*.

ASCOMYCETES

Apiosporina morbosa (black knot), *Botryosphaeria obtusa*, *Botryosphaeria ribis* (B. *dothidea*, B. *berengeriana*), *Claviceps purpurea*, *Cymadothea trifolii* (sooty blotch), *Diaporthe phaseolorum*,

Gaeumannomyces graminis, *Gibberella zeae*, *Glomerella cingulata*, *Leptosphaerulina trifolii*, *Monilinia fructicola* (*Sclerotinia fructicola*), *Nectria cinnabarina*, *Ophiostoma ulmi* (*Ceratocystis ulmi*), *Pseudopeziza medicaginis*, *Pseudopeziza trifolii*, *Sclerotinia sclerotiorum* (*Whetzelinia sclerotiorum*), *Sclerotinia trifoliorum*, *Valsa ambiens*, *Venturia inaequalis* (apple scab), *Xylaria polymorpha*.

Powdery Mildews

Erysiphe graminis, *Microsphaera vaccinii* (on Ericaceae), *Podosphaera clandestina* (on Rosaceae), *Sphaerotheca* Asteraceae, Cucurbitaceae, Cucurbitaceae, Scrophulariaceae), *Sphaerotheca macularis* (on hops and strawberry), *Unicinula viticola*.

Coelomycetes

Colletotrichum acutatum, *Colletotrichum coccodes*, *Colletotrichum destructivum*, *Colletotrichum fragariae*, *Colletotrichum gloeosporioides*, *Colletotrichum graminicola*, *Colletotrichum trifolii*, *Macrophomina phaseolina* (*Macrophoma phaseolina*, *M. phaseoli*, *Botryodiplodia phaseoli*), *Phoma medicaginis*, *Phomopsis juniperovora*, *Phomopsis sojae*, *Phomopsis viticola*, *Septoria rubi*, *Septoria tritici*, *Sphaeropsis sapinea* (*Diplodia pinea*), *Stagonospora nodorum* (*Septoria nodorum*), *Stenocarpelia maydis* (*Diplodia zeae*, *D. zeae-maydis*).

Hyphomycetes

Alternaria alternata, *Alternaria solani*, *Bipolaris maydis* (*Heminthosporium maydis*, *Drechslera maydis*), *Bipolaris sorokiniana* (*Helminthosporium sorokiniana*, *Drechslera sorokiniana*), *Bipolaris victoriae* (*Helminthosporium victoriae*, *Drechslera victoriae*), *Botrytis cinerea*.

Cercospora medicaginis, *Cercospora zeae-maydis*, *Cladosporium herbarum*, *Drechslera avenae* (on oats, other grasses), *Drechslera graminea* (on barley, other grasses), *Drechslera poae* (on grasses), *Drechslera teres* (on barley, other grasses), *Drechslera tritici-repentis* (on cereals, other grasses), *Exserohilum turcicum* (*Helminthosporium turcicum*, *Bipolaris turcicum*), *Fusarium acuminatum*, *Fusarium avenaceum*, *Fusarium culmorum*, *Fusarium equiseti*, *Fusarium graminearum*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Fusarium oxysporum*, *Fusarium roseum*, *Fusarium solani*, *Penicillium expansum*, *Rhynchosporium secalis*, *Thielaviopsis basicola*, *Verticillium albo-atrum*, *Verticillium dahliae*.

HEMIASCOMYCETES

Taphrina caerulescens (leaf blister on oak, *Ostrya*, *Rhus*), *Taphrina communis* (plum pocket on *Prunus*), *Taphrina deformans* (peach leaf curl).

BASIDIOMYCETES

Wood rotters and root-collar rotters

Armillaria mellea, *Ceratobasidium cerealea*, *Daedaleopsis confragosa* (*Daedalea confragosa*), *Ganoderma applanatum* (*Fomes applanatus*), *Ganoderma lucidum*, *Hirschioporus pargamenus* (*Trichaptum biformis*, *Polyporus pargamenus*), *Laetiporus sulphureus* (*Polyporus sulphureus*), *Phellinus gilii*, *Phellinus robiniae*, *Schizophyllum commune*, *Stereum ostrea*, *Trametes versicolor* (*Polyporus versicolor*, *Coriolus versicolor*).

