Stevens Institute of Technology

Biological Safety Guide

Prepared by: Stevens Environmental Health and Safety

November, 2014

A Guide for those Stevens' laboratories engaged in the use, storage, handling, manipulation, or destruction of biological materials.

Stevens Institute of Technology Biological Safety Guide

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I. **Introduction**

The Biological Safety Guide has been developed by the Stevens Institute of Technology's Environmental Health and Safety Department. This guide is intended as a laboratory supplement to the previously approved Biological Safety Protocol. The purpose of the guide is to assist Stevens' investigators in developing sound biological safety practices in their laboratories and to help the Institute comply with applicable guidelines and regulations.

Stevens' investigators seeking to conduct research utilizing potentially infectious biological material should submit a Registration Document for Biohazards to the Stevens Institutional Biosafety Committee for evaluation and approval. The completed registration document should include a risk assessment related to the proposed work.

Questions concerning the implementation of the guide should be directed to the Stevens Environmental Health and Safety Department.

This guide has been implemented in the following Stevens laboratory facility:

Principal Investigator (P.I.):

_____

Laboratory Manager (may be P.I. or designee):

_____

Building:

_____

Rooms Covered by this Guide:

_____

II. **Regulatory Review**

Tier I Regulations and Guidelines:
The principal guidelines governing biological safety in the academic research laboratory include:

- The CDC/NIH Guidelines, entitled: Biosafety in the Microbiological and Biomedical Laboratory (BMBL), US Department of Health and Human Services, 5th Edition;
- The NIH Recombinant DNA Guidelines, entitled: NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), November 2013; and
- The OSHA Bloodborne Pathogen Standard entitled: Occupational Exposure to Bloodborne Pathogens, US Department of Labor, 29 CFR 1910.1030.

Tier II Regulations and Guidelines:
Additionally, there are a variety of regulations that indirectly affect activities in an academic research laboratory. Principal among them are:

- Biological Agents Provisions of the Antiterrorism and Effective Death Penalty Act of 1996 (and revisions)
- Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (The USA Patriot Act)
- Federal Select Agent Program, US Department of Health and Human Services and US Department of Agriculture, Final Rule on Select Agents and Toxins: 7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73
- Additional Requirements for Facilities Transferring or Receiving Select Infectious Agents; **PHS** 42 CFR part 72
- Hazardous Materials: Revision to Standards for Infectious Substances and Genetically Modified Micro-Organisms; **DOT** 49 CFR parts 171, 172, 173, 177, and 178

Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) Guidelines on: Biosafety in Microbiological and Biomedical Laboratories (BMBL).
In 1984, the CDC/NIH published the first edition of the BMBL. This document describes combinations of standard and special microbiological practices, safety equipment, and facilities that constitute Biosafety Levels 1-4, which are recommended for working with a variety of infectious agents in various laboratory settings. This document also outlines requirements for animal biosafety levels. The BMBL has been revised several times and is commonly seen as the standard for biosafety. Stevens, like most other universities, is using the BMBL as the basis for this biosafety guide.

National Institutes of Health (NIH): Guidelines for Research Involving Recombinant and Synthetic DNA Molecules.
These guidelines address the safe conduct of research that involves construction and handling of recombinant DNA molecules and organisms containing them. In 1974, a recombinant DNA Advisory Committee (RAC) was established to determine appropriate biological and physical containment practices and procedures for experiments that potentially posed risks to human health and the environment. As a result of the committee's activity, the initial version of the NIH Guidelines was published in 1976. It has been amended and revised many times since then. Included in the Guidelines is a requirement for the institution to establish an Institutional Biosafety Committee (IBC) with authority to approve or disapprove proposed research using the NIH Guidelines as a minimum standard.

OSHA Bloodborne Pathogens Standard.
The United States Department of Labor requires all employers having employees who may be reasonably anticipated to come into contact with blood or other potentially infectious materials (OPIM) to establish and follow a written Exposure Control Plan (ECP). These requirements are stipulated in 29 CFR 1910.1030 (OSHA Bloodborne Pathogens Standard).

III. Stevens Biological Safety Policy on Research Involving Recombinant DNA, Potentially Infectious Microorganisms, Human-Derived Materials, and Other Potentially Infectious Materials

In an effort to comply with applicable biological safety regulations and guidelines, Stevens Environmental Health and Safety Department has instituted the following biosafety program elements:

Registration Document for Biohazards

Stevens Environmental Health and Safety Department has developed a formal Registration Document for Biohazards. The completion of this document is required of all investigators conducting research involving recombinant DNA, potentially infectious microorganisms, human-derived materials, or other potentially infectious materials. This document will be the mechanism by which biological protocols are presented to the Stevens IBC for review and approval. The Registration Document is attached as Appendix I.

Institutional Biological Safety Committee (IBC)

Stevens Institute of Technology has appointed additional members to the its Institutional Biosafety Committee (IBC) including a new member from the Center for Healthcare Innovations, a community representative, and an adequate number of scientists to ensure that all biological research disciplines at Stevens are represented.

The IBC may approve Potentially Biohazardous Protocols for:

- Biosafety Level 1 protocols are approved for a period of three (3) years
- Biosafety Level 2 protocols are approved for a period of two (2) years
- Approved protocols may be amended at any time

The IBC shall meet periodically, but not less than two meetings per year. A quorum is achieved when half of the voting IBC membership is present. IBC minutes shall be maintained. Written approval/disapproval letters shall be provided for each protocol submitted documenting assigned biosafety level, providing any specific guidance documents or conditions of approval.

At this time we do not anticipate any research protocol to require approval beyond Biosafety Level 2. If an investigator seeks approval for a protocol requiring BL 2+ containment, specific written approval must be granted by the IBC.

Completion and Approval of a Laboratory Biological Safety Guide

Stevens Environmental Health and Safety Department has developed a hands-on Biological Safety Guide that includes:

- policies by which biological protocols are reviewed and approved
- registration documents, forms, and educational materials; and
- description of how biological materials may be safely handled in the laboratory.

This guide will serve as a supplement to the existing Stevens Biosafety Protocol.

Biological Safety Training

Stevens Environmental Health and Safety Department will ensure that all laboratory staff, students, as well as investigators, who may potentially come into contact with biological materials in the laboratory attend a yearly biological safety training session.

IV. Risk Assessment

Risk assessment is a process used to examine various factors associated with a laboratory procedure involving biological materials in order to identify:

- the hazardous characteristics of the material
- the activities that can result in a person's exposure to an infectious agent,
- the likelihood that exposure will cause a laboratory acquired infection, and
- the probable consequences of an infection.

The information identified by risk assessment will provide a guide for the selection of biosafety levels, microbiological practices, safety equipment and facility safeguards that can prevent laboratory acquired infections and reduce environmental contamination risk. Factors to consider in a risk assessment include both agent hazards and laboratory procedure factors.

The CDC/NIH Guidelines recommend the laboratory directors, principal investigators, biosafety committees, and others; perform a risk assessment process when determining the appropriate safeguards to employ to ensure that potentially biohazardous protocols are conducted safely.

Agent Hazards:

- Capability to infect and cause disease in a susceptible host
- Virulence as measured by the severity of disease
- Availability of preventive measures and effective treatments for the disease
- Probable routes of transmission of laboratory infection. The predominant routes of transmission in the laboratory include:
    - mucous membrane exposure,
    - parenteral inoculation,
    - ingestion and
    - inhalation of infectious aerosols
- Infective dose
- Stability in the environment
- Host range
- Its endemic nature
- Reports of laboratory acquired infections
- Origin of the agent

Classification of Infectious Agents on the Basis of Hazard (Risk Groups)
Risk groups (RG) are a method used by the World Health Organization (WHO) and by the National Institutes of Health (NIH) to classify human etiological agents based on hazard to both the individual and to the community. There are four risk groups. These correlate to but are not equivalent to biosafety levels. Determining the risk group of a biological agent can be part of the biosafety risk assessment and helps in assigning the correct biosafety level for containment. In general, RG-2 agents are handled at BSL- 2, and RG-3 agents at BSL-3. However, the use of certain RG-2 agents in large quantities might require BSL-3 conditions, while some RG-3 agents may be safely manipulated at a BSL-2, under certain conditions.

Basis for the Classification of Biohazardous Agents by Risk Group
Risk Group: Risk to the individual and the community

Risk Group 1 (RG-1)
Agents that are not associated with disease in healthy adult humans (no or low individual and community risk).

Risk Group 2 (RG-2)
Agents that are associated with human disease which are rarely serious and for which preventive or therapeutic interventions are often available (moderate individual risk but low community risk).

Risk Group 3 (RG-3)
Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).

Risk Group 4 (RG-4)
Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk)

Examples of RG-1 agents include microorganisms like *Escherichia coli*-K12 or *Saccharomyces cerevisiae*. A list of Risk Group 2, 3 and 4 agents can be found in the appendix to this guide. It is important to note however, that no list is all inclusive. Also, those agents not listed in RG-2, RG-3 or RG-4 are not automatically classified in RG-1. Those unlisted agents need to be subjected to a risk assessment based on the known and potential properties of the agents.

Hazards of Genetically-Modified Agents
When conducting a risk assessment of genetically modified agents, consideration of the same factors used in risk assessment of the wild-type organism should be done. However, it is important to address the possibility that the genetic modification could alter (i.e., increase or decrease) the pathogenicity of the agent or affect its susceptibility to antibiotics or other treatments. Sometimes, important information may not be available for a newly engineered agent and the risk assessment may be difficult or incomplete. In these cases, due diligence should be practiced and the biosafety level assignment should be made conservatively. Once more information is available another risk assessment should be completed.

Hazards of Cell Cultures
Human and animal cells and tissues have the potential to harbor latent infectious agents and personnel that handle these materials are at risk for possible exposure. The Centers for Disease Control and Prevention (CDC) and OSHA require that all cell lines of human origin be handled at BSL-2. All personnel working with or handling these materials need to be included in Steven's Bloodborne Pathogen Program and/or biological safety program.

Primate cell lines derived from lymphoid or tumor tissue, all cell lines exposed to or transformed by a primate oncogenic virus, all clinical material (e.g., samples of human tissues and fluids obtained after surgical resection or autopsy), all primate tissue, all cell lines new to the laboratory (until shown to be free of all adventitious agents) and all virus and mycoplasma-containing primate cell lines are classified as RG-2 and should be handled at a Biosafety Level 2.

