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Facility Safety Plan B360 Complex Biohazardous Operations CMLS-412r0

G.A. Cooper

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CMLS-412r0: Building 360 Complex Biohazardous Operations

Building 360 Complex

Biohazardous Operations

December 2006

Lawrence Livermore National Laboratory

Disclaimer

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Preface

Lawrence Livermore National Laboratory's (LLNL) Environment, Safety, and Health (ES&H) policy is that operations must be planned and performed safely, with full consideration for the law and for the protection of employees, the public, and the environment. In addition to observing LLNL policies contained in the *Environmental, Safety, and Health (ES&H) Manual,* LLNL employees shall comply with all federal, state, and local Codes, Standards, & Regulations (CS&Rs) when conducting any activity. Management has determined that the controls specified within this FSP Addendum must also be followed in order to perform operations efficiently and safely within this facility. Any operation conducted in this facility that does not conform to the *ES&H Manual* or to this FSP Addendum must be approved by an Integration Worksheet/Safety Plan (IWS/SP) that specifically assesses responsibilities, hazards, and the necessary controls to conduct the operation safely.

Everyone who enters this area (including employees, visitors, consultants, etc.) must follow this FSP Addendum. Each person is expected to make every reasonable effort to protect himself/herself and others from injury or illness. Regular facility occupants are expected to guide and govern visitors and to assist new or temporary occupants to understand and follow this procedure. When there are any doubts regarding the safety of any phase of work, personnel shall check with the Responsible Individual for the affected area.

Changes to this FSP Addendum shall be approved by the Associate Director (AD) for Chemistry, Materials, and Life Science Directorate.

This FSP Addendum was reviewed and concurred by:

Tracey Simpson — Date 12/19/06
ES&H Team 2 Leader
Hazards Control Department

Greg Cooper — Date 12/22/06
Facility Manager
CMLS Directorate

This FSP Addendum was approved by:

Thomas Diaz De La Rubia — Date 10/29/06
Associate Director
CMLS Directorate

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1.0 Introduction

1.1 Purpose

This Addendum to the Facility Safety Plan (FSP) 360 Complex describes the safety requirements for the safe conduct of all biohazardous research operations in all buildings within the 360 complex program areas. These requirements include all the responsibilities and authorities of building personnel, operational hazards, and environmental concerns and their controls. In addition, this Addendum prescribes facility-specific training requirements and emergency controls, as well as maintenance and quality assurance requirements for ES&H-related building systems.

The controls established in this FSP addendum conform to FSP 360 and the *ES&H Manual.*

1.2 Applicability

This Addendum covers biohazardous operations in facilities assigned to 360 complex programs.

Safety requirements in this FSP Addendum and the *ES&H Manual* shall apply to all participants in 360 complex program operations and individual users of 360 complex facilities. This includes staff members, contract workers, support and service staff, participating guests, short-term & long-term visitors, and summer students. Floor plans for all buildings occupied or controlled by the CMLS Directorate are available from the Facility Point of Contact (FPOC).

1.3 Changes

Any changes in operations that increase the hazard level, introduce additional hazards, or decrease safety shall not be made until a revision of or supplement to this FSP addendum has been reviewed and approved consistent with the review and approval process for the original FSP. Proposed changes in building safety and environmental control systems require a design review and must be approved by the Facility Manager.

1.4 Review

This FSP addendum will be formally reviewed and reissued at a minimum of every three years to ensure that its contents are appropriate and adequate for current operations. If changes are necessary before the triennial review date, the FSP will be amended and the changes approved by the Associate Director for CMLS Programs.

2.0 Scope

2.1 Authorized Operations

This addendum to FSP 360 approves biohazardous operations that are limited to Biosafety Level (BSL) 1 or 2 activities and are limited to small-scale, non-production research-laboratory scale amounts. For complete definitions of biosafety levels, refer to Document 13.6, "Safe Handling and Use of Biological Research Materials," of the *ES&H Manual*.

3.0 Hazards Analysis

3.1 General

The hazards associated with working with biohazardous agents will vary from personal exposure to accidental environmental releases. Personal exposure may result from the handling of toxic chemicals, infectious agents, animals, toxic gases, or extremely hot or cold objects. The degree of personal exposure will depend on infectious-contaminated sources, immune status of the host, and efficiency of the transmission of infection.

Accidental releases into the environment may cause an imbalance of the normal established micro-flora for a specific area. This may have an impact on specific food industries, sewage treatment facilities, and may pose a threat to the public health of the general public and to those individuals who may be immunologically compromised. Because of the serious ramifications to employee exposure and accidental releases into the environment, control measures required to prevent such events from occurring are now mandated by law.

3.2 Biohazards (Etiological Agents)

Biohazards are often referred to as etiological agents. Biohazards include both biohazardous materials and biological agents. Biohazards mean any biological material or their components that present a real potential risk of illness or injury to humans, plants, and animals. A summary of materials and controls are listed in Table 1 in Section 3.2.2.2.

3.2.1 Biohazardous Materials

Biohazardous materials are not capable of self-replication and are the components of biological agents that present a real or potential risk of causing illness or injury to humans, plants, and animals.

3.2.1.1 Biological Toxins or Biotoxins

Biological toxins or biotoxins are non-living biohazardous (toxic) proteins that are naturally produced by many different types of living organisms and are thousands of times more toxic by weight than chemical agents. Toxins (themselves) are not infectious or contagious to other host after exposure. However, the presence of toxin producing organisms may be infectious or contagious to a host after exposure.

3.2.1.2 Endotoxins

Endotoxins are associated with the outer membrane of certain gram-negative bacteria and are released only when the cells are disrupted. Endotoxins are not secreted. They are composed of complex

polysaccharides molecules responsible for eliciting an antigenic response resulting in fever, and altered resistance to bacterial infections. Exposure to large quantities may result in toxic hemorrhagic shock and severe diarrhea.