Rusts

Gymnosporangium clavipes (cedar-quince rust), *Gymnosporangium globosum* (cedar-hawthorn rust), *Gymnosporangium juniperi-virginianae* (cedar-apple rust), *Puccinia coronata* (on Rhamnaceae, Eleganaceae/Poaceae), *Puccinia graminis* (on Berberis/Poaceae), *Puccinia recondita* (on Ranunculaceae/Poaceae), *Pucciniastrum americanum* (late leaf rust on raspberry).

Smuts

Tilletia caries (*Tilletia tritici*), *Tilletia laevis* (*Tilletia foetida*), *Ustilago avenae*, *Ustilago hordei*, *Ustilago tritici*, *Ustilago zaeae*.

Other Basidiomycetes

Rhizoctonia solani (*Thanatephorus cucumeris*), *Sclerotium rolfsii*.

F.2.3 Viruses (Regulated by the State of California)

Alfalfa mosaic, barley yellow dwarf, bean common mosaic, bean yellow mosaic, beet curly top, beet mosaic, cactus virus X, camellia yellow mottle, carnation mottle, cauliflower mosaic, chrysanthemum mosaic, chrysanthemum virus B, cucumber mosaic, cymbidium mosaic, dasheen mosaic, fig mosaic, impatiens necrotic spot, lettuce big vein, lettuce mosaic, lily symptomless, maize dwarf mosaic, odontoglossum ringspot, papaya ringspot, pepper mottle, plum line pattern, potato leafroll, potato virus S, potato virus X, potato virus Y, prune dwarf, prunus necrotic ringspot, squash mosaic, sugarcane mosaic, tobacco etch, tomato mosaic, tomato spotted wilt, turnip mosaic, watermelon mosaic virus 2, zucchini yellow mosaic.

Appendix G

Universal Precautions

Universal precautions shall be followed to prevent contact with blood and other potentially infectious materials and thereby reduce the risk of occupational exposure. Universal precautions are an approach to infection control in which all human blood, certain human body fluids and unfixed tissues and cell cultures of human origin are treated as if known to be infectious for HIV, HBV, and other bloodborne pathogens. The following precautions are advocated by CDC for healthcare workers and are adopted as policy at LLNL:

- a. All healthcare and laboratory employees shall use appropriate barrier precautions to prevent skin and mucous membrane exposure when contact with blood, body fluids, unfixed tissues, or cell cultures is anticipated.
- b. Gloves shall be worn when touching or working with blood, body fluids, cell cultures, mucous membranes, or non-intact skin.
- c. Gloves shall be worn when handling items or surfaces contaminated with blood, body fluids, unfixed tissues, or cell cultures.
- d. Gloves shall be worn while performing venipuncture and other vascular access procedures.
- e. Gloves shall be changed after contact with each patient, after each spill management procedure, or whenever the integrity of one or both of the gloves becomes questionable.
- f. During procedures that are likely to generate droplets of blood, other body fluids, or cell culture liquids, a mask and protective eyewear (or a face shield) shall be worn to prevent exposure of the mucous membranes of the mouth, nose, and eyes.
- g. Gowns, aprons or laboratory coats shall be worn during procedures that are likely to generate splashes of blood or other body fluids.
- h. Hands and other skin surfaces shall be washed immediately and thoroughly with water and antiseptic cleanser if contaminated with blood, other body fluids, or cell culture liquids.
- i. Hands shall be immediately washed after gloves are removed.
- j. Employee shall take precautions to prevent injuries caused by needles, scalpels, and other sharp instruments or devices during or after medical procedures, when cleaning instruments, and during disposal of used needles.