Laboratory Procedure Hazards
- Parenteral inoculations
    - Injection of potentially hazardous materials can occur by a needle, other contaminated sharp or by bites from infected animals or arthropod vectors.
- Spills and splashes into skin and mucous membranes
    - Mucous membranes include the eyes, nose and mouth.
- Ingestion through mouth pipetting
- Animal bites and scratches
- Inhalation exposures to infectious aerosols

Aerosols, or respirable sized particles, are extremely hazardous because they are generated in many lab procedures and are usually undetected. The creation of infectious aerosols places the person carrying out the procedure and others in the laboratory at risk. Any procedure that breaks the surface tension of a liquid will produce aerosols. Pipetting, blenders, non-self-contained centrifuges, sonicators and vortex mixers all produce aerosols. Procedures and equipment that create aerosols also create larger droplets that rapidly settle out of the air. These droplets can settle on surfaces and may therefore contaminate gloved hands, work spaces and potentially mucous membranes via hand to face contact.

Conducting a Biological Safety Risk Assessment

Investigators planning to work with potentially infectious biological materials should perform a risk assessment to ensure the appropriate biosafety containment level, work practices, and administrative controls are selected to ensure that laboratory personnel are adequately protected. The 5th edition of the CDC/NIH Guidelines recommends the following five-stage risk assessment process:

1. Agent Hazards

First, identify agent hazards and perform an initial assessment of risk. Consider the principal hazardous characteristics of the agent, which include its capability to infect and cause disease in a susceptible human host, severity of disease, and the availability of preventive measures and effective treatments.

2. Laboratory Procedure Hazards

Second, identify laboratory procedure hazards. The principal laboratory procedure hazards are agent concentration, suspension volume, equipment and procedures that generate small particle aerosols and larger airborne particles (droplets), and use of sharps. Procedures involving animals can present a number of hazards such as bites and scratches, exposure to zoonotic agents, and the handling of experimentally generated infectious aerosols.

3. Select Appropriate Biosafety Containment Level

Third, make a determination of the appropriate biosafety level and select additional precautions indicated by the risk assessment. The selection of the appropriate biosafety level and the selection of any additional laboratory precautions require a comprehensive understanding of the practices, safety equipment, and facility safeguards described in Sections III, IV, and V of this guide.

4. Evaluate Laboratory Staff Proficiencies

Fourth, evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment. The protection of laboratory workers, other persons associated with the laboratory, and the public will depend ultimately on the laboratory workers themselves. In conducting a risk assessment, the laboratory director or principal investigator should ensure that laboratory workers have acquired the technical proficiency in the use of microbiological practices and safety equipment required for the safe handling of the agent, and have developed good habits that sustain excellence in the performance of those practices. An evaluation of a person's training, experience in handling infectious agents, proficiency in the use of sterile techniques and the biosafety cabinet, ability to respond to emergencies, and willingness to accept responsibility for protecting one's self and others is important insurance that a laboratory worker is capable of working safely.

5. Consult with Biosafety Officer and Biosafety Committee

Fifth, review the risk assessment with a biosafety professional, subject matter expert, and the IBC. A review of the risk assessment and selected safeguards is always beneficial and sometimes required by regulatory or funding agencies, as is the case with the NIH Guidelines.

Principals of Biological Safety

The CDC/NIH Guidelines entitled Biosafety in Microbiological and Biomedical Laboratories (BMBL) are currently in their fifth edition and are updated periodically. The descriptions of biosafety levels (BL) 1 - 4 in the 5th Edition parallel those established in the NIH Guidelines for Research involving Recombinant DNA as well as those originally used in the Classification of Etiologic Agents on the Basis of Hazard. The BMBL describes various combinations of work practices, safety equipment, and facilities recommended for work with various microorganisms in the laboratory setting. These advisory recommendations are intended to assist laboratory managers in establishing sound biological safety programs.

Exposure Determination
Laboratory personnel are considered to have occupational exposure to infectious microorganisms, or other potentially infectious materials, if they are directly engaged in research or other activities which utilize materials regulated by the CDC/NIH Guidelines at BL-2 containment or higher.

Transmission of Infectious Agents
As described by the American Public Association in "Control of Communicable Diseases in Man", transmission of an infectious agent may occur by four main routes, as follows:

Contact Transmission may be divided into three subgroups.
- **Direct Contact**: Direct physical transfer between a susceptible host and an infected individual, e.g., sexually transmitted diseases.
- **Indirect Contact**: Personal contact of a susceptible person with a contaminated inanimate object (fomite) such as used surgical instruments, contaminated transfer pipette, needles, medical equipment, soiled clothes and bedding.
- **Droplet Contact**: Direct projection of droplet spray on the conjunctiva or mucous membranes of the eyes, nose, or mouth of a susceptible person. Droplet contact involves droplets greater than 5 microns in size and distances of 1 meter of less.

Vehicleborne Transmission: Transfer of an infectious agent to a susceptible host via contaminated items such as water, food, milk, or biological products such as blood, plasma, serum, tissues, and organs.

Airborne Transmission: The dissemination of microbial aerosols to a susceptible host via the respiratory tract.
- **Droplet Nuclei**: The dried residue of respiratory droplets resulting from the evaporation of fluid from droplets emitted by an infectious host, e.g., tuberculosis. Droplet nuclei are generally 1 to 5 microns in size.
- **Dust**: Small particles of various sizes which may arise from clothes bedding, contaminated floors, and soil, e.g., fungal spores separated from dry soil by air currents or by mechanical agitation.

Vectorborne Transmission: Transfer of an infectious agent from an infected host to a susceptible individual via an arthropod or insect.

Laboratory Acquired Infections
The majority of laboratory acquired infections are attained from incidents involving indirect and droplet contact. Needle sticks or other puncture wounds involving contaminated laboratory equipment may be considered examples of indirect contact. Aerosol droplets projected onto horizontal work surfaces in the laboratory may be transferred to the conjunctiva or mucous membranes of laboratory workers. This type of droplet contact may occur when a laboratory worker touches a contaminated surface in the laboratory and then touches his or her face with their contaminated hand. Eliminating these types of exposure incidents will be discussed in the Work Practice Control section.

Containment
In the field of biosafety, the term containment is used to describe proper management of infectious materials. Successful containment of biological material will result in reduced or eliminated exposure to the laboratory worker, ancillary personnel, and the environment. The two principal types of containment include primary and secondary containment.

Primary containment refers to the protection of laboratory workers and the laboratory from contamination. An example of primary containment is confining a manipulation with the potential to generate infectious aerosols within a biological safety cabinet.

Secondary containment refers to the protection of the environment external to the laboratory from contamination. Examples of secondary containment include the physical structure of the laboratory (walls, floors, ceiling) and the non-recirculating ventilation system servicing the laboratory.

In addition to primary and secondary containment, laboratory workers must employ adequate engineering and work practice controls to further reduce or eliminate potential exposure to infectious materials. Engineering and work practice controls are discussed in subsequent sections of this guide. Laboratory supervisors are required to initiate sufficient administrative policies to ensure the safety of laboratory workers. Such administrative policies may cover working in the laboratory during off hours, emergency response, waste disposal, accident reporting, training requirements and supervision of contract employees.

Biosafety Levels
Laboratory biosafety levels 1 through 4 have been established in the current edition of the joint CDC/NIH Guidelines entitled Biosafety in Microbiological and Biomedical Laboratories (BMBL), U.S. Department of Health and Human Services, Public Health Services, Centers for Disease Control and Prevention and the National Institutes of Health. The levels are designated in ascending order, by the degree of protection provided to the worker, the environment, and the community. Each level builds upon the preceding level with BL-1 being the least stringent and BL-4 being the most stringent. This guide will concentrate on discuss BL-1 and BL-2 and briefly discuss BL-3 and BL-4.

The terms risk group and class are often used synonymously with the term biosafety level. The terms risk group and class refer to the risk of infection associated with a particular agent, while the term biosafety level refers to the level of containment required to work safely with a particular agent. The term biosafety level replaced the term physical containment level (P-1 to P-4) originally used by the federal government in discussions of biosafety. The term physical containment level may still be encountered in the recombinant DNA guidelines.

Biosafety Level 1 (BL-1)
BL-1 is appropriate for work with organisms/agents presenting minimal potential hazard to laboratory personnel and the environment. In the laboratory, work is usually conducted on the open bench top without the use of special containment equipment; however, standard microbiological techniques are used. All potentially contaminated waste materials are decontaminated using an approved decontamination technique such as autoclaving. Scientists with microbiological expertise provide laboratory workers with adequate training and supervision.

Biosafety Level 2 (BL-2)
BL-2 is appropriate for work with organisms presenting moderate potential hazard to laboratory personnel and the environment. BL-2 is more stringent than BL-1. In addition to BL-1 requirements, BL-2 requires that all manipulations with the potential to create aerosols be confined within a properly certified and maintained biosafety cabinet. Additional BL-2 requirements include specific training and supervision by

competent scientists, restricted laboratory access, appropriate personal protective equipment (PPE), and careful handling of potentially contaminated sharp items.

Biosafety Level 3 (BL-3)
BL-3 is appropriate for work with organisms that may cause serious or potentially lethal disease resulting from exposure via the inhalation route. All manipulations are contained within a properly certified and maintained biosafety cabinet. Laboratory workers must wear enhanced personal protective clothing and equipment when working in the BL-3 laboratory. The BL-3 laboratory is equipped with specialized engineering and design characteristics including directional airflow, restricted access, anteroom, sealed surface penetrations, and a double-door or two-sided (clean and dirty) autoclave.

Biosafety Level 3 (BL-4)
BL-4 is appropriate for work with dangerous and exotic agents that pose high individual risk of life threatening disease resulting from exposure via the aerosol route. There are usually no effective therapy and/or vaccines for BL-4 agents. The primary hazards to individuals manipulating BL-4 agents are from respiratory exposure, mucous membrane or broken skin exposure, and autoinnoculation. BL-4 agents are contained by working in a Class III biological safety cabinet or in a full body positive pressure suit. The BL-4 facility is usually a separate building or zone with elaborate ventilation and waste management characteristics.

Biosafety Laboratory Facilities at Stevens
Currently, Stevens Institute of technology has no biohazard protocols that require more than BL-2 containment. Further Steven's laboratories are not equipped to accommodate biohazard protocols that require more than BL-2 containment. In rare instances investigators may wish to work at BL-2+ containment. The Stevens IBC must provide written approval for any investigator to work at BL-2+ containment.