3.2.1.3 Clinical and Diagnostic Specimens

A diagnostic specimen is any human or animal material including but not limited to, excreta, secreta, blood, components, tissue, and tissue fluids, being handled for the purposes of diagnosis. Clinical and diagnostic specimens from human and animal sources may be hazardous. Typically, the infectious nature of clinical material is unknown and may contain potential human pathogens requiring specific handling procedures. For further description, see Document 13.2, "Exposure Control Plan - Working Safely with Blood and Blood borne Pathogens," of the ES&H Manual.

3.2.2 Biohazardous Agents

Biohazardous agents are biological in nature, usually capable of self-replication and have the capacity to produce deleterious effects on other biological organisms, particularly humans. They include, but are not limited to, various viruses, prions, chlamydia, bacteria, fungi, yeast, algae, plants, animals, and any products that may contain any of these agents.

Factors to be considered in determining the level of containment include agent factors such as virulence, pathogenicity, infectious dose, environmental stability, route of spread, communicability, operations, quantity, availability of vaccine or treatment, and gene product effects such as toxicity, physiological activity, and allergenicity.

3.2.2.1 CDC and NIH's Classification of Etiologic Agents

The CDC and NIH have classified etiological agents into 4 Risk Groups. An organism of higher classification would indicate a higher level of hazard or higher risk group.

Risk Group (RG) 1: Agents not associated with disease in healthy human adult.

Risk Group (RG) 2: Agents that are associated with human disease that is rarely serious for which preventive or therapeutic interventions are often available.

Risk Group (RG) 3: Agents that are associated with serious or lethal human disease for which preventive or

therapeutic intervention may be available (high individual risk but low community risk).

Risk Group (RG) 4: Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic intervention are not available (high individual risk and high community risk). Prohibited at LLNL.

TABLE 1: CLASSIFICATION OF CURRENT RESEARCH IN 360 complex		
Examples	**Biosafety Level 1**	**Biosafety Level 2**
Laboratories	Basic Teaching	Human Blood, Blood Products & Body Fluids
		Hospital Diagnostic
		Teaching and Public Health
Laboratory Work practices	Good Microbiological Practices	Good Microbiological Practices, Protective Clothing, and Biosafety Signs
Safety Equipment	None, Open Bench Work Permitted	Open Bench Work Plus Biological Safety Cabinets for Potential Aerosols
Etiological Agents, bacterial Viruses and Plasmids - Some with Mammalian DNA Inserts	Risk Group 1 Agents	Risk Group 2 Agents
	Lamda	
	M13	
	P1	
	pBR322	
Mammalian Cell Lines	CHO, V79	Epstein-Barr Transformed Lymphoblastoid Cells
	CHO-Human Hybrids	
	Mouse Myeloma	Viable Human Tumor, or Human Tumor Cells
	Mouse Tumor Cells	
	Mouse Lymphoma	
	Mouse Tissues	
		Viable Samples of Human and Primate, Bone, Marrow, Serum, Semen, Blood, Urine, and Other Potentially Infectious Cells/ Secretions
		Untransformed Normal Human Cell Lines (Except for Lymphoblastoid Cells)

TABLE 1: CLASSIFICATION OF CURRENT RESEARCH IN 360 complex		
Examples	**Biosafety Level 1**	**Biosafety Level 2**
	Fixed Human/Primate Tissues	Unfixed Human/Primate Tissues
Oncogenic Viruses		Low Risk and Moderate Risk Oncogenic Virus

3.2.2.2 NCI Classification of Oncogenic Viruses

The National Cancer Institute (NCI) has defined oncogenic viruses as a special class of etiologic agents that have been categorized into 3 classifications of risk: Low Risk, Moderate Risk, and High Risk (Section 11, Ref. 11.3).

3.3 Human Blood and Bodily Fluids

Human blood and human body fluids have been determined by the Occupational Safety and Health Administration (OSHA) to be potentially contaminated with blood borne pathogens (4). As listed in Table 2, blood borne pathogens include, but are not limited to, the Hepatitis B Virus, Hepatitis C Virus, and the Human Immuno-deficiency Virus. See Document 13.2, *"Exposure Control Plan: Working Safely with Blood and Bloodborne Pathogens,"* of the *ES&H Manual* for more information regarding blood borne pathogens.

TABLE 2: LIST OF BLOODBORNE PATHOGENS (1,2,5)				
Disease/ Agent(s)	**Common Names**	**Risk**	**Incubation**	**Sources (*)**
Serum Hepatitis/ Hepatitis B Virus	Hepatitis B (42 nm) Hepadnavirus dsDNA	6-10% 1-2%Fatal	11 weeks	Blood, cerebral spinal fluid (CSF), Semen, Saliva
Transfusion Hepatitis/ Hepatitis C Virus	Hepatitis C, Non A-Non B (40-60 nm) Flaviviridae (ss RNA)	0.5-1% Fatal	7 weeks	Blood, Serum
AIDS: Retroviridae(100 nm) (Oncornavirus - RNA)	HIV - 1, LAV (formally HTLV 3)	< 0.5% 100% Fatal	Adults: 8 yrs Infanst: 2 yrs	Blood, Serum, CSF Saliva, Tears, Urine, Breast Milk, Amniotic Fluid.,
	HIV-2 (West Africa) HTLV IV	<8.9% 100% Fatal	Unknown	Blood, Serum, CSF Saliva, Tears, Urine, Breast Milk, Amniotic Fluid
Leukemia/ Lymphomas Human T - Lymphotropic Virus	Retroviridae(Oncornavirus 100 nm) HTLV I	18-49%	Unknown	Blood
	HTLV II	52%	Unknown	Blood
	HTLV V	Unknown	Unknown	Blood

* - Human sources are noted unless otherwise stated.
These human materials are not considered biohazardous unless either diagnosed with disease or there is strong reason to suspect disease is present

3.4 Recombinant DNA Experiments

Recombinant DNA experiments include any experiments involving the construction and handling of recombinant DNA molecules, organisms, and viruses containing recombinant DNA molecules and work involving gene therapy. Recombinant DNA molecules is defined as either:

(i) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or

(ii) molecules that result from the replication of those described in (i) above.

The molecules that are expressed from synthetic or recombinant DNA 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. They are considered to be in the same risk group as those of the original host organism in which the DNA was found. Recombinant DNA experiments may involve microbial, animal, or plant host. In accordance with NIH guidelines (Section 11, Ref. 11.8), all work involving recombinant DNA must be submitted to the Institutional Biosafety Committee (IBC).