- k. To prevent needle-stick injuries, needles shall not be recapped, bent or broken, removed from disposable syringes, or otherwise manipulated by hand.
- l. After use, disposable syringes, needles, scalpels blades, and other sharp items shall be placed in puncture-resistant sharps waste containers for disposal. These containers shall be placed as close as practical to the procedure being performed or to the area where disposable sharps are used.
- m. Sharps used for procedures involving blood, body fluids, unfixed tissues, or cell cultures shall incorporate engineered sharps injury protection (ESIP) features whenever possible.
- n. Laboratory employees who have exudative lesions or weeping dermatitis shall refrain from handling clinical samples or specimens and contaminated equipment until the condition is resolved.
- o. Pregnant employees shall review safe work procedures with supervisors, the ES&H Team industrial hygienist and the Biosafety Officer.
- p. Regulated waste shall be managed as specified in Document 36.1.

Appendix H

Disinfectants

H.1 Chemical Disinfectants

This appendix summarizes the pertinent characteristics and potential application of several types of commonly used chemical disinfectants. Because of the inherent hazards involved, use of these substances shall be reviewed in advance by the ES&H Team. For more information regarding disinfectants and required contact times, see Document 13.1. Table G-1 summarizes liquid, gaseous, and physical decontaminating agents for sanitation, disinfection, and sterilization.

H.1.1 Alcohol

A concentration of 70%–80% by weight of ethyl or isopropyl alcohol is commonly used as a general disinfectant. Alcohol denatures proteins and is a somewhat slow-acting germicide. Although a good general disinfectant, alcohol exhibits no activity against bacterial spores.

H.1.2 Chlorine

This halogen is a universal disinfectant that is active against all microorganisms and bacterial spores. Free, available chlorine is an active element. Chlorine combines with protein (e.g., organic matter) and rapidly decreases in concentration in its presence. It is a strong oxidizing agent that is corrosive to metals. If chlorine is used directly on a stainless surface, rinse thoroughly with water after decontamination to prevent tarnishing.

Chlorine solutions gradually lose their strength over time and therefore have a limited shelf life. Fresh solutions shall be prepared frequently. Sodium hypochlorite is usually used as a base for chlorine disinfectant. For disinfecting purposes, a concentration of between 5,000 and 10,000 ppm of available chlorine is sufficient. Household or laundry bleach usually contains 5.25% available chlorine or 52,500 ppm. A 1:10 diluted solution contains 5,250 ppm of available chlorine. Decontamination with this concentration requires a 30-minute contact time. Do not autoclave chlorine solutions or materials treated with chlorine, because residual chlorine vaporizes, creating an inhalation hazard.

Table H-1. Summary of liquid, gaseous, and physical decontamination agents for biological agents and toxins.

Decontaminating agent	No effect on	Sanitize ^a	Disinfect ^a	Sterilize ^a
Liquids				
Alcohol, ethyl (70-95% v/v)	Bacterial spores ^b	—	TB ¹ at 95% for 0.25 min	—
			TB ² at 70% for 0.5 min	—
	Nonlipid viruses ^c	—	Lipoviruses for 1.0 min	—
	—	—	At 50% for 2.0 min	Medical instruments ^b at 70% for 15 min
Alcohol, isopropyl (60-90% v/v)	Bacterial spores ^d	At 70% for <2 min	At 70% for 2 min	At 70% for >5 min
Alcohol, Isopropyl +5% propylene oxide	—	—	—	Bacterial spores ^b for 1.0 min
Glutaraldehyde ^b	—	At <2% for 15 min	Bacillus anthrax at 2% for 15 min	At >2% for 15 min
	—	—	Cl. and Bacillus spores ^b at 2-3% for 180 min	—
Hydrogen peroxide	—	At <3% for 10 min	At 3-6% for 10 min	At 6-25% for 10
	—	—	10 ⁸ bacterial spores ^b at 10% for 60 min	—
Phenol (1-5%) ^c	Bacterial spores, ^c Nonlipid viruses	At 0.5% for <30 min	Broad spec. at 0.5-3% for 30 min C. burnetti, F. turalensis, P. mallei, Bacillus anthracis, and Rickettsia at 5% for 30 min	At >3% for 30 min