Designation of Biosafety Levels
The CDC/NIH Guidelines, referenced above, and the NIH Guidelines, entitled Guidelines for Research Involving Recombinant and Synthetic DNA Molecules (Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard, included as an appendix to this guide), recommend appropriate biosafety levels, practices, and facilities (laboratory and vertebrate animal) to reduce or minimize laboratory acquired infections. The two guidelines described above are not inclusive documents; rather, they describe those organisms most frequently encountered in clinical and research microbiology, biomedical, and biotechnology laboratories. These guides offer the minimal acceptable containment criteria based on the most recent epidemiological data available to the CDC/NIH. Individual laboratory directors may impose more stringent requirements.

The table below is intended to illustrate general relationships between the classification of microorganisms into risk groups and the required biosafety levels, laboratory type, laboratory practices, and required safety equipment.

Relationship of Risk Groups to Biosafety Levels, Practices, and Equipment				
Risk Group	**Biosafety Level**	**Laboratory Type**	**Laboratory Practices**	**Safety Equipment**
RG-1	BL-1	Basic Teaching	GMT	Open Bench
RG-2	BL-2	Clinical, Research, Diagnostic	GMT plus Protective Clothing and Biohazard Symbol	Open Bench plus BSC to Contain Potential Aerosols
RG-3	BL-3	Special Research or Diagnostic	Level 2 plus special clothing, PPE, Controlled Access, and Directional Airflow	BSC and/or Other Primary Containment for All Activities
RG-4	BL-4	Maximum Containment Facility	Level 3 plus Airlock Entry, Shower Exit, Special Waste Treatment	Class III BSC or Positive Pressure Suits, Double-Ended Autoclave, Filtered Air, Water Treatment

GMT - Good Microbiological Techniques
BSC - Biological Safety Cabinet

Oncogenic Viruses

On October 3, 1974 the National Cancer Institute (NCI) published the "NCI Safety Standards for Research Involving Oncogenic Viruses". The standard classifies oncogenic viruses into three risk categories, as follows: low risk, moderate risk, and high risk. In 1974 all known oncogenic viruses were classified as low or moderate risk agents. The current edition of the NIH Guidelines, November 2013, has classified both low and moderate risk oncogenic viruses as Class 2 agents, requiring BL-2 containment, and as Animal Viral Etiologic Agents in Common Use and specifies BL-1 containment (unless the agent is infectious to human cells, then BL-2 containment is appropriate). In the absence of clear regulatory direction many laboratory researchers and host institutions designate those agents described above as BL-2.

Containment of Oncogenic Viruses

The original containment recommendations made by the NCI in 1974 specified the practical equivalents of BL-2 containment for low risk oncogenic viruses, BL-3 containment for moderate risk oncogenic viruses, and BL-4 containment for high risk oncogenic viruses (although no viruses were classified as high risk). As previously stated, both low and moderate risk oncogenic viruses may be classified as Class 2 agents, requiring BL-2 containment, and as Animal Viral Etiologic Agents in Common Use requiring BL-1 containment (unless the agent is infectious to human cells, then BL-2 containment is appropriate).

Viral Vectors

Viral vectors have become indispensable tools of the molecular biology and it is important for users to understand the origins of these tools and potential implications of their use. Suggested biosafety containment levels are provided for various viral vector systems. Use of a higher-level containment may be required in some cases, depending on the specific properties of the vector and/or insert. Special care should be given to the design and handling of viral vectors containing genes that make growth-regulating products, products released into the circulation, and products that may have a general effect on the host immune system. The table below, adapted from Rutgers University, Stanford University, Michigan State University, and University of Iowa sources, provides information concerning many common viral vectors and general guidance on appropriate biosafety levels. Please note that investigators submitting a Registration Document for Biohazards that includes work with viral vectors will need to provide risk assessment rationale for their protocol's anticipated biosafety level.

Biosafety Levels and Characteristics of Current Viral Vector Systems

Vector	BL	Notes
Adenovirus	2	Adenoviruses are infectious human viruses which often cause mild respiratory illness, pink eye or gastroenteritis. Rare cases of severe disease can occur, and its use as a genetic vector therefore requires the use of adequate containment equipment and practices.
Adeno-Associated Virus (AAV)	1	AAV are infectious human viruses with no known disease association. The NIH Guidelines state that "adeno-associated virus (AAV) types 1 through 4, and 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" can **in most cases** be handled at BL-1.
Epstein - Barr Virus (EBV)	2	EBV is a member of the herpesvirus family and one of the most common human viruses. The virus is found worldwide and most people become infected with EBV sometime during their lives. In the United States, as many as 95% of adults between 35 and 40 years of age have been infected. Among persons not infected with EBV in their childhood years, EBV infection during adolescence may cause infectious mononucleosis 35% to 50% of the time. A late event in a very few carriers of this virus is the emergence of Burkitt's lymphoma and nasopharyngeal carcinoma. EBV is a transforming virus and is often used to produce immortalized cell lines. BSL-2 is appropriate for most experiments.
Herpesviruses	1 2	Herpesviruses include infectious human viruses such as herpes simplex virus type-1 (HSV-1), which is the most commonly used vector system. HSV-1 is common in the general population, but can cause encephalitis in rare cases; its utility as a vector system stems from its broad host cell range, ability to transduce neurons, and its large insert capacity. Biosafety Level 2 is appropriate for many constructs.
Retrovirus	1 2 2+	Retrovirus are infectious viruses which can integrate into transduced cells with high frequency, and which may have oncogenic potential in their natural hosts. Retrovirus vector systems are typically based on murine viruses - most commonly, these systems include ecotropic viruses (which can infect only murine cells), amphotropic viruses (which can infect human cells) or pseudotyped viruses, when vector particles express glycoproteins (GPs) derived from other enveloped viruses (which can also infect human cells). The most common GP currently used is VSV-g; however there are newer pseudotypes being derived from viruses such as measles (Rubeola), Ebola and Marburg. Pseudotyping vectors often results in a higher Biosafety level. Containment for vectors with the ability to infect human cells (amphotropic) will usually be recommended at BSL-2/2+, whereas for ecotropic vectors with no ability to infect human cells, BSL-1 containment may be appropriate.
Lentiviruses	2 2+	Lentiviruses are a subset of retroviruses, with the ability to integrate into host chromosomes, and to infect non-dividing cells. These viruses can cause severe immunologic and neurologic disease in their natural hosts. Lentivirus vector systems can include viruses of non-human/non-primate origin (feline immunodeficiency virus, equine infectious anemia virus) as well as simian viruses (simian immunodeficiency virus) and human viruses (HIV). The more recent generation vectors have been designed to significantly diminish the possibility for recombination to occur resulting in a wild type- potentially infectious virus. Typical lentivirus vectors are packaged using pseudotyped enveloped proteins. The most common envelope protein used for this purpose is from vesicular stomatitis virus (VSV). It is usually recommended that work with

		nonhuman lentiviruses that are incapable of establishing productive infections in humans be conducted at BSL-2. Work with simian or human lentiviruses (SIV, HIV) is typically conducted at a higher containment level.
Moloney Murine Leukemia Virus (MMLV)	2 2+	The host range of recombinant MMLV vectors is dependent on the specificity of the viral envelope. The ecotropic env gene produces particles that infect only rodent cells. Amphotropic env gene allows infection of murine and nonmurine cells, including human cells. VSV-G envelope allows infection in a wide range of mammalian and non-mammalian cells. Biosafety Level 2 is appropriate for many constructs, while higher levels may be required depending upon the construct.
Poxvirus	2	Poxvirus vectors include avian viruses (avipox vectors) such as NYVAC and ALVAC, which cannot establish productive infections in humans, as well as mammalian poxviruses, which can productively infect humans -such as vaccinia virus and modified vaccinia viruses (MVA). Poxviruses are highly stable, and vaccinia virus can cause severe infections in immunocompromised persons, persons with certain underlying skin conditions, or pregnant women. Such individuals should not work with vaccinia virus. The use of BSL-2 is appropriate for many poxviruses and constructs.
Baculovirus	1	Baculovirus are non-mammalian virus vectors that infect insects; these are very stable and may remain dormant in the environment for years before infecting insects. Work is mostly done at the BSL-1 level.
Rabies virus	2	Rabies virus is a member of the Rhabdoviridae family and is a common zoonotic infection from bats and other wild mammals. Infection results in encephalitis or paralysis, and is often fatal. Due to its neuronal tropism, pseudotyped rabies virus vectors can be used to study neuronal trafficking or express endogenous genes efficiently in neurons. Biosafety Level 2 (BSL-2) is appropriate for many constructs.
Sendai virus (SeV)	2	Sendai virus (SeV) causes respiratory disease in rodents and sometimes swine. There is limited evidence of zoonotic transmission to humans. However, the virus is capable of infecting human cell lines and is similar to human parainfluenza virus type 1. For these reasons, SeV work is usually classified as BSL-2

Note: Currently, Stevens' laboratory facilities are equipped to accommodate protocols at BL-1 and BL-2. In special circumstances and with written approval from the Stevens' Institutional Biosafety Committee arrangements may be made to conduct approved protocols at the BL-2+ level of containment.

Human Blood, Blood Products, and Primary Tissue Explants

The OSHA Bloodborne Pathogen Standard, 29 CFR 1910.1030, specifies BL-2 containment for all laboratory manipulations of human blood and blood products. Additionally, the concept of **Universal Precautions** is introduced which instructs all those who may come in contact with human blood, blood products, and other potentially infectious materials to assume that these materials are contaminated with the Hepatitis-B Virus (HBV) and the Human Immunodeficiency Virus (HIV), or other potential bloodborne pathogens and to take the appropriate precautions.

Long Term Cell and Tissue Culture

Appendix H of the most recent edition of the BMBL Guidelines, published in 2009, recommend BL-2 practices, containment, waste decontamination and PPE when handling human and non-human primate cells and tissues. These recommendations are based on the potential for these materials to harbor latent viral infectious agents including: HBV, HIV, HCV, HTLV, EBV, HPV, and CMV. Further risk may be

associated with cell lines immortalized with viral agents including: SV-40, EBV, adenovirus, and HPV. Based on these potential risks, the Stevens' IBC recommends BL-2 containment for protocols involving human and non-human cell and tissue culture.