3.5 Cell (Tissue) Culture Lines

Cell culture lines are known to harbor latent viruses adventitiously or from deliberate experimental infections and pose a potentially undetected hazard. Established cell lines of sub-primate or normal primate origin that do not harbor a primate virus (and are not contaminated with bacteria or fungi of greater than BSL-1) may be handled in a BSL-1 containment facility. Primary and permanent human or animal cell lines may be regarded as carrying Class 1 oncogenic viruses unless they are known to be infected with a more hazardous agent(s).

Permanent human lymphocyte cell cultures or primate cell lines derived from lymphoid or tumor tissue should be handled with the assumption that they harbor the Risk Group 2 agent, Epstein barr virus, (EBV). Additionally, all cell lines that have been converted, exposed, or transformed by a primate oncogenic viruses; and all new cell lines must be handled in a BSL2 containment facility. Under no circumstances shall anyone work with cells derived from themselves or from first-degree relatives, since the host immune system may not provide adequate protection.

3.6 Human Subjects

Work with human subjects requires approval from the Institutional Review Board (IRB). The IRB ensures that the rights of human subjects are protected and to assure their informed voluntary consent to participate in human subjects research is also protected.

3.7 Animals

Working with animals poses unique hazards to researchers, the animals themselves, and to Animal Care Facility personnel handling the animals. Special care should be taken to limit exposure to zoonotic agents that exist as animal flora. Experimental work with any animal requires the approval from the Institutional Animal Care and Use Committee (IACUC) to ensure the humane care of animals and experimentation (Section 11, Ref. 11.6).

3.8 Hazardous Materials

Working with biohazardous materials often requires working with radioactive and hazardous materials. The hazards dealing with radioactive material and/or hazardous materials are discussed in Section 5.0, "General Building Safety Limits and Controls," of FSP 360.

4.0 Responsibilities and Authorities

Overall responsibility within the Biosciences Program is discussed in Section 4.0, "Responsibilities and Authorities," of FSP 360.

5.0 Health and Safety Controls

5.1 Administrative Controls

5.1.1 Protocol Review Process

All research activities involving the use of human subjects, animal subjects, and biohazardous materials or DNA technology will require institutional review with the LLNL Biosafety Operations Committee (LBOC), and approved by the Institutional Review Board (IRB), Animal Care and Use Committee (IACUC), and the Institutional Biosafety Committee (IBC), respectively. Contact the area Industrial Hygienist supporting for more information on working with these committees.

- The LLNL Biosafety Operations Committee (LBOC) provides experimenters with an initial single point of contact, determines the level of required review, identifies the associated hazards, recommends the level of training required, recommends the need for medical surveillance, proposes changes to the *ES&H Manual*, and addresses biosafety issues requested by Council of Biosciences and Biotechnology

- Institutional Review Board (IRB): Federal regulations and Laboratory policy require that approval by the IRB be obtained before any research project involving use of human subjects or human tissue be initiated in order to protect the rights and welfare of individuals who volunteer to become subjects in research projects.

- Institutional Animal Care and Use Committee (IACUC): Approval by the IACUC shall be obtained prior to initiating any research involving vertebrate animals. The IACUC must determine if the proposed work meets acceptable standards for the care and use of animals in research.

- Institutional Biosafety Committee (IBC): The IBC shall review all research protocol involved in the use of recombinant DNA technology, artificial gene transfer, biological agents (bacteria, viruses, protozoa, fungi, etc.), toxins (natural and synthetic), and biological materials from human and animal sources (e.g., tissues, body fluids, cell cultures, etc.). Federal regulations under 29 CFR 1910.1030 require that any work with human blood, other human materials, or human blood borne pathogens and under 42 CFR Part 72 for any work with infectious materials be reviewed to determine exposure potential for workers. The IBC approves and/or registers all biological research at LLNL. In addition, the IBC is responsible for determining whether a protocol complies with applicable rules and regulations and that it meets appropriate biosafety containment standards as

set forth by the National Institutes of Health (NIH) Guidelines on recombinant DNA.

CMLS review. The administrative control first used to mitigate hazards is the initial Integration Worksheet (IWS) that is prepared by the RI and reviewed by Program Management with assistance from the ES&H Team. Operations covered under this FSP will require an IWS to determine if a supplemental SP will be required.

5.1.2 Work Practice Controls

Work with biohazardous agents and/ or materials on site and will be limited to Biosafety Level (BSL) 1 or 2 Containment Levels. Appropriate BSL work practices and containment guidelines will be followed and are summarized in Section 3.2.2.2, Table 1 (Section 11, Refs. 11.8 & 11.9).

Universal precaution is an approach to infection control in which all human blood and certain human body fluids are treated as contaminated with blood borne pathogens. Universal Precaution work practices are listed in Appendix B of this document.

Frequent hand washing is encouraged. Hands should be washed with soap and water after removing gloves, after handling potentially infectious materials, and before leaving the laboratory.

Animals: All research animals are routinely handled in accordance with existing regulation guidelines (Section 11, Ref. 11.6), and LLNL Animal Care Facility Standard Operating Procedures. As a result, all animals are quarantined for a period of 7-14 days before being used as part of the research protocol. Appropriate animal biosafety levels are assigned in accordance to the hazard.

Decontamination: All surfaces must be decontaminated before initiating any work and at the end of each workday. There are 3 levels of biological decontamination of microorganisms (see below). The preferred method for activities covered under this FSP is disinfection unless working in a sterile environment.

1. Sanitization: Involves the general reduction of microorganisms by use of general cleaning agents.

2. Disinfection: Involves the destruction of targeted organisms with the use of chemicals or physical agents. For example, table tops and surfaces can be decontaminated with disinfectants such as amphyl or broccal.

3. Sterilization: Sterilization is the complete destruction of all microbes and is generally used in the total decontamination process.