**Table H-1. Summary of liquid, gaseous, and physical decontamination agents for biological agents and toxins.
(cont'd)**

Decontaminating agent	No effect on	Sanitize ^a	Disinfect ^a	Sterilize ^a
Liquids (continued)				
Soap and water ^e	—	C. burnetti ^{f,g} for 10 min. The toxins will be diluted if washed for more than 10 min.	Synergistic with certain phenol derivatives	—
Sodium hypochlorite, (household bleach) undiluted	—	—	—	Cl. botulinum toxin, SEB, and Ricin ^g at 250 ppm (0.5%) for 15 min
	—	At <50 ppm (<0.1%) for 30 min	Most bacterial toxins at 50 ppm (0.1%) for 30 min TB at 50 ppm (0.1%) for for 30 sec at pH of 8.4 at 50-60°C General bacteria and viruses at 500 ppm (1.0%) for 30 min Bacillus anthracis ^b at 2500 ppm (5%) for 30 min	Lipoviruses at 500 ppm (1.0%) for 30 min
	—	—	—	SEB at 250 ppm (0.5%) for 72 min T2 mycotoxin at 2500 ppm (5%) for 72 min
Gaseous Agents				
Paraformaldehyde ^b	—	At <1% for 60 min	At 2% for 60 min	SEB ⁵ at >2% for 60 min
	—	—	Cl. spores and Bacillus spores at 8% for 180 min	—

**Table H-1. Summary of liquid, gaseous, and physical decontamination agents for biological agents and toxins.
(cont'd)**

Decontaminating agent	No effect on	Sanitize ^a	Disinfect ^a	Sterilize ^a
Gaseous Agents (continued)				
Ethylene oxide (12% EtO: 88% propellant)	—	—	—	At 130°F for 1200 mg/L for 120 min At 130°F at 650 mg/L for 240 min At 130°F at 470 mg/L for 320 min At 100°F at 470 mg/L for 480 min
Vapor-Phase Hydrogen Peroxide at 2 mg/L (20%)	Porous materials	—	—	B. globigii for 0.17 min Cl. sporogenes for 0.20 min B. Stearothermophilus for 0.30 min
Physical Agents				
Steam heat				
At 80°C	—	—	VEE, SEB, and Ricin for 30 min	—
At 121°C	(SEB) E. coli enterotoxin, Saxitoxin, and Tetrodotoxin	—	—	All bacteria, lipoviruses, protein-based toxins, and viruses for 15 min
At >500°C	—	—	T2 mycotoxin for 30 min	—
Gamma radiation (Cobalt 60)	—	—	—	S. faecium: <50 organisms/item at 3.2 Mrad; 50–500 organisms/item at 4.5 Mrad; and 500–5000 organisms/item at 5.0 Mrad

**Table H-1. Summary of liquid, gaseous, and physical decontamination agents for biological agents and toxins.
(cont'd)**

Decontaminating agent	No effect on	Sanitize ^a	Disinfect ^a	Sterilize ^a
Physical Agents (continued)				
Ultraviolet (UV) light (180–280 nm UV-C)	Cl. botulinum toxin, bacterial spores, Ebola virus, slow-growing viruses, lipoviruses, Micrococcus radiodurans, and non-protein-based toxins	At 0.5 mW/cm ² for 1440 min (24 h) (Ref. 1)	Biological safety cabinets, for example, at 35– 40 m/W/cm ² for 30 min	T2 mycotoxin and protein- based toxins at >40 mW/cm ² for 30 min
X ray	Bacterial spores, Micrococcus radiodurans, and Micrococcus radiophilus	—	Salmonella typhimurium at 0.65 Mrad (6500 Gy)	Medical devices ^b at 2.5 Mrad (25,000 Gy)

^a Contact times may vary and are dependent on the concentration of agent used.

^b Seymour S. Block, *Disinfection, Sterilization, and Preservation*, 3rd edition, Lea & Febiger (1983), p. 1053.