Summary of Biosafety Levels Assigned to Common Biological Materials

The table below depicts biosafety containment levels typically assigned to some common biological materials. Currently at Stevens, it is not anticipated that investigators will be working with materials that require more than BL-2 containment. Further, current Steven's laboratory facilities are not equipped to accommodate research protocols requiring greater than BL-2 containment. However, it may be possible for investigators to work at BL-2+ containment (BL-2 facilities plus BL-3 work practices and administrative controls). Approval of a BL-2+ protocol requires specific approval from the Stevens' Institutional Biosafety Committee.

Material	Risk Group	Biosafety Level	Notes
Potentially Infectious Microorganisms	1, 2, 2+	1, 2, 2+	CDC/NIH Guidelines (BMBL)
Recombinant DNA	1, 2, 2+	1, 2, 2+	NIH rDNA Guidelines
Low and Moderate Risk Oncogenic Viruses	1, 2	1, 2	NIH rDNA/NCI Guidelines
Animal Viral Etiologic Agents in Common Use	2	2	NIH rDNA/NCI Guidelines
Human Blood, Blood Products, Serum, Plasma	2	2	OSHA BBP Std.
Other Human Body Fluids Described in BBP Std.	2	2	OSHA BBP Std.
Human and Non-Human Primate Cell Lines	2	2	OSHA BBP Std.
Viral Vectors	1, 2, 2+	1, 2, 2+	NIH rDNA Guidelines

Note: Currently, Stevens' laboratory facilities are equipped to accommodate protocols at BL-1 and BL-2. In special circumstances and with written approval from the Stevens' Institutional Biosafety Committee arrangements may be made to conduct approved protocols at the BL-2+ level of containment.

Control of Infectious Materials

Engineering Controls

Engineering controls refer to devices, mechanical or otherwise, that may be used to eliminate, minimize, or reduce occupational exposure to biological material. Engineering controls are usually designed to control contamination at the source thereby preventing the release of the contaminant into the worker's environment (example: biological safety cabinet). Additionally, engineering controls may be designed to minimize the effect of an accidental release of a contaminant into the work environment (example: laboratory ventilation system).

Examples of common biosafety engineering controls include:

Biological Safety Cabinet (BSC)

A ventilated and HEPA filtered cabinet that provides laboratory workers with protection from potentially infectious aerosols and provides a clean surface on which to perform microbiological manipulations (protects laboratory workers, the product, and the environment from contamination). All manipulations of potentially infectious materials that have the potential to produce aerosols should be confined within a biological safety cabinet. Biological safety cabinets should be certified by an approved vendor upon set-up, whenever moved or repaired, and annually thereafter. Biological Safety Cabinets should not be placed in laboratory locations where their air flow patterns will be disrupted, for example: BSCs should not be located directly below HVAC supply and/or exhaust ducts, adjacent to laboratory entrances and exits, chemical fume hoods, open windows, etc.

Sharps Container

Sharps containers are closable, leak-proof, puncture-resistant containers into which sharps are deposited for disposal. Disposable syringes, scalpel blades, and other sharp items should be deposited directly into an appropriately labeled sharps container immediately after use. Disposable needles should never be recapped, bent, broken, sheared, or removed from disposable syringes.

Steam Autoclave

Steam autoclaves are generally considered to be the method of choice for decontaminating infectious laboratory waste. Gravity displacement autoclaves operate at 121 degrees C. (15 lbs/in^2 pressure) while vacuum-type autoclaves operate at 132 degrees C. (27 lbs/in^2 pressure). It is important to consider appropriate load characteristics and autoclave operating parameters in order to determine adequate decontamination time. Further, autoclave bags must not be over-filled in order to allow proper steam/heat penetration into the bag during processing. In some instances it may be necessary to add a small amount of water to the load being decontaminated to assure steam penetration into the center of the load.

Mechanical Pipetting Device

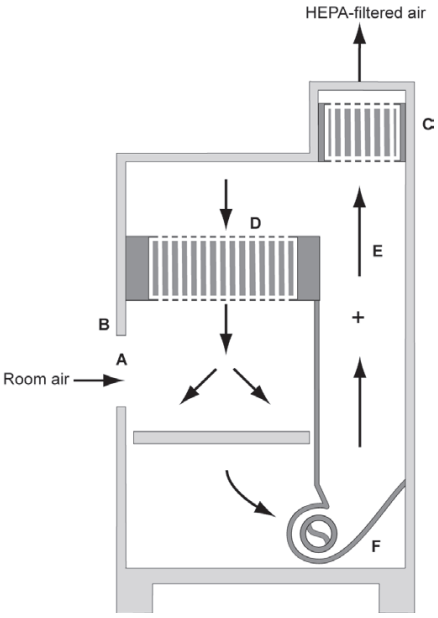
A wide variety of mechanical pipetting devices are commercially available which allow for the measurement and transfer of potentially infectious liquids while eliminating the need for mouth pipetting. Many of these devices utilize disposable tips and a high efficiency filtration system.

Other examples of engineering controls designed to eliminate or minimize occupational exposure to potentially infectious biological material may include: laboratory bench splash guards, self-sheathing needles, and centrifuge safety caps. An example of an engineering control designed to minimize the effect of an accidental release of contamination in the work environment is a non-recirculating (single pass) ventilation system. Specialized laboratory ventilation systems may have other specialized infection control characteristics including uni-directional air flow and air pressure differentials.

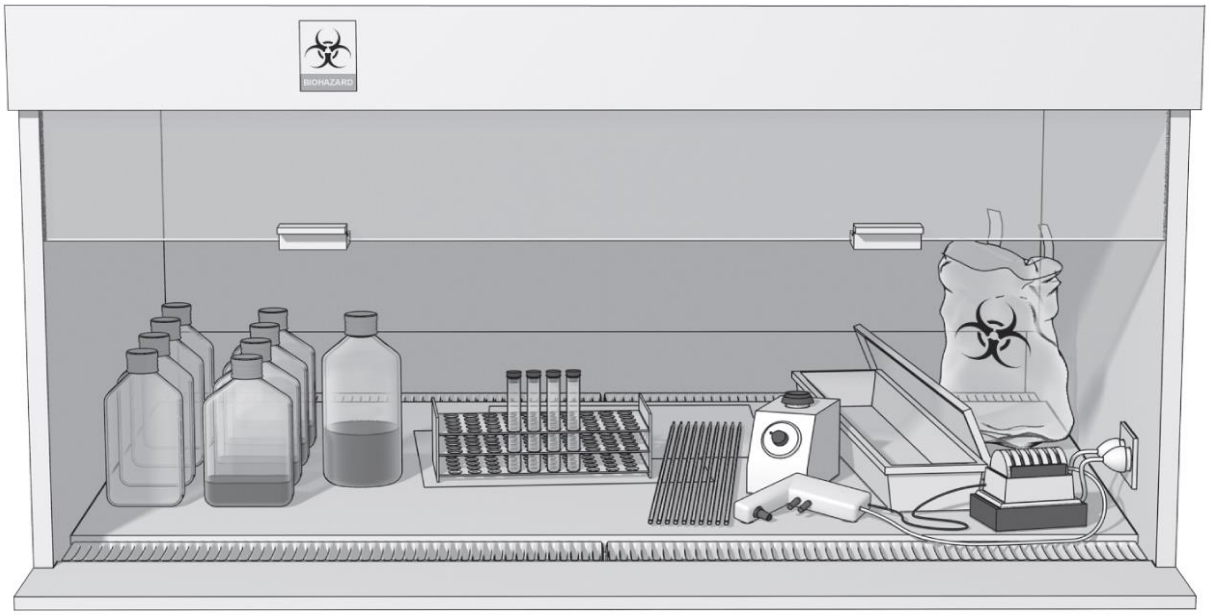
Biological Safety Cabinets

As previously described Biological Safety Cabinets (BSC) are used in microbiology laboratories to contain manipulations of potentially infectious materials with the potential to produce aerosol droplets. The BSC is the primary means of aerosol containment and performs three main functions:

- protection of the laboratory worker by providing a negative pressure enclosure that draws air into the cabinet and away from lab personnel;
- protection of the laboratory procedure by providing a sterile work area; and
- protection of the environment by discharging HEPA filtered air.



The Class II, Type A1 Biological Safety Cabinet (BSC): (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) common plenum; (F) blower. This cabinet is the most common BSC encountered in the academic research laboratory. The Class II, Type A BSC returns HEPA filtered air to the laboratory environment and is not appropriate for the manipulation of volatile chemical hazards.
(Source: CDC/NIH BMBL, 5th Edition)



A typical layout for working "clean to dirty" within a Class II BSC. Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded in the shallow pan and other contaminated materials can be placed in the biohazard bag (right). This arrangement is reversed for left-handed persons.
(Source: CDC/NIH BMBL, 5th Edition)

Working in a Biological Safety Cabinet
Conduct all BL-2 procedures with the potential to produce aerosol droplets within a properly functioning and certified biological safety cabinet (BSC). When working in the BSC:

- Decontaminate the BSC work surfaces both before and after each use.

- Allow the BSC to run for several minutes prior to use so that appropriate air flow patterns may be established.

- Appropriate personal protective equipment shall be worn while working in the BSC.

- Laboratory workers should move their arms, material, and equipment into and out of the BSC gently and as infrequently as possible in an attempt to minimize air flow disruption.

- The front air intake grill must never be blocked with paper, lab supplies, or equipment.

- Equipment, large items, aerosol generating devices should be placed toward the rear edge of the work surface without blocking the rear air intake grill.

- Work should flow from the clean side of the cabinet to the contaminated side of the cabinet.

- The autoclavable biohazard collection bag and pipette collection tray should not be placed outside the BSC as the frequent in-and-out movement needed to use these containers is disruptive to the integrity of the cabinet's air barrier, and can compromise both personnel and product protection.

- Ultraviolet lights are not required in BSCs, however, if they are used, they should be cleaned weekly to remove any dust and dirt that may block the germicidal effectiveness of the light. Ultraviolet light intensity should be checked when the cabinet is recertified to ensure that light emission is appropriate. Ultraviolet lights must be turned off while the room is occupied, to protect eyes and skin from inadvertent exposure.

- Open flames should not be used in a BSC.