Biohazard Signs must be placed at the entry of each laboratory where Risk Group 2 biohazardous materials are handled or stored. The biohazard symbol (see Figure 1 below) is used as a warning sign. The sign should indicate the biohazard present, the person(s) responsible for the work, and any restrictions on access to the room. The biohazard symbol is normally red on a white field. The sign is not to be placed at the entry of laboratories where only Risk Group 1 are handled or stored.

Figure 1

5.1.3 Medical Waste Management

Medical waste is a unique category of waste, which is regulated by California's Department of Health Services (DHS) and enforced by Alameda County Health Care Services Agency for the LLNL Main Site and enforced by the San Joaquin County Public Health Services for Site 300. In addition to the guidance described below on management of these wastes, refer to Section 5.8, *"Environmental Hazards and Controls"* of the Building 360 FSP, and the *ES&H Manual,* Document 36.1, *"Hazardous, Radioactive, and Biological Waste Management Requirements,"* Section 3.0, *"Administrative Controls."*

Medical waste consists of biohazardous waste, sharps waste contaminated with a biohazardous component, and trauma scene waste. Definitions of each waste type are included in the following sections.

5.1.3.1 Biohazardous Waste (includes any of the following)

- Animal parts, tissues, fluids, or carcasses that are known to be infected with diseases that are highly communicable to humans.
- Laboratory wastes containing human or animal specimen cultures, cultures and stocks of infectious agents, and waste from

the production of bacteria, viruses, and spores, discarded human or animal vaccines, and culture dishes.

- Recognizable human blood, in fluid form, and items containing enough human blood that the item releases the blood upon compression (i.e., soaked bandages or clothing).
- Human or animal excretion, exudate, or secretions required to be isolated by infection control staff.
- Human surgery specimens or tissues removed at surgery or autopsy suspected of being contaminated with infectious agents communicable to humans.

5.1.3.2 Sharps Waste

Sharps waste is any device having acute, rigid corners, or edges capable of cutting or piercing. Sharps wastes contaminated with biohazardous components are regulated as medical wastes. Examples of regulated sharps wastes include the following:

- Broken glass items, such as blood vials and pipettes, contaminated with biohazardous waste.
- Discarded hypodermic needles, syringes, blades, and needles with attached tubing, and scalpels that have been contaminated with biohazardous wastes.

5.1.4 Medical Waste Accumulation and Storage

5.1.4.1 Biohazardous Waste

- Biohazardous wastes must be collected in red biohazard bags labeled with the words "biohazardous waste" or with the international biohazard symbol and the word "biohazard" and placed inside rigid containers with appropriate labeling. Double-bagging is often a best management practice to prevent leakage of the waste into the rigid container.
- The rigid leak-proof container must be labeled with the words "biohazardous waste" or with the international biohazard symbol and the word "biohazard" on the lid and on all the sides.
- Biohazardous waste should be disposed on a daily basis, however, for large quantity generators (all programs at the LLNL main site), biohazardous waste may be accumulated for up to 7 calendar days after the first waste article is placed in the container if the waste is accumulated above 0°C. (Biohazardous waste may be accumulated for up to 90 days below 0°C if freezer space is available).

- For small quantity generators (Site 300), biohazardous waste may be accumulated for up to 30 calendar days after the first waste article is placed in the container if the waste is accumulated above 0°C. (Accumulate such waste for 90 days if accumulated below 0°C).

- If the waste contains hazardous and/or radioactive components, as well as medical waste components, the waste may be subject to hazardous, radioactive, or mixed waste regulations, rather than the medical waste regulations. Contact the area Environmental Analyst for assistance in the characterization of waste and decontaminating procedures to be performed prior to Radioactive and Hazardous Waste Management's (RHWM) receipt of non-medical wastestreams described in the section above.

5.1.4.2 Sharps Waste

- Biohazardous sharps wastes must be collected at the site of generation and accumulated and stored in red, leakproof puncture resistant sharps containers that are labeled as "Sharps Waste" or with the international biohazard symbol and the word "biohazard."

- Non-biohazardous sharps waste must also be collected in rigid sharps containers. However, these sharps containers should be of any color other than red and should not contain the embossed biohazard symbol on the container, but rather the appropriate label that represents the characterization of the waste (i.e., hazardous waste label, radioactive waste label, mixed waste label, or nonhazardous waste label).

- Sharps wastes contaminated with biohazardous components which are accumulated above 0°C (at room temperature) in the red sharps containers may accumulate until the container reaches the 3/4 fill-line. Sharps wastes which are accumulated above 0°C must be treated within 7 calendar days after the waste container is 3/4 full. However, if freezer space is available, sharps wastes may be transferred to the freezer and stored below 0°C for up to 90 days after the container becomes 3/4 full following the accumulation time at room temperature. The waste must then be treated by the end of the 90-day storage period. *Note that once the container becomes 3/4 full at room temperature, the waste must be transferred to the freezer within seven days from that day so that the treatment time-limit is not exceeded. If the container is stored at room temperature for any time once the container becomes 3/4 full prior to transfer to the freezer, the*

period of time the container is stored at room temperature must be included as part of the 90 day storage timeframe in the freezer.

- Home-generated sharps (i.e., syringes used for self-medicating purposes) must also be disposed of in rigid sharps containers. However, syringes used for self-medicating purposes may not be disposed of through LLNL. LLNL currently does not supply individuals who generate such syringes with red rigid sharps containers. These individuals are responsible for obtaining their own personal impervious containers for home-generated sharps wastes and disposing the containers appropriately at home. The local waste management service at home can provide individuals with home-generated sharps disposal guidance.

5.1.5 Medical Waste Disposal

Medical waste generated at various locations at the LLNL main site are transported to the B-360 Complex every Wednesday before 9:00 a.m. where the waste is treated by steam sterilization (autoclaving). Note: waste containing a medical waste component and hazardous, radioactive, mixed (radioactive and hazardous), or California combined (radioactive and regulated as hazardous only by California) is not considered medical waste and is not autoclaved. Instead, it is managed through Radioactive and RHWM using a waste disposal requisition (WDR) as described in FSP 360. The biohazardous component must be eliminated through disinfection prior to disposal through RHWM. Contact the area Industrial Hygienist prior to adding disinfectant to ensure the disinfectant will not adversely react with any chemical components in the waste. Specific type, concentration, and volume of the disinfectant used must be noted on the WDR prior to submittal of the WDR to RHWM for approval.