^c American Industrial Hygiene Association, *Biosafety Reference Manual*, 2nd edition, Publication No. 204-RC-95 (1995), p. 175.

^d 59 FR 34496, *Guidelines for Research Involving Recombinant DNA Molecules*, July 5, 1994. Also see the following Internet address: <http://www.nih.gov/od/oba/rdna.htm/>

^e Soap and water will often dilute contaminants from equipment and skin.

^f Synergistic with sodium palmitate, sodium stearate, sodium ricinate, coconut soap, and castile soap.

^g Franz, David, *U.S. Army Defense Against Toxin Weapons*, 2nd edition, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD (August 1996).

^h *U.S. Army Field Manual 3-9, "Potential Military Chemical/Biological Agents and Compounds"* (December 12, 1990).

H.1.3 Iodophor

The most widely used groups of disinfectants in laboratories are iodophors (e.g., Wescodyne). Manufacturers recommend dilution ranges from 1 oz in 5 gal of water (i.e., 25 ppm) to 3 oz in 5 gal of water (i.e., 75 ppm or 0.0075%). The disinfectant characteristics of iodophor are similar to chlorine in that small amounts are rapidly taken up by extraneous protein. Clean surfaces or clear water can be effectively treated by 75 ppm available iodine, but difficulties may be experienced if an appreciable amount of protein is present or if the pH is below 6 or above 7. For washing hands or for use as a sporicide, Wescodyne should be diluted to a concentration of 1:10 in 50% ethyl alcohol to yield 1,600 ppm of available iodine, for relatively rapid inactivation of all microorganisms.

H.1.4 Quaternary Ammonium Compounds

After years of testing and use, there is still considerable controversy about the efficiency of quaternary ammonium compounds (commonly referred to as "quats") as disinfectants. These cationic detergents are strongly surface-active and are therefore good surface cleaners. Quats attach to protein and tend to clump microorganisms. Quats lose effectiveness in the presence of organic matter and are neutralized by anionic detergents such as soap. Quats are bacteriostatic, tuberculostatic, sporostatic, fungistatic, and algistatic at low concentrations. The compounds are biocidal against lipophilic viruses at medium concentrations but are virucidal only against hydrophilic viruses, even at high concentrations. Quats have the advantages of being odorless, nonstaining, noncorrosive to metals, stable, inexpensive, and relatively nontoxic. Caution should be used when handling concentrated quats; even a small droplet splashed into the eyes may cause damage to tissues. Be sure to wear safety glasses and proper PPE when handling these disinfectants. The concentration of the disinfectant used should be consistent with the manufacturer's specification on the label.

H.1.5 Formaldehyde

Formaldehyde for use as a disinfectant is usually marketed as formalin solution (37% concentration of the gas in water) or as paraformaldehyde, a solid polymerized compound. Formaldehyde with an active ingredient of at least 5% is an effective liquid disinfectant but loses its disinfectant activity at 4°C or less. It is pungent with an irritating odor and is a suspected human carcinogen. Formaldehyde vapor generated from formaldehyde solution is an effective space disinfectant for sterilizing rooms or buildings. Formaldehyde gas can be generated by heating paraformaldehyde crystals. Formaldehyde vapors and gas are toxic and can elicit hypersensitivity and irritation. Dilution should be prepared in a chemical fume hood. Respiratory protection may be necessary. Formaldehyde gas is flammable and may be explosive under certain conditions.

H.1.6 Phenol

Phenol itself is not often used as a disinfectant. The odor is somewhat unpleasant, and a gummy residue remains on treated surfaces, especially when used in steam sterilization. Although phenols themselves may not be in widespread use, phenol homologs and phenolic compounds are the bases of a number of popular disinfectants. Phenolic compounds are effective disinfectants against some viruses, rickettsiae, fungi, and vegetative bacteria. The phenolics are not effective in ordinary use against bacterial spores.

Concentrated phenolics should be used carefully; even a small droplet splashed into the eyes may cause damage to tissues. Phenolics are readily absorbed by the skin, and splashes can cause local irritations, severe burns, and systemic poisoning, leading possibly to death. Therefore, safety glasses and other appropriate PPE should be worn. Contaminated skin should be washed aggressively with water.