- Any spills within the BSC should be cleaned up immediately while the BSC continues to run. Decontamination solution should be applied in a way to minimize splash, splatter, and spray within the BSC.

- Pay attention to all alarms while working in the BSC. Sash position and air flow alarms are common. A sash position alarm may indicate that the sash window is in an inappropriate position. An air flow alarm may indicate that there is inadequate airflow into or out of the BSC. Either alarm may indicate that the BSC is not functioning properly. Work within the BSC should discontinue, cultures and materials secured, work surfaces decontaminated, and arrangements should be made for BSC evaluation and repair.

Work Practice Controls
Work practice controls refer to practices and procedures which reduce or eliminate the chance of occupational exposure to potentially infectious materials. Examples of work practice controls include:

- Always wear the appropriate personal protective equipment for the task being performed.

- Contain all manipulations of potentially infectious materials with the potential to create aerosol droplets within a properly functioning and certified biological safety cabinet.

- Perform manipulations of potentially infectious materials in a manner designed to minimize splash, spray, or splatter whenever possible.

- Maintain an appropriate biological spill kit in all laboratories where potentially infectious materials are stored or manipulated.

- Use Universal Precautions when handling human-derived materials.

- Wash hands promptly after removal of gloves, prior to exiting a laboratory, and prior to eating and or drinking.

- Do not eat, drink, smoke, apply lip balm or makeup, and handle contact lenses in areas where occupational exposure potentially infectious material may occur.

- Handle and dispose of contaminated sharps carefully. Minimize the use of sharps whenever possible.

- Never store food or drink in refrigerators, freezers, cabinets, or on shelves, countertops, and benchtops, where potentially infectious materials may be (or have been) present.

- Observe hazard warning signs and labels applied to all biological safety cabinets, laminar flow hoods, incubators, and other pieces of laboratory equipment where potentially infectious materials may be (or have been) stored (see Hazard Communication, below).

- Ensure that laboratory personnel remove all potentially infectious material, contaminated equipment, and sharp objects from any piece of laboratory equipment that is to be serviced.

- Ensure that all laboratory equipment that was used to store or manipulate potentially infectious material is appropriately surface decontaminated by laboratory personnel prior to moving or servicing.

- Promptly decontaminate all laboratory benches, work surface or equipment following exposure to potentially infectious material.

- Know the most suitable disinfectant for the materials being manipulated in your laboratory.

- Do not lean or sit on laboratory bench tops or other laboratory equipment where blood or other potentially infectious materials may have been stored or manipulated.

- Laboratory paperwork should not be prepared on laboratory bench tops, on top of centrifuges, refrigerators, freezers, or other pieces of laboratory equipment where blood or other potentially infectious materials may have been stored or manipulated.

Handling of Sharps

Contaminated sharps such as needles, scalpel blades, broken test tubes, and other sharp instruments present the greatest risk of transmission of biological agents in the laboratory. Disposable syringes, scalpel blades, and other sharp items should be deposited into an appropriate leak-proof, puncture-resistant, and labeled sharps container immediately after use. Disposable needles should never be recapped, bent, broken, sheared, or removed from disposable syringes.

Sharps containers should be located in all work locations where it is reasonably anticipated that sharps may be used. Sharps containers should only be filled to within one inch of the top of the container. Sharps containers should never be overfilled. Never attempt to force additional material into a full container.

Laboratories engaged in research utilizing human-derived materials are regulated by the OSHA Bloodborne Pathogen Standard (BBP). The BBP standard requires laboratories engaged in research utilizing human-derived materials to develop a safe sharps program, seek to eliminate sharps from their research protocol, and encourage the use of non-sharp alternatives wherever possible.

Personal Protective Equipment

Personal protective equipment (PPE) are items that are worn to protect workers from exposure to potential occupational hazards. PPE is especially important when exposure can not be prevented by other means, e.g., engineering and work practice controls. These items provide protection by establishing a barrier between the employee and the potentially infectious material. PPE must be accessible and available in sizes that fit all employees. PPE will be repaired or replaced as needed. Examples of PPE worn to protect workers from occupational exposure to potentially infectious material include:

Gloves

Non-sterile single use nitrile examination gloves are appropriate for most, if not all, activities and procedures related to pathogenic microorganisms performed in Stevens' laboratories. This section of the safety manual does not discuss gloves worn for purposes other than protection from infectious agents, e.g., formaldehyde. Gloves must be worn when there is the potential for exposure to blood or other potentially infectious material.

Disposable gloves must be changed periodically throughout the work day and after any overt incident where the glove may have been contaminated. Gloves should be changed at the completion of each experimental protocol or at various stopping points in longer more complex protocols. Hands must be washed each time disposable gloves are removed. Employees with non-intact skin should cover affected area with a suitable bandage prior to donning gloves. Hypoallergenic gloves, glove liners, powderless gloves or other alternatives shall be made available to those Stevens employees who are allergic to the typical gloves provided.

To Remove Potentially Contaminated Disposable Gloves:

- Pinch with two fingers the outside of one glove (near the inner wrist) with the other gloved hand.
- Turn the glove inside out as it is pulled off.
- Hold removed glove loosely in the still gloved hand.
- Reach inside second glove with two fingers of the bare hand and pinch it.
- Turn the glove inside out as it is removed, enclosing the first glove.
- Properly discard the entire package.
- Wash hands.

Stevens' employees may also use heavier non-disposable utility gloves for activities where less tactile sensitivity and/or more protection is required such as waste decontamination or laboratory clean-up activities. Unlike disposable gloves, which must be discarded after a single use, non-disposable utility gloves may be washed or otherwise decontaminated and used again. It is important that non-disposable utility gloves are inspected often to ensure that they maintain their integrity.

Protective Eyeware
Protective eyeware must be worn during procedures which generate aerosols or splatter or splash potentially infectious materials. **When working with potentially infectious material, safety glasses must be worn at all times**. Protective eyeware include items such as safety glasses with solid side shields, goggles, and full length face shields.

Reusable protective eyeware issued to Stevens' employees meet the criteria established by the American National Standard Institute Standard Z87.1-1989 entitled Practice for Occupational and Educational Eye and Face Protection.

Face Masks
It is not anticipated that typical laboratory manipulations require the use of face masks when working at BL-1 or BL-2 containment. When engaged in certain higher risk activities, laboratory workers may consider the use of a face mask. These activities may include the cleanup of a spilled biological culture or the packaging of biological waste prior to decontamination.

Only if deemed necessary by the Environmental Health and Safety Department, face masks may be worn during procedures which generate aerosols or splatter or splash potentially infectious materials. Face masks may include single use disposable surgical masks, dust masks, and higher levels of respiratory protection. Higher levels of respiratory protection require employees to participate in Stevens' Respiratory Protection Program that included training, medical evaluation, and respirator fit testing.

Lab Coats, Gowns and Aprons
Protective gowns, aprons, lab coats, clinic jackets or similar outer garments must be worn during procedures which generate aerosols or splatter or splash potentially infectious materials. Any gown or other protective outer garment that is visibly soiled with blood or other potentially infectious material should be immediately removed and disposed of properly. Gowns, lab coats and other protective outer garments should not be worn out of the clinic, lab, or other applicable work location. Reusable cloth gowns or other protective outer garment shall be cleaned and laundered on a regular basis at no cost to the employee.

Cleaning, Disinfection, and Sterilization
Cleaning refers to the physical removal of organic material or soil from objects. Cleaning is generally considered to be the first step when disinfecting or sterilizing reusable instruments or equipment. Organic materials may contain high concentrations of microorganisms. Additionally, organic materials may protect the microorganisms from the decontamination or sterilization process. The preferred method of cleaning is soap and water. A brush may be used to help remove foreign matter adhering to the surface being cleaned. An example of items that require periodic cleaning include reusable PPE such as safety glasses, goggles, and face shields.

Disinfection refers to the destruction of most pathogenic organisms but not bacterial spores. Prior to disinfection, equipment and work surfaces should be thoroughly cleaned. Commercial germicides approved for use and EPA registered as "hospital disinfectants", which are also tuberculocidal, are recommended by the CDC for disinfecting environmental surfaces. A 10% solution of household bleach: approximately 1 1/2 cups of household bleach in 1 gallon of tap water may also be used for disinfection. Household bleach contains 5.25% sodium hypochlorite by weight. Once the bleach solution is mixed, the container should be

affixed with a label stating the ingredients, the concentration, and the date. Reusable personal protective equipment soiled by blood or other potentially infectious material shall be cleaned and disinfected prior to reuse.

Sterilization refers to the destruction of all microbial life, including a high percentage of bacterial spores. Sterilization is necessary for instruments, equipment, or objects that penetrate skin, come into contact with the bloodstream or other normally sterile areas of the body, and equipment used in certain clinical and research laboratory procedures. Autoclaving is the preferred method of sterilization for small laboratory equipment, laboratory reagents, and potentially infectious waste materials. Autoclave tape, bacterial culture vials, and chemical indicator strips may be used to assure adequate sterilization. Dry heat and immersion in EPA approved chemical sterilants are alternative sterilization methods that may be acceptable. Disposable (single-use) items have eliminated the need to reprocess and sterilize equipment in many instances.

Housekeeping

Environmental surfaces such as walls, floors, and ceilings are not normally associated with the transmission of infections to employees because they do not routinely come into contact with susceptible tissue (e.g., mucous membranes, conjunctiva of the eye). However, since dirt is a reservoir for disease and a potential vehicle for the transmission of infection, cleaning and removal of dust, dirt, and soil should be done routinely by laboratory personnel. The type of area, the type of surface being cleaned, and the level of dirt or contamination present will determine cleaning schedules and methods of decontamination.

Work surfaces contaminated by infectious material shall be cleaned and decontaminated by laboratory personnel as soon as possible after the completion of a procedure. Protective coverings such as plastic wrap, aluminum foil, lab table soakers, or other materials used to cover environmental surfaces and equipment shall be removed and replaced as soon as possible after contamination. Additionally, these materials will be removed and replaced by laboratory personnel on a regular basis (e.g., after each shift, daily, or weekly) depending on the frequency of contamination. Bins, pails, cans, and other similar receptacles, which may become contaminated and are intended for reuse, shall be frequently inspected, cleaned and decontaminated as required.