5.1.5.1 Biohazardous Waste

- Solid biohazardous waste is autoclaved in special autoclaves permitted by Alameda County Health Care Services Agency and then placed in the municipal trash.
- Liquid biohazardous waste may be disposed of into the sanitary sewer on a case-by-case basis following proper decontamination procedures and prior approval from the area Environmental Analyst.

5.1.5.2 Sharps Waste

Sharps waste containing only biohazardous components are first autoclaved in special autoclaves permitted by Alameda County Health Care Services Agency to eliminate the biohazardous

component and are then sent offsite for incineration through RHWM. Generators are required to complete waste disposal requisition forms for all sharps wastes generated at LLNL

5.1.5.3 Liquid Biohazardous Waste

Liquid biohazardous waste must have the biohazard destroyed by adding bleach at a concentration agreed to by the area Industrial Hygienist (typically a 10% final concentration of household bleach). Once decontaminated, the waste shall be placed in an appropriately labeled (nonhazardous or radioactive waste label) waste container. Contact the RHWM technician to provide the waste containers and for more information regarding disposal of specific items. Note: Use of disinfectant to destroy the biohazard in a waste that is also hazardous requires special procedures be followed. Contact the area environmental analyst in such a case.

5.1.6 Medical Waste Documentation

LLNL main site medical waste generators are required to maintain an updated waste treatment record to ensure that their waste was successfully sterilized (by permitted autoclave) within the regulatory timeframes. Current generators use a standard log to maintain such information.

Operators of autoclaves used onsite to sterilize medical waste are responsible for ensuring that the following autoclave recordkeeping requirements are met:

- Annual autoclave calibration with records,
- Monthly *Bacillus stearothermophilus* ampule test with records,
- Records of heat-sensitive tape results for each waste load that is autoclaved.

5.1.7 Decontamination

Reusable rigid secondary containers used to hold biohazardous waste shall be thoroughly washed and decontaminated at least monthly and whenever a work contamination is noted (e.g., leaky bags), unless the surfaces of the container have been completely protected from contamination by disposable liners, bags, or other devices removed with the waste. Approved methods of decontamination include the following:

- Exposure to hot water of at least 82 degrees centigrade (180 degrees Fahrenheit) for a minimum of 15 seconds, or
- Exposure to chemical sanitizers (i.e., rinsing with or immersion in) a solution containing one of the following for at least 10 minutes:
    - Quaternary ammonium solution (400 ppm active agent)

- Iodoform solution (100 ppm available iodine)
- Phenolic solution (500 ppm active agent)
- Hypochlorite solution (10% aqueous solution of household bleach that contains 5% hypochlorite)

5.1.8 Environmental Protection Department Contacts

Please 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 RHWM field technician can assist in ensuring receipt of proper biohazardous and sharps containers, labels, and waste disposal requisition forms which must accompany all sharps wastes and biohazardous wastes with a hazardous or radioactive component.

5.1.9 Transportation

Transportation of biological material must be performed in a manner that does not pose a threat or injury to personnel and property. Transportation of such materials involves the use of both proper packaging and shipping.

Onsite transportation of biological materials is performed using labeled, leak-proof secondary containers. Hazardous and biohazardous materials, substances, and/or wastes, excluding analytical samples, may not be transported in bicycle baskets, lab coats, automobiles, or personal vehicles. For more information on the transportation of chemical and biological materials, 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 (DOT), International Civil Aviation Organization (ICAO), U.S. Department of Agriculture (USDA), and the Centers for Disease and Control (CDC). Offsite shipping of biological and chemical materials must be done in accordance with Document 21.1, "Acquisition, Receipt, Transportation, and Tracking of Hazardous Materials," in the *ES&H Manual*. For specific packaging and shipping procedures, contact either the LLNL Transportation Office or the Materials Management Department.

5.1.10 Medical Surveillance

Staff who work with pathogens, human blood, or other human bodily fluids will be offered vaccination (i.e., the Hepatitis B Vaccine: 3 injections over 6 months) to prevent illness in the event of accidental infection. Additional information regarding the vaccine can be obtained from the Health Services Department.

5.2 Engineering Controls

5.2.1 Engineering controls required for work with biohazardous materials include all requirements associated with working with hazardous materials. Engineering controls, in combination with safe work practices that alter the manner in which tasks are performed, are expected to be primary means of eliminating or minimizing the risk of occupational exposure. Engineering controls are used to isolate or remove hazards from the workplace in order to reduce the potential for exposure. Such engineering controls include, but not limited, to mechanical aids, dead air boxes, laboratory-type fumehoods, biological safety cabinets (BSC), and negative air flow units, which are discussed in detail on the following page.

- *Mechanical Aid*– Mouth-pipetting of any substance is prohibited and the use of mechanical aids to transfer potentially harmful substances (i.e., biohazardous) is strictly enforced. Use of aerosol-resistant tips and aerosol-reducing practices shall be implemented whenever practicable. Personnel shall use these devices for all pipetting.

- *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 must 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. Fumehoods are available in most laboratories within CMLS and must not be used for long-term storage of materials and equipment merely as a matter of convenience. Fume hoods are not to be used for biohazardous operations.

- *Negative Air-Flow Unit*–All animals being injected with infectious materials, receiving mutagens or carcinogens supplemented into their food or water will be housed in negative air-flow animal units. Cages will be labeled as to the type of hazard present, amount and route of exposure, and the date of administration. Signs placed on each unit will indicate if (1) animals are currently being exposed, (2) animals were exposed in the past, or (3) animals that were not exposed.

- *Biological Safety Cabinets*–Biological safety cabinets provide sterile laminar airflow onto the work surface, containment of aerosols or droplets, and protection to the user. There are 3 different classifications of BSC: Class 1 to Class 3. The performance of BSC must be verified at the time of installation, whenever they are moved, and annually thereafter. For more information regarding BSC, see Appendix E and F of this plan and

Document 13.1, *"Biological Controls and Operations*," of the *ES&H Manual*.