H.1.7 Other Vapors and Gases

Ethylene oxide, peracetic acid, beta-propiolactone, methyl bromide, hydrogen peroxide, and ethylene amine are compounds that, when vaporized, are an effective disinfectant. When used in closed systems and under controlled conditions of temperature and humidity, excellent decontamination results can be obtained. Ethylene oxide, although a suspected carcinogen, is convenient to use, versatile, and noncorrosive. Peracetic acid is corrosive for metal and rubber. Beta-propiolactone, although a suspected human carcinogen, in the vapor state acts rapidly against bacteria, rickettsia, and viruses. It has a half-life of 3–5 hours when mixed with water, is easily neutralized with water and lends itself to removal by aeration.

H.2 Summary of General Precautions to Follow When Using Chemical Disinfectants

When handling concentrated stock solutions of certain disinfectants, be aware of the potential hazards, and exercise caution. For general information about chemical safety, see Document 14.1, "Chemicals," in the *ES&H Manual*. Concentrated quaternary and phenolic disinfectants are particularly harmful to the eyes. Even a small droplet splashed in the eyes may cause blindness. Absorption of phenolic compounds by the skin can lead to local irritation, severe burns, and systemic poisoning, leading possibly to death. Constant or prolonged exposure to phenol may cause headache, dizziness, difficulty in swallowing, diarrhea, vomiting, shock, convulsions, and death. Safety glasses and proper personal protective clothing should be worn to avoid corrosion and depigmentation of the skin. Good ventilation is required when working with phenol to minimize inhalation.

Vapors of formaldehyde can elicit hypersensitivity and irritation; they are also toxic, and working in a chemical fume hood is highly recommended. Respiratory protection may be necessary.

Pertinent characteristics and potential applications for several categories of chemical disinfectants most likely to be used in the biological laboratory are summarized in Table G-1.

The suggested practical concentrations and contact times may differ markedly from the manufacturer's recommendations. The efficacy of any of the disinfectants should be conclusively determined by individual investigators. In addition, wastes associated with the use of disinfectants shall be managed according to the requirements in Document 36.1.

Appendix I

Biological Safety Cabinets

Ventilation control of infectious agents or other biologically derived molecules is usually achieved with a BSC. The three primary classes of BSC are Class I, II, and III, which differ in design and containment capability.

The selection of an appropriate BSC for a given operation shall be approved by the area ES&H Team industrial hygienist according to the operation and an evaluation of the BSL classification. In addition, all BSCs shall be tested and evaluated by certified personnel after purchase and installation but before use; after being moved, relocated or serviced; and at least annually thereafter. Each class of BSC is described below (see Table I-1 for performance guidelines):

Class I Biological Safety Cabinets. Class I cabinets are similar to a conventional laboratory hood with an open-face, negative-pressure design. These cabinets are most suitable for BSL 1 and some BSL 2 and 3 containment.

Class II (Laminar Flow) Biological Safety Cabinets. Class II cabinets are used to protect the user from research materials and to protect the research materials from external contamination. Class II cabinets use HEPA filters as contamination controls. The downward flow of laminar air creates a protective barrier and prevents room air from contaminating the sterile work surfaces inside the cabinet. Room air is exhausted through the front grille. The internal cabinet air is exhausted through the front and rear grilles. Class II cabinets are further divided into four basic types (Type A, B1, B2, and B3), which differ in such respects as the:

- Proportion of air recirculated into the work area.
- Airflow velocities into the work opening and down toward the work surface.
- Manner of exhaust air discharge.
- Air plenum pressure relative to the room.

Class III Biological Safety Cabinets. Class III cabinets are hermetically sealed enclosures for the handling of extremely infectious materials.

Table I-1. Performance guidelines for biological safety cabinets.