Broken glassware, which may be contaminated, shall never be picked up by hand. Rather, mechanical means such as forceps will be used. When picking up this type of material care must be taken not to aerosolize the blood or other potentially infectious contaminant. Additionally, adequate personal protective equipment shall be worn to protect the employee from accidental contamination. Spills of blood or other potentially infectious materials will be cleaned and decontaminated immediately.

Spill Clean Up Procedures

In the event of a spill of a potentially infectious biological material, including human blood, human cell cultures, recombinant materials, or OPIM the following emergency response procedures shall be followed:

- Notify: All spills of potentially infectious biological material shall be reported to the Laboratory Director (typically the Principal Investigator) and to the Environmental Health and Safety Department.

- Retrieve and Don: Retrieve laboratory biological spill kit and don appropriate PPE.

- Spill Inside Biological Safety Cabinet:
  - Keep biosafety cabinet running
  - Cover affected area with absorbent material
  - Gently apply a liberal amount of disinfectant on top of covered spill
  - Allow at least 20 minutes of contact time

- Collect absorbent material and place into plastic biohazard bag within the BSC
- Clean affected area again with disinfectant
- Placed soiled cleaning materials into biohazard bag
- Remove potentially contaminated PPE and place in biohazard bag within the BSC
- Wash hands before removing safety glasses
- Seal biohazard bag and place in medical waste box for ultimate disposal
- Wash hands

- Spill Outside Biological Safety Cabinet:
  - Attend to any injured persons and follow appropriate emergency response protocols
  - Evacuate affected area or entire laboratory if necessary
  - Allow 30 minutes for aerosol droplets to settle
  - Follow spill protocol for spills inside biosafety cabinet:
    - Cover affected area with absorbent material
    - Gently apply a liberal amount of disinfectant on top of covered spill
    - Allow at least 20 minutes of contact time
    - Collect absorbent material and place into plastic biohazard bag within the BSC
    - Clean affected area again with disinfectant
    - Placed soiled cleaning materials into biohazard bag
    - Remove potentially contaminated PPE and place in biohazard bag within the BSC
    - Wash hands before removing safety glasses
    - Seal biohazard bag and place in medical waste box for ultimate disposal
    - Wash hands
  - Contact the Stevens Environmental Health and Safety Department if spill is beyond the capacity of properly trained and equipped Stevens laboratory employees
  - Document incident

Responsibility for Spill Clean Up

The Stevens Institute of Technology advocates a tiered approach to the clean-up of biological spills in Stevens' laboratories. Laboratory workers must exercise judgment when deciding if they have the appropriate training and equipment to safely clean up the spill.

- Small spills of BL-1 and BL-2 material occurring within the BSC: appropriate for the laboratory worker to clean up with standard PPE, laboratory biological spill kit, and appropriate decontamination solution.

- Moderate spills of BL-1 and BL-2 material occurring within the BSC: appropriate for the laboratory worker to clean up with standard PPE, laboratory biological spill kit, and appropriate decontamination solution.

- Large spills BL-1 and BL-2 occurring within the BSC: not appropriate for the laboratory worker to clean up; contact Stevens Environmental Health and Safety Department for assistance.

- Small spills of BL-1 material occurring outside the BSC: appropriate for the laboratory worker to clean up with standard PPE, laboratory biological spill kit, and appropriate decontamination solution.

- Small, moderate and large spills of BL-2 material occurring outside the BSC: not appropriate for the laboratory worker to clean up; contact Stevens Environmental Health and Safety Department for assistance.

Responsibility for Spill Clean Up

Biosafety Level	Spill Size	Biosafety Cabinet	Clean Up Responsibility
BL-1	Small	Inside	Trained Lab Personnel
BL-2	Small	Inside	Trained Lab Personnel
BL-1	Moderate	Inside	Trained Lab Personnel
BL-1	Moderate	Inside	Trained Lab Personnel
BL-1	Large	Inside	Trained EHS Personnel
BL-2	Large	Inside	Trained EHS Personnel
BL-1	Small	Outside	Trained Lab Personnel
BL-2	Small	Outside	Trained EHS Personnel
BL-2	Moderate	Outside	Trained EHS Personnel
BL-2	Large	Outside	Trained EHS Personnel

Any laboratory worker who is uncomfortable engaging in spill clean up activities shall have the option to contact more experienced laboratory colleagues or the Stevens Environmental Health and Safety Department.

Biological Spill Kits

All Stevens laboratories engaged in research activities involving potentially infectious materials will be equipped with a biological spill kit. Biological spill kits may be custom made or purchased commercially. At a minimum biological spill kits must include:

- Appropriate PPE (gloves, eye protection, surgical mask, laboratory coat or tyvek suit)
- Approved decontamination solution
- Absorbent material
- Autoclavable waste bag and sharps container

Biological/Regulated Medical Waste Disposal

Section 2.4 of the Stevens Waste Management Plan describes the handling of Red Bag/Regulated Medical Waste. Laboratories working with recombinant materials, potentially infectious microorganisms, human and non-human primate cell lines, viral vectors, and other potentially infectious materials are required to decontaminate their liquid and solid biological waste materials prior to discarding the sealed bags in Regulated Medical Waste (RMW) box located in individual laboratories. Properly sealed and labeled RMW boxes will be disposed through a licensed medical waste vendor in accordance with federal, state, and local regulations. Please refer to Appendix III (Characteristics of Common Laboratory Disinfectants) and Appendix IV (Biological Waste Management) for more information.

Transport of Biological Material

During the course of research activities, it may become necessary to transport biological material between labs, between floors, or between buildings at Stevens. In terms of transportation regulations, biological materials are typically defined as any materials taken from humans or animals, living or dead, fresh or preserved (cells, tissues, organs, blood and body fluids), viruses, DNA, or parasites used for diagnostic or research purposes. In order to eliminate potential exposure to biological material during transport, lab workers must adhere to the following guidelines:

- Transport of biological materials may occur by hand or with a cart.
- Individual samples must be stored in a sealed and labeled primary container.
- Sealed and labeled primary containers must be placed in leak proof secondary containers during transport.

- When transporting liquids, secondary containers must be large enough to contain more than the total volume of liquid being transported and must be lined with an absorbent material.
- Examples of some common transport methods are described below:

An example of a secondary container, lined with an absorbent material, suitable for transporting liquid biological materials between labs or floors. It is recommended that this secondary container be transported on a cart as an additional safeguard.

An example of a commercially available specimen transport container. This container has a tight fitting, sealable lid, with a rubber gasket. This container may be hand carried or placed on a cart. When used to transport biological materials, this container should be properly labeled.

An example of a commercially available cooler being utilized for specimen transport. In this example, individual sample tubes are placed within a zip lock and labeled plastic bag. The transport cooler is properly labeled and contains absorbent material.

If evidence of a leak or spill is detected when opening a container used to transport biological material, it is important to ensure that all potentially contaminated items are adequately decontaminates with a suitable disinfectant.

Shipping of Biological Material

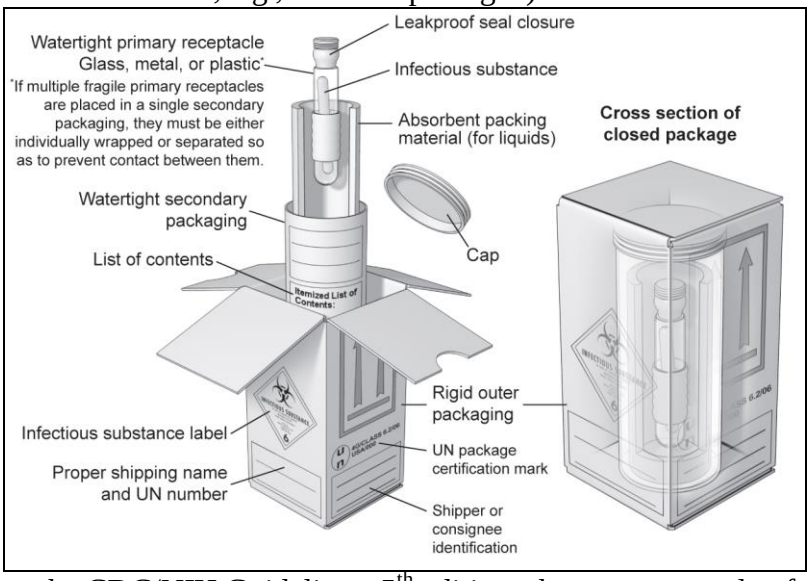
The guidelines and examples described above pertain only to the transport of biological materials between labs, floors, or building at Stevens. When it becomes necessary to ship biological materials to locations outside of Stevens, additional guidelines and regulations must be adhered to. The following regulations apply to the packaging and shipment of biological materials:

- U.S. Department of Transportation, 49 CFR Parts 171-180 and amendments
- U.S. Public Health Service, 42 CFR Part 72, Interstate Shipment of Etiologic Agents
- U.S. Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne Pathogens
- International Air Transport Association (IATA), Dangerous Goods Regulations

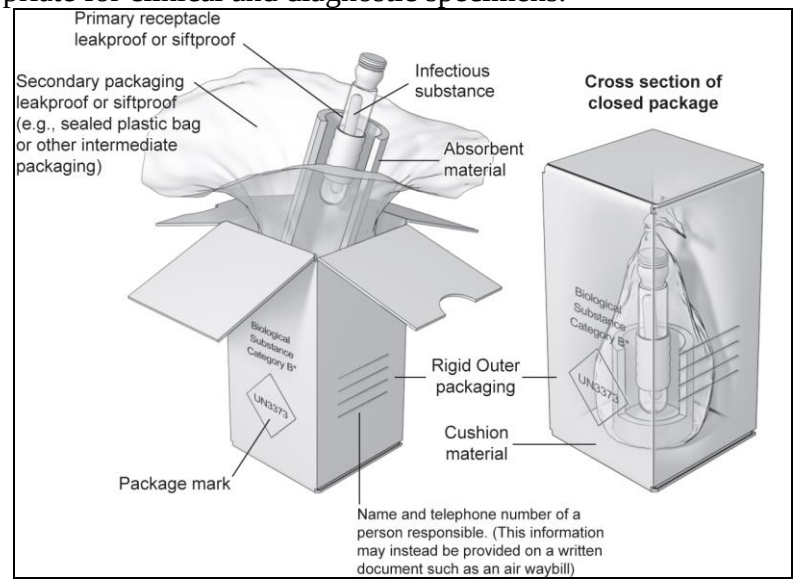
- U.S. Postal Service, 39 CFR Part 111, Mailability of Etiologic Agents, Mailability of Sharps and Other Medical Devices, and Publication 52, Acceptance of Hazardous, Restricted or Perishable Matter

All Stevens personnel involved in the shipping of biological specimens will complete semi-annual training as required by the IATA Dangerous Goods Regulations; as the IATA Dangerous Goods Regulations are the primary regulation governing the shipment of biological materials by air. Laboratory groups that are uncertain regarding the required protocols to follow when shipping biological materials shall contact the Stevens Environmental Health and Safety Department for guidance.