- *Containment Protection For Vacuum Systems*–The aspiration of tissue culture media from monolayer cultures or of supernatants from centrifuge samples into primary collection flasks are a common laboratory procedure. Protection should be provided against drawing aerosols of hazardous chemical or biological materials or overflow fluid into the house vacuum system. This protection is provided by the use of an air filter (e.g., vacuum protection filter, 0.2 μm) 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 more information regarding the assembly of this device, see Appendix A of this document.

- *Sharps Containers*– "Sharp Containers" that have become 3/4 full should be disposed. See Section 5.1.5 for more information regarding waste disposal.

- *Engineered Sharps Injury Protection (ESIP)*– If hypodermic syringe needles, surgical scalpels or other sharps must be used, they shall incorporate ESIP features whenever possible (e.g., re-sheathable, retractable scalpels, intrinsically-safe needles, blunt-tip needles). If a procedure using a needle does not require the piercing function, then a blunt-tipped needle should be used. All needles are to be disposed of in a properly labeled sharps container.

5.3 Personal Protective Equipment

Identifying and understanding the hazard and then matching the needed personal protective equipment to the workplace hazard is the key to selecting effective and appropriate protection. Respirators are not recommended and unnecessary when biological safety cabinets are available for use.

Laboratory coats minimize skin exposure and protect street clothes from being contaminated. Laboratory coats must be worn while working in the laboratory and are not to be worn in non-laboratory areas (e.g., offices). The use of gloves and a face shield (in conjunction with eye protection) is highly recommended when splashing is anticipated and will be designated in appropriate safety documentation, such as the IWS, HAC, etc. Glove selection is important when dealing with a variety of chemicals and biological materials. Contact the ES&H Team Industrial Hygienist for more information regarding selection. Eye protection is required to be worn at all times while working in the laboratory.

6.0 Emergency Controls

6.1 Handling Spills

In any laboratory operation, the possibility of a spill exists. A thorough understanding of the potential hazards of the experiment, as well as careful planning of a spill response protocol, will help minimize personal injury and property damage. See Appendix D for more information regarding the clean up of small-scale spills.

7.0 Environmental Concerns and Controls

Environmental concerns and controls are discussed in Section 5.8, "Environmental Hazards and Controls," of FSP 360 and Document 36.1, "Hazardous, Radioactive, and Biological Waste Management Requirements," of the *ES&H Manual*. Medical and biohazardous waste management is discussed in Section 5.1.3 of this addendum.

8.0 Emergency Response

Emergency Response procedures are discussed in Section 8.0 of FSP 360.

9.0 Maintenance, Inspection, and Quality Assurance of Safety Systems and Equipment

All facilities included in this FSP are also included in Section 6.0 of FSP 360, which covers these issues. Specific to this FSP are BSCs, which are maintained by CMLS personnel. All BSCs receive yearly inspections and preventive maintenance by an approved vendor (Section 11, Ref. 11.7).

10.0 Training Requirements

All training requirements are discussed in Section 7.0 of FSP 360.

11.0 References

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

11.2 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.

11.3 National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses, 1974 (DHEW, Government Printing Office - 2975, Pub. No. NIH 75-790).

11.4 Occupational Safety and Health Administration - Occupational Exposure to Bloodborne Pathogens (29 CFR 1910.1030) Federal Register Vol 56. No. 235, December 6, 1991.

11.5 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.

11.6 U.S. Department of Health and Human Services, Public Health Service National Institutes of Health, Animal Welfare. 9 Code of Federal Regulations.

11.7 U.S. Department of Health and Human Services, Public Health Service Centers for Disease Control and Prevention. Additional Requirements for Facilities Transferring or Receiving Select Agents (CDC), 42 CFR 72.6.

11.8 U.S. Department of Health and Human Services, Public Health Service Centers for Disease Control and National Institutes of Health, Guidelines for Research Involving Recombinant DNA Molecules. Revised October 2000, (65 FR 60328).

11.9 U.S. Department of Health and Human Services, Public Health Service Centers for Disease Control and National Institutes of Health, Biosafety in Microbiological and Biomedical Laboratories (BMBL) (U.S. Government Printing Office, Washington, DC, 1993-DHHS Pub. No. 93-8395).

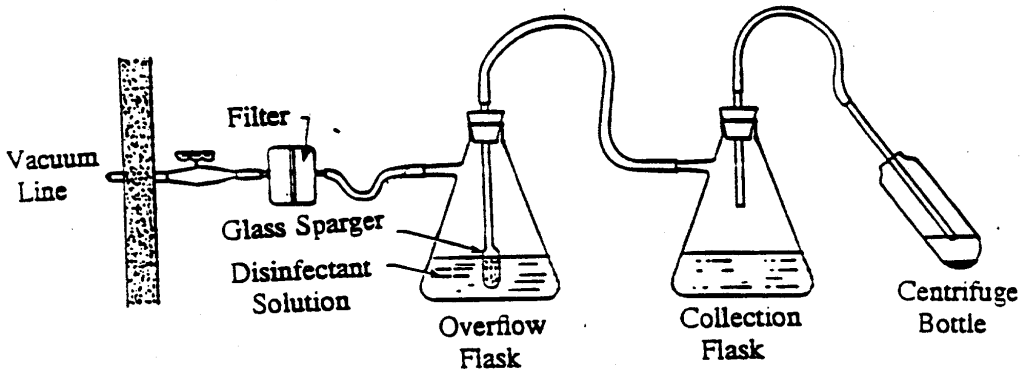
Appendix A: Vacuum Line Filter

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 drawing aerosols of hazardous chemical or biological materials or overflow 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, 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, such as Dow Corning Antifoam A, be added to the overflow flask, since 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. The filter and flask should be autoclaved before the filter is discarded. A new filter can be installed and the assembly replaced.

This protective apparatus for the vacuum system is shown in the figure below. A cartridge-type HEPA filter provides an effective barrier to passage of aerosols into the house vacuum system. The filter has a capacity to remove airborne particles 300 nm (0.30 mm) or larger in size.