Class	Description	Minimum face velocity (fpm) ^a	Duct/Plenum Pressure (inches w.g.) ^a	Hazard/risk level
Class I	Front panel not in place	75	NA	None to low
	Front panel without gloves	150	NA	None to low
	Front panel with gloves	NA	0.5 inch	None to low
Class II, Type A1	30% of air is HEPA filtered and exhausted into the room or thimble-ducted out. 70% of air is HEPA filtered and recirculated into the work area.	min avg 75 fpm	Positive pressure	Low to moderate risk with absence of volatile toxic chemicals and radionucleotides
Class II, Type A2	30% of air is HEPA filtered and exhausted into the room or thimble-ducted out. 70% of air is HEPA filtered and recirculated into the work area.	min avg 100 fpm	Negative pressure	Low- to moderate-risk agents; minute quantities of toxic chemicals or radionucleotides only if ducted
Class II, Type B1	Most of contaminated air is hard-ducted out of building without recirculated; both supply and exhaust are HEPA-filtered.	min avg 100 fpm	Negative pressure	Low- to moderate-risk agents; minute quantities of toxic chemicals or radionucleotides ^b
Class II, Type B2	All of contaminated air is hard-ducted out of building without recirc; both supply and exhaust are HEPA-filtered.	min avg 100 fpm.	Negative pressure	Low- to moderate-risk agents; toxic chemicals or radionucleotides ^b
Class III	Supply air is HEPA filtered. 100% of the air is doubled HEPA filtered.	NA	Negative pressure with 0.5-inch pressure drop	Very high risk. Access through double door sterilizer and decontaminant dunk bath.

^a Manufacturers provide specifications for BSCs with National Sanitation Foundation certification. The information in this table is not always applicable. Face velocity is measured in feet per minute (fpm).

^b The sliding sash is adjustable. Experiments should be performed with an 8-inch opening for proper face velocity.

General Operating Information

Understand how the BSC works, and plan your work. Protect yourself, your research, and your coworkers.

Keep your laboratory meticulously clean. Minimize storage of boxes and supplies, particularly near the BSC. Wash your hands thoroughly before and after working in the BSC. Wearing a clean laboratory coat and gloves while working in a BSC increases your safety and helps reduce contamination of research materials.

BSC effectiveness depends on proper directional airflow (i.e., inward and downward, through high efficiency filters) and can be reduced by anything that disrupts the air flow patterns, e.g., rapid movement of arms in and out of the BSC, people walking rapidly past the opening of the BSC, down-drafts from ventilation systems, and opened laboratory doors.

How to Use a Biological Safety Cabinet Effectively

- Turn off the BSC ultraviolet lamp if in operation and turn on the BSC. Wipe the work surface and the front window glass with the appropriate germicide (e.g., 70% ethanol or a 1:10 bleach solution). Wipe off each item you need for your procedure and place in the cabinet. Allow the cabinet to run for at least 5 minutes before beginning work.
- Do not place any objects over the front grille, and do not block the rear exhaust grille. Work should be performed at least 6 inches back from the front grille.
- Segregate contaminated items away from clean items. Minimize movement of contaminated items over clean ones. Remember to work from the clean side to the dirty side.
- Put on a laboratory coat, and thoroughly wash hands. Wear gloves, as appropriate.
- Follow good microbiological techniques, such as holding open tubes and bottles as horizontal as possible. Use convenient mechanical pipetting aids. Do not mouth pipette. Place horizontal pipette discard pans containing appropriate disinfectant or water inside the BSC. Do not place vertical pipette discard canisters on the floor outside the BSC.
- The heat from the open flame creates turbulence in airflow and compromises sterility; heat buildup may damage the filters. If flaming is required, use a Bunsen burner with a pilot light or an electric loop incinerator.
- If you need to remove items from the BSC or add new items, move your arms slowly in and out of the cabinet to minimize airflow disruption.
- If you need to use a piece of equipment that creates turbulence in the BSC (e.g., centrifuge, blender, and sonicator), place equipment in the back third of the cabinet, and stop work while equipment is operating.