The diagram below, from the CDC/NIH Guidelines, 5th edition, shows an example of the UN standard triple packaging system for materials known or suspected of being a Category A infectious substance (a material that is transported in a form that is capable of causing permanent disability or life-threatening or fatal disease to otherwise healthy humans or animals, e.g., a known pathogen).



The diagram below, from the CDC/NIH Guidelines, 5th edition, shows an example of the UN standard triple packaging system for materials known or suspected of being a Category B infectious substance. This type of packaging is also appropriate for clinical and diagnostic specimens.



Hazard Communication
In order to communicate the existence of a potential biological hazard to others, all containers of regulated medical waste must be labeled with the Universal Biohazard Symbol. These labels shall be fluorescent orange or orange-red with lettering and symbols printed in a contrasting color. These labels are commercially available from a variety of sources. A sample biohazard warning label is depicted below.

Biohazard warning labels shall also be affixed to biological safety cabinets, refrigerators, freezers, incubators, and other equipment used to manipulate, store, transport, and ship blood or other potentially infectious material.

Entrance doors to work areas in clinical, academic and research laboratories where potentially infectious materials are in use shall be posted with the biohazard warning label. In addition to the biohazard symbol, these labels shall include the name of the infectious agent in use, any special requirements for entrance to the area, and the name and telephone number of the laboratory director or other responsible person, see attached label.

Laboratories engaged in recombinant DNA manipulations must also post areas and containers where recombinant materials are manipulated, transported, and stored. The Universal Biohazard Symbol is not required for recombinant DNA laboratories operating at BL-1 containment. An alternative labeling method that includes the words "r-DNA/BL-1" may be used. However the Universal Biohazard Symbol is required for recombinant DNA laboratories operating at BL- 2 or higher.

Laboratory Access
In order to control unnecessary and unintended foot traffic through the laboratory, laboratory doors will be closed whenever BL-2 work is in progress. Further, BL-2 "Experiment in Progress" signs will be affixed to laboratory doors when potentially infectious materials are being manipulated.

HIV and HBV Research Laboratories and Production Facilities
All research laboratories and production facilities engaged in the culture, production, concentration, experimentation, and manipulation of HIV or HBV shall meet the criteria set forth in 29 CFR 1910.1030(e) (Section (e) of the Bloodborne Pathogen Standard). Additionally, these laboratories shall conform to BL-2 standards, practices, equipment and facilities established by the U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health in Biosafety in Microbiological and Biomedical Laboratories (BMBL), HHS Publication No. (CDC) 93-8395. Further, these laboratories will follow operational guidelines established by the Centers for Disease Control and Prevention's Agent Summary Statement for Human Immunodeficiency Virus and Report on Laboratory-Acquired Infection with Human Immunodeficiency Virus, MMWR, April 1, 1988, Vol 37, No.S-4. In some instances, depending on the concentration of the virus being grown, BL-3 standards, practices, equipment, or facilities may be required.

Training
Stevens' employees engaged in the manipulation of biological materials regulated at BL-2, or higher, shall attend a biological safety training session on an annual basis. At the end of a Biological Safety training session Stevens' employees will be able to:

- Obtain a copy of the Biological Safety Guide and be aware of the primary regulations;
- Define potentially infectious material and cite examples;
- Understand modes of transmission of infectious materials especially those which apply to the laboratory setting;
- Understand the basic principals of Biosafety and the containment criteria established for BL-1 and BL-2 laboratories;
- Identify tasks and situations that may involve exposure to potentially infectious material;
- Understand control measures to eliminate, minimize, or reduce exposure to potentially infectious material by using appropriate engineering controls, work practice controls, and PPE;
- Understand how biological safety cabinets function to protect laboratory personnel, maintain product sterility, and protect the environment;
- Take appropriate measures in response to an exposure incident or a spill of potentially infectious material. Additionally, employees will understand the post-exposure medical evaluation and follow-up required after an exposure incident;
- Recognize the universal biohazard symbol and understand its appropriate use; and
- Understand the process by which biohazard protocols are reviewed and approved at Stevens Institute of Technology.

Recombinant DNA
BL-1 through BL-4 have been established in the joint CDC/NIH Guidelines primarily to minimize the potential of laboratory acquired infections among microbiological and biomedical laboratory workers and ancillary personnel. Other guidelines and regulations have been established to regulate laboratory activities involving recombinant DNA manipulations. The NIH Guidelines for Research Involving Recombinant DNA Molecules has been updated regularly since 1978 to keep pace with technological changes in molecular genetics as well as the application of this technology.

All experiments, research, and other activities involving recombinant DNA manipulations performed in Stevens facilities should be conducted in accordance with applicable sections of the NIH Guidelines, cited above. The NIH Guidelines contain an appendix entitled, "Classification of Human Etiologic Agents on the Basis of Hazard". This supplementary information is included as an Appendix to this Biosafety Guide. Because the NIH updates the Recombinant DNA Guidelines often, this listing is usually the most current classification of infectious organisms into risk groups and/or biosafety levels published by a regulatory agency. Stevens' employees may consult it in the event that a risk assessment involving a particular infectious agent or procedure is required.

The purpose of the NIH Guidelines is to "specify practices for constructing and handling recombinant DNA molecules and organisms and viruses containing recombinant DNA molecules". The NIH Guidelines define

recombinant DNA molecules as "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 molecules that result from the replication of those described above". Like the joint CDC/NIH Guidelines, the NIH guidelines are intended to minimize the potential of laboratory acquired infections among laboratory workers. Additionally, the NIH Guidelines are intended to protect the environment from potentially adverse or unknown affects associated with genetically manipulated plants, organisms, and products.

The NIH Guidelines classify microorganisms into 4 Risk Groups (RG) according to their relative pathogenicity for healthy adult humans as follows:

Risk Group 1
Well characterized agents that are not associated with disease in healthy adult humans and of minimal potential hazard to laboratory personnel and the environment. [No or very low individual and community risk].

Risk Group 2
Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available and the risk of spread of infection is limited. [Moderate individual risk, low community risk].

Risk Group 3
Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available and the risk of spread of infection is limited. [High individual risk, low community risk].

Risk Group 4
Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available and can be readily transmitted from one individual to another, directly or indirectly. [High individual and community risk].

The following table is intended to illustrate general relationships between the classification of microorganisms into Risk Groups and the required biosafety level. The table is presented to instill in MRL employees an appreciation of the equivalent biological hazards associated with recombinant DNA technology.

Relationship of Risk Groups to BL-s, Practices, and Equipment				
Risk Group	**Biosafety Level**	**Laboratory Type**	**Laboratory Practices**	**Safety Equipment**
RG-1	BL-1	Basic Teaching	GMT	Open Bench
RG-2	BL-2	Clinical, Research, Diagnostic	GMT plus Protective Clothing and Biohazard Symbol	Open Bench plus BSC to Contain Potential Aerosols
RG-3	BL-3	Special Research or Diagnostic	Level 2 plus special clothing, PPE, Controlled Access, and Directional Airflow	BSC and/or Other Primary Containment for All Activities
RG-4	BL-4	Maximum Containment Facility	Level 3 plus Airlock Entry, Shower Exit, Special Waste Treatment	Class III BSC or Positive Pressure Suits, Double-Ended Autoclave, Filtered Air, Water Treatment

GMT - Good Microbiological Techniques
BSC - Biological Safety Cabinet

Biological Containment

A previous section of this guide (Principles of Biosafety) has introduced the term containment to describe those techniques used to manage or "contain" infectious materials. Generally, containment techniques are of two types: primary containment and secondary containment. Additionally, containment techniques can be divided into two categories: laboratory practices and safety equipment. Recombinant DNA experiments lend themselves to a third category of containment known as biological containment. Biological containment refers to the specific natural biological barriers which exist that limit either the infectivity of a vector or vehicle (plasmid or virus) for specific hosts or its dissemination and survival in the environment. Host-vector systems should be chosen or constructed to minimize the survival of the vector in its host outside the laboratory and to minimize the transmission of the vector from the propagation host to other non-laboratory hosts.

Disposal of Recombinant-DNA Waste

All wastes generated when conducting recombinant DNA manipulations require adequate decontamination prior to disposal. Recombinant DNA wastes are to be treated as biohazardous. The same techniques and equipment shall be used when decontaminating recombinant DNA waste as when decontaminating biohazardous waste. Please refer to section on biohazardous waste disposal, above, and Appendix IV, attached, for additional information. Although generally non-infectious, BL-1 recombinant DNA waste requires adequate decontamination prior to disposal. Similarly, transgenic plant materials require adequate "decontamination" prior to disposal.

Bloodborne Pathogen Standard

In 1991 OSHA promulgated Occupational Exposure to Bloodborne Pathogens, US Department of Labor, 29 CFR 1910.1030; commonly referred to as the Bloodborne Pathogen or BBP Standard. This standard is intended to protect employees by eliminating or minimizing occupational exposure to blood and other potentially infectious materials. The standard applies to all employees who may be reasonably anticipated to come into contact with blood or other potentially infectious materials during the performance of their assigned duties. The BBP standard requires that employers implement a written exposure control plan designed to eliminate or minimize potential employee exposure to blood, other body fluids, and other potentially infectious materials. One key component of the exposure control plan is the concept of **Universal Precautions**. This concept instructs employees to assume that all blood and certain other body fluids, no matter the source, are known to be contaminated with bloodborne pathogens and handled appropriately. The exposure control plan is the main component of an overall BBP Program that includes engineering controls, work practice controls, personal protective equipment, information and training, Hepatitis B vaccination, post exposure medical evaluation and follow-up, and hazard communication.