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

Appendix B: Universal Precautions

Universal precautions shall be followed to prevent contact with blood and other potentially infectious materials to reduce the risk of occupational exposure. Universal precaution is an approach to infection control where all human blood and certain human body fluids are treated as if known to be infectious for HIV, HBV, and other blood borne pathogens. The following precautions are advocated by CDC for health care workers:

1. All healthcare and laboratory workers will use appropriate barrier precautions to prevent skin and mucous membrane exposure when contact with blood or bodily fluids are anticipated.
2. Gloves must be worn when touching blood, bodily fluids, mucous membranes, or non-intact skin.
3. Gloves must be worn when handling items or surfaces contaminated with blood and bodily fluids.
4. Gloves must be worn while performing venipuncture and other vascular access procedures.
5. Gloves must be changed after contact with each patient.
6. Mask and protective eyewear or face shields should be worn during procedures that are likely to generate droplet of blood or other bodily fluids in order to prevent exposures of the mucus membranes of the mouth, nose, and eyes.
7. Gowns or aprons should be worn during procedures that are likely to generate splashes of blood or other bodily fluids.
8. Hands and other skin surfaces should be washed immediately and thoroughly with water and antiseptic cleanser if contaminated with blood or other bodily fluids.
9. Hands should be immediately washed after gloves are removed.
10. Employee must 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.
11. To prevent needle-stick injuries, needles should not be recapped, bent or broken by hand, removed from disposable syringes, or otherwise manipulated by hand.
12. After they are used, disposable syringes, needles, scalpels blades, and other sharp items must be placed in puncture-resistant containers for disposal. These containers should be as close as practical to the area where disposable sharps are used.
13. Laboratory personnel who have exudative lesions or weeping dermatitis must refrain from handling clinical samples or specimens and contaminated equipment until the condition is resolved.
14. Pregnant employees should review safe work procedures with supervisors & HCD.

15. Management of regulated waste shall be managed according to guidance in Section 5.1.7 of this addendum and Section 5.8 of the Building 360 Complex FSP.

Appendix C: Disinfectants

Chemical Disinfectants

Pertinent characteristics and potential application for several categories of chemical disinfectants most likely to be used are summarized below.

Alcohol

A concentration of 70 - 80% by weight of Ethyl or isopropyl alcohol is commonly used as a general disinfectant. Alcohol will denature proteins and is somewhat slow in their germicidal action. Although they are good general disinfectants, they exhibit no activity against bacterial spores. Concentrations greater than 80% are less effective.

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 used directly on a stainless surface, rinse thoroughly with water after decontamination to prevent tarnishing.

Chlorine solutions have a limited shelf life and will gradually lose its strength over time. Fresh solutions must 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 to 10 diluted solution will contain 5,250 ppm of available chlorine. Decontamination with this concentration will require a 30-minute contact time. **Do not autoclave chlorine solutions or materials treated with them.** The residual chlorine will vaporize and result in an inhalation hazard.

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 (25 ppm) to 3 oz in 5 gal (75 ppm or 0.0075%) of water. The disinfectant characteristics of iodophor are similar to chlorine in that small amount of iodophors can be 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 any appreciable amount of protein is present or when the pH is below 6 or above 7. For washing hands or for use as a sporicide, it is recommended that Wescodyne be diluted 1 to 10 in 50% ethyl alcohol. This will yield 1,600 ppm of available iodine; a relatively rapid inactivation of all microorganisms will occur.

Appendix C: Disinfectants (continued)

Quaternary Ammonium Compounds or Quats

After years of testing and use, there is still considerable controversy about the efficiency of the "Quats" as disinfectants. These cationic detergents are strongly surface-active, and this detergent property makes them good surface cleaners. They will attach to protein and will lose effectiveness in the presence of proteins. The Quats tend to clump microorganisms and are neutralized by anionic detergents such as soap. Quats are bacteriostatic, tuberculostatic, sporostatic, fungistatic, and algistatic at low concentrations. They are biocidal against lipophilic viruses at medium concentrations, but they are only biostatic virucidal against hydrophilic viruses even at high concentrations. The Quats have the advantages of being odorless, nonstaining, noncorrosive to metals, stable, inexpensive and relatively non-toxic. 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 personal protective equipment when handling these disinfectants. The concentration of the disinfectant used should be made in accordance to manufacturer's specification on the label.

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 containing a >5% active ingredient is an effective liquid disinfectant but loses its disinfectant activity at 4˚C or less. It is pungent and irritating odor and is a 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/or irritation. Dilution should be made in a chemical fume hood. Respiratory protection may be necessary. Formaldehyde gas is flammable and may be explosive under certain conditions.

Phenol

Phenol itself is not often used as a disinfectant. The odor is somewhat unpleasant, and a gummy residue remains on treated surfaces. This is especially true during steam sterilization. Although phenols itself may not be in widespread use, phenol homologs and phenolic compounds are bases of a number of popular disinfectants. The 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 lead to local irritations, severe bums, and systemic poisoning leading possibly to death. Consequently, safety glasses and other proper personal protective equipment should be worn. Contaminated skin should be washed aggressively with water.

Appendix C: Disinfectants (continued)

Other Vapors and Gases

Vaporous Hydrogen Peroxide (VHP) is found to be an effective disinfectant. When used in closed systems and under controlled conditions of temperature and humidity, excellent decontamination results can be obtained.

Cautions Required When Applying Chemical Disinfectants

When handling concentrated stock solutions of certain disinfectants, be aware of the potential hazards and exercise caution. 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 to 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 the ES&H Manual, Document 13.1, *"Biological Controls and Operations,"* Section 3.0, Table-2 *"Summary of liquid, gaseous, and physical decontamination agents for biological agents and toxins"* (http://www.llnl.gov/es_and_h/hsm/chapter_3.0/chap3.0.html#3.0.2). 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.

**Wastes associated with the application of disinfectants will be managed according to the guidance in Section 5.1.7 of this addendum and Section 5.8 FSP 360.*

Appendix D: Emergency Response

Handling Spills

In any laboratory operation, the possibility of a spill exists. A thorough understanding of the potential hazards of the experiment, as well as careful planning of a spill response protocol, will help minimize personal injury and property damage. While the severity of the accident will dictate the response required, the general recommended approach listed below should be followed.