- Protect the building vacuum system from contamination by placing a cartridge filter between the vacuum trap and the source. See Appendix H for more information.
- Clean up any spills in the BSC immediately. See Appendix F for more information and guidance.
- Remove all materials and wipe all interior surfaces with 70% alcohol when you finish work. Let the cabinet run for 10 minutes, then turn it off. Examine the tray under the work surface. Disinfect and clean as necessary.
- Segregate waste by category and then properly dispose.
- Remove laboratory coat and wash hands thoroughly before leaving the laboratory.

Reference: NIH Laboratory Safety Monograph, "A Supplement to the NIH Guidelines for Recombinant DNA Research," July 1978.

Appendix J

Vacuum Line Filters

The aspiration of tissue culture media from monolayer cultures or of supernatants from centrifuge samples into primary collection flasks is a common laboratory procedure. Protection should be provided against aerosols of hazardous chemical or biological materials and against the overflow of fluid into the vacuum system. This protection is provided by the use of an air filter in the line immediately leading into the house vacuum line and an overflow flask for liquids between the collection flask and the air filter.

To assemble this protective apparatus (see Figure J-1), use flexible tubing of appropriate inside diameter for the flask and filter fittings and of sufficient wall thickness for the applied vacuum. Filter flasks of capacities from 250 to 4000 ml may be used for the overflow flask, depending on the available space and the amount of fluid that could be accidentally aspirated out of the collection flask. The overflow flasks should contain a disinfectant solution appropriate for the biohazardous material under study. It is essential that an antifoam agent (e.g., Dow Corning Antifoam A) be added to the overflow flask, because bubbling of air through the disinfectant will cause considerable foam that, if allowed to reach the filter, will shut off the vacuum.

If the filter becomes contaminated or requires changing, the filter and flask can be safely removed by clamping the line between filter and vacuum source. Before the old filter is discarded, both the old filter and flask should be autoclaved, after which a new filter can be installed and the assembly replaced. A cartridge-type HEPA filter removes airborne particles 300 nm (0.30 micron) in size or smaller and therefore provides an effective barrier to the passage of aerosols into the house vacuum system.

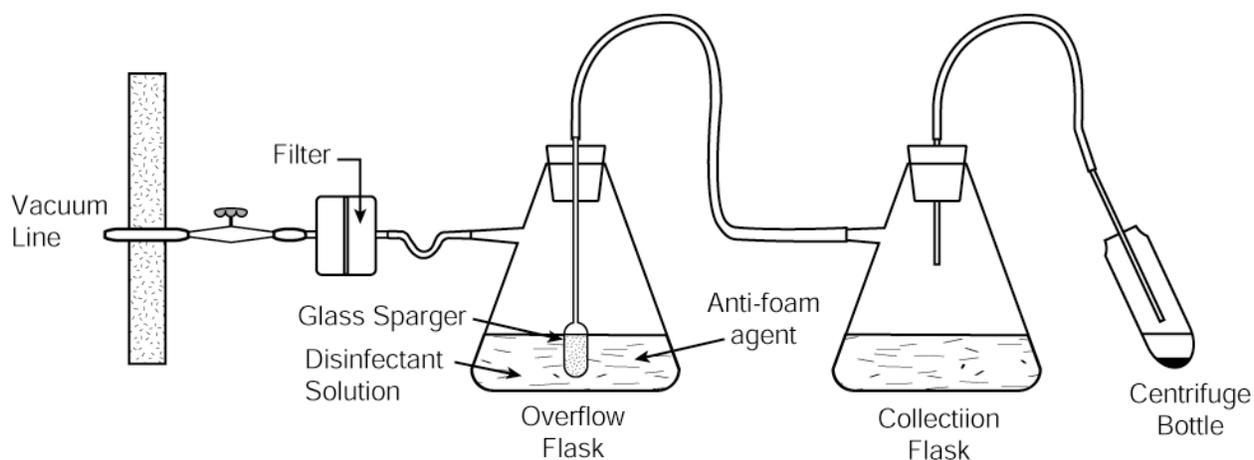


Figure J-1. Protective apparatus for vacuum system.