First Priority: Protect Personnel

- Notify your supervisor and Hazards Control Department personnel from the nearest phone. Do not contaminate the phone. If the spill is large or Hazards Control Department personnel fail to respond quickly enough, call 911 and report the spill.

- Notify all personnel in the immediate area. Evacuate the immediate room or area if the accident is hazardous to anyone or if there is doubt about the extent of the hazard.

- When the agent involved may be hazardous when inhaled, hold your breath as much as possible while evacuating the area. Administer first aid when necessary. Remove contaminated clothing and shoes and leave the area. Wash hands, face and other contaminated portions of the body with appropriate disinfectant and soap. If eyes or other part of the body has been contaminated, flush the affected areas with water for fifteen minutes.

- Secure the area of the spill and prevent people from entering the area. Post the entrance specifying (1) the type of accident, the agent(s) involved, (2) date and time of the accident, and (3) the name of person(s) to contact prior to entering the area. Report to LLNL Health Services for evaluation.

Second Priority: Protect Equipment and Facility through Proper Cleanup and Decontamination.

- Proceed only after consultation with, and approval from, Hazards Control.

- Reenter the area after allowing at least 30 minutes for aerosols to settle.

Appendix D: Emergency Response (Continued)

- If the spill took place in the open laboratory, decontamination may range from flooding the area with an appropriate disinfectant to fumigating the entire room with disinfectant chemical vapor, depending on the nature and scale of the spill. The disinfectants used should be applied from the outside into the spill to prevent further contamination. Use of appropriate personal protective equipment varies according to the level of hazard involved. In general, it may involve some or all of the following: coveralls, gloves, shoe covers, head cover, and respiratory protection.

- If the spill took place within a biological safety cabinet, initiate chemical disinfection procedures while operating the cabinet ventilation system to prevent the escape of the contaminants from the cabinet. Appropriate gloves should be worn. Use sufficient disinfectant to assure the disinfection of the drain pans and catch basins below the work surface. Lift the front exhaust grill and tray and wipe all surfaces. Wipe the catch basin and drain the disinfectant into a container. This procedure will not disinfect the filters, blower, air ducts or other interior parts of the cabinet. Contact Hazards Control for decontamination of the entire cabinet.

- If the spill involved a radioactive biohazardous material, the cleanup procedure may have to be modified depending on the relative risk assessment of the biological and the radiological hazards involved. Contact Hazards Control for specific details.

- If the spill involved a hazardous biohazardous material, special equipment and/or reagents may be required. Contact Hazards Control for specific details.

- Regulated wastes generated from emergency response activities will be managed according to guidance described in the Building 360 Complex FSP.

Appendix E: Biological Safety Cabinets
Procedures for Effective Use

General Operating Information

Understand how the cabinet works, and plan work accordingly. Protect yourself, your research, and your co-workers.

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

The effectiveness of the BSC is a function of directional airflow (inward and downward, through high efficiency filters). Anything that disrupts the air flow patterns can reduce cabinet effectiveness. Some examples are: (1) rapid movement of arms in and out of the BSC, (2) people walking rapidly past the opening of the BSC, down-drafts from ventilation systems; and (3) opened laboratory doors.

Operational Procedures

- Turn off the BSC ultraviolet (UV) lamp if in operation and turn on the BSC. Wipe work surface and the front window glass with the appropriate germicide (70% ethanol, 1:10 bleach solution). Wipe off each item needed for the procedure and place in the cabinet. Allow the cabinet to run for at least 5 minutes before beginning work. Use of UV lamps for “disinfection” is discouraged. If used, turn off prior to other 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. Put on 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 use vertical pipette discard canisters on the floor outside the BSC.
- The heat from the open flame creates turbulence in airflow and will compromise 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 items need to be removed from the BSC or new items introduced, arms shall be slowly moved in and out of the cabinet to minimize airflow disruption.

Appendix E: Biological Safety Cabinets
Procedures for Effective Use (continued)

- If there is a need to use a piece of equipment that creates turbulence in the BSC (e.g., centrifuge, blender, sonicator), place equipment in the back 1/3 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 A for more information.

- Clean-up any spills in the BSC immediately. See Appendix B for more information and guidance.

- Remove all materials and wipe all interior surfaces with 70% alcohol when finished work. Let the cabinet run 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. Refer to Section 6.0 of FSP 360 for detailed environmental controls.

- 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 F: Biological Safety Cabinets
Necropsy Rules for Infected Animals

1. Necropsy of infected animals should be carried out in biological safety cabinets by trained personnel.

2. Surgeons' wrap around gowns should be worn over laboratory clothing during necropsy.

3. Rubber gloves should be worn when performing necropsies.

4. The inside of biological safety cabinets and other potentially contaminated surfaces should be disinfected with a suitable germicide at the initiation and termination of the necropsy.

5. The fur of the animal should be wetted with a suitable disinfectant.

6. Small animals should be pinned down or fastened on wood or metal in a tray.

7. Upon completion of necropsy, all potential biohazardous materials should be placed in suitable containers and sterilized. Contaminated mixed waste is segregated and stored for appropriate disposal.

8. Contaminated instruments should be placed in a horizontal bath, located within the biological cabinet containing suitable disinfectant.

9. Grossly contaminated rubber gloves should be cleaned in disinfectant before removal from the hands, remove to check for holes, and then sterilize for reuse or decontaminate prior to disposal. Wearing gloves is not a substitute for hand-washing; hands should be washed after necropsy and carcass disposal.

10. Dead animals are to be placed into a plastic bag, tied into a knot or taped closed at end. The bag is placed into a second plastic bag and closed. A cardboard tag, with string, is filled out with date, name of investigator, species of animal, number of animals, name of agent animals are infected with, amount of agent animals received and tied around neck of plastic bag. The tagged bag is placed into the appropriate ACF freezer, plastic lined, properly labeled cardboard box. All information is entered into freezer logbook. Filled boxes will be properly packaged for removal by RHWM and shipped to contract agency for incineration.

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