Application of the BBP Standard

The BBP Standard applies to all employees who may have reasonably anticipated contact with blood or other potentially infectious materials as a result of performing their normally assigned duties. Laboratories utilizing human-derived materials are required to adhere to applicable sections of the Bloodborne Pathogen Standard. These requirements are in addition to compliance with the NIH/CDC Guidelines (BMBL) or the NIH Guidelines for recombinant DNA. Included below is the text of the regulatory compliance letters written between the US Department of Labor and the President of the American Biosafety Association. These letters serve as the regulatory basis for including human materials, including cell lines, manipulated in academic research laboratories within the purview of the OSHA Bloodborne Pathogen Standard.

June 21, 1994 OSHA Standards Interpretation and Compliance Letters entitled "Applicability of 1910.1030 to Established Human Cell Lines"

> The Bloodborne Pathogens standard (BPS) provides protection to employees who have occupational exposure to human blood or other potentially infectious materials (OPIM). Established human cell lines* which are characterized** to be free of contamination from human hepatitis viruses, human immunodeficiency viruses, and other recognized bloodborne pathogens, are not considered to be OPIM and are not covered by BPS. Established human or other animal cell lines which are known to be or likely infected/contaminated with human microbes or agents classed as bloodborne pathogens, especially hepatitis viruses and human immunodeficiency viruses are covered by the BPS. The final judgment for making the determination that human or other animal cell lines in culture are free of bloodborne pathogens must be made by a Bio-safety Professional or other qualified scientist with the background and experience to review such potential contamination and risk, in accordance with the requirements of the BPS. Documentation that such cell lines are not OPIM should be a matter of written record and on file with the employer for OSHA review.
>
> All primary human cell explants from tissues and subsequent in vitro passages of human tissue explant cultures (human cell "strains" ***) must be regarded as containing potential bloodborne pathogens and should be handled in accordance with the BPS. Non-transformed, human cell "strains", characterized by documented, reasonable laboratory testing as described in the attachment, to be free of human immunodeficiency virus, hepatitis viruses, or other bloodborne pathogens may be exempted from the standard's requirements. However, if such tissue explants or subsequent cultures are derived from human subjects known to carry bloodborne pathogens, such as hepatitis viruses or human

immunodeficiency viruses or are deliberately infected with bloodborne pathogens, they must be handled in accordance with the precautions noted in the BPS. Likewise, animal tissues, explants or cell cultures known to be contaminated by deliberate infection with human immunodeficiency virus or Hepatitis B virus are also subject to the BPS.

All laboratory work with primary human tissues or body fluids is covered by the BPS.

Definitions:

Human Cell Line*
A Human Cell Line is defined as **in vitro** or animal passaged (e.g., nude mouse) cultures or human cells that fulfill traditional requirements of a **cell line** designation. That is, the cells are **immortalized** cells, transformed by spontaneous mutation or natural or laboratory infection with an immortalizating agent such as Epstein-Barr virus (EBV). EBV is a bloodborne pathogen. It should be noted that human cervical carcinoma cells or other transformed human cell lines like HeLa cells are sometimes adulterated with laboratory pathogens accidentally introduced by cultivation with other cell cultures, or physically contaminated by other cell cultures handled in the same lab. In order to handle human HeLa cells, without having to comply with the requirements of the bloodborne pathogens standard (BPS), human HeLa cells should be documented to be pure HeLa cells and shown to be free of bloodborne pathogens by testing.

Characterization of Human Cells**
Characterization of human cells, for inclusion or exclusion from compliance with the BPS, would include screening of the cells lines or "strains" for viruses characterized as bloodborne pathogens by the Standard, including human immunodeficiency viruses, hepatitis viruses or EBV, if the cells are capable of propagating such viruses. Most cell lines are screened for human mycoplasmas and are free of bacterial and mycotic contaminants. Testing may include antigenic screening for viral or agent markers, co-cultivation with various indicator cells that allow contaminants to grow, or using molecular technology (polymerase chain reaction or nucleic acid hybridization) to identify latent viruses capable of infecting humans such as Herpesviruses(e.g., EBV), or papilloma members of the **Papovavirus group**, etc. Cell lines that are procured from commercial vendors or other sources with documented testing to be free of human bloodborne pathogens and which have been protected by the employer from environmental contamination may be excluded from the BPS.

Human Cell Strains***
Human cell strains are defined as cells propagated in vitro from primary explants of human tissue or body fluids which have finite lifetime (non-transformed) in tissue culture for 20-70 passages. Human cell "strains" must be handled as potential biohazards unless characterized by testing to be free of bloodborne pathogens (i.e., WI-38 cells are often so documented).

Exposure Determination
The following Stevens job classifications in which employees may have laboratory exposure to bloodborne pathogens include: Principal Investigators, post-doctoral fellows, graduate students, undergraduate students, and other laboratory workers who may be reasonably anticipated to come into contact with human-derived materials should be covered by the Stevens' Bloodborne Pathogens Program.

Assignment of Responsibility
The laboratory aspects of the Stevens' Bloodborne Pathogens Program are under the direction of the Stevens' Director of Environmental Health and Safety.

Stevens Institute Laboratory Workers Shall:

- become familiar with the Stevens' Exposure Control Plan;
- follow safe work practices as described in the Work Practice section of the Exposure Control Plan;
- be cognizant of the presence of potentially infectious materials in the laboratories in which they work;
- attend required training;
- wear appropriate personal protective equipment (PPE);
- maintain and PPE in an acceptable manner;
- report all accidents, injuries, exposure incidents, and hazardous conditions to their supervisor;
- seek prompt treatment for work related injuries and exposures; and
- comply with other pertinent sections of the Exposure Control Plan.

The Bloodborne Pathogen Standard and the Steven's Biological Safety Guide

There are many aspects of the Bloodborne Pathogens Standard that are similar to laboratory biosafety issues which have been previously described in various sections of this guide. Previous sections of this guide have described:

- Transmission of Infectious Agents
- Laboratory Acquired Infections
- Personal Protective Equipment
- Gloves
- Protective Eyewear
- Face Masks
- Labcoats, Gowns and Aprons
- Cleaning, Disinfection, and Sterilization
- Sharps
- Housekeeping
- Handwashing
- Regulated Medical Waste Disposal
- Hazard Communication
- HIV and HBV Research Laboratories and Production Facilities

Since the above mentioned subjects have been addressed in previous sections of this guide as they apply to potentially infectious microorganisms, recombinant DNA, oncogenic viruses, viral vectors, human cell lines, long term cell culture, etc., there is no need to repeat these subjects in the BBP section of the guide.

However, where the BBP Standard has specific regulatory requirements beyond those biosafety criteria addressed by the CDC/NIH Guidelines (BMBL) and the NIH rDNA Guidelines; these requirements will be addressed in the subsequent sections of the guide.

Transmission of Bloodborne Pathogens

The BBP standard is principally concerned with eliminating or minimizing the occupational transmission of the Hepatitis B Virus (HBV) and the Human Immunodeficiency Virus (HIV). HIV and HBV are transmitted in a similar manner; by sexual contact, by needle sharing, and by perinatal transmission. In the workplace, however, both viruses have been transmitted only by contaminated needle stick, other contaminated puncture wound, and by contact of an open wound, non-intact skin, or mucous membrane with contaminated blood, body fluids, or concentrated virus. Blood is the most important source of HIV and HBV in the occupational

setting. It may be reasonably anticipated that Stevens' employees may come into contact with potentially contaminated materials when performing laboratory manipulations involving human body fluids (see list below) or with potentially contaminated human-derived materials, e.g., human cell or tissue culture and human cell lines. Eliminating potential exposure incidents will be discussed in the Work Practice section of the Exposure Control Plan.

Environmental Survivability

One milliliter (ml) of blood from a person infected with HBV may contain more than 100 million infectious virus particles. In a dried state HBV may remain viable on work surfaces (e.g., laboratory bench or within a biosafety cabinet) for one week or longer. In contrast, one ml of blood from an individual infected with HIV may contain several hundred to 10,000 infectious viral particles. Experiments conducted by the Centers for Disease Control and Prevention (CDC) have shown that viral concentrations of HIV have been reduced by up to 99% by drying (air exposure) within several hours. The above data indicates that HBV is significantly hardier than HIV. Although the consequences of an HIV infection are obviously severe, occupational HBV exposure and infection are more common, easier to acquire, and harm more workers than occupational HIV infection.

Exposure Control Plan/Methods of Compliance

Universal Precautions

In 1993 the CDC introduced the concept of "Universal Blood and Body Fluid Precautions" (Universal Precautions) to be applied in the care of all patients and in the handling of blood and body fluid specimens. This approach is based on the concept that all patients, blood, and body fluid specimens are to be handled as if they are known to be infected with HIV, HBV, or other bloodborne pathogens. Universal Precautions require that adequate safeguards, e.g., barrier precautions, be taken to eliminate or minimize occupational exposure to blood and body fluids. The OSHA BBP standard requires the use of Universal Precautions in occupational settings where contact with blood or other body fluids may be reasonably anticipated.

Body Fluids to Which Universal Precautions Apply

Blood is the most important source of HIV, HBV, and other bloodborne pathogens in the occupational setting. Other body fluids, in addition to blood, and laboratory materials to which Universal Precautions apply include:

- semen,
- vaginal secretions,
- cerebrospinal fluid
- synovial fluid,
- pleural fluid,
- pericardial fluid,
- peritoneal fluid,
- amniotic fluid,
- saliva in dental procedures,
- any body fluid that is visibly contaminated with blood,
- all body fluids in situations where it is difficult to differentiate between body fluids,
- unfixed human primary tissue explants,
- HIV, HBV, HCV cell, tissue, and organ cultures,
- potentially contaminated culture media or other laboratory solution,
- blood, organs, or tissues from experimental animals infected with HIV, HBV, or HCV,
- human cell lines
- other potentially infectious material

Other Potentially Infectious Material (OPIM)
Other Potentially Infectious Material (OPIM) is a term commonly used to describe various materials that are not specifically included on the list of body fluids covered by the BBP, but may still be considered potentially infectious. Examples may include a bloody bandage encountered in a doctor's office or a contaminated paper towel used to clean up a spill of contaminated cell culture media, etc.

Body Fluids to Which Universal Precautions Do Not Apply
Unless visibly contaminated with blood, the following body fluids are not considered as potentially infectious materials under the BBP standard:

- saliva,
- urine,
- feces,
- vomit.

Field Settings
The handwashing requirements, described above, generally apply to clinical or laboratory settings which may be termed "housed settings". Handwashing requirements specified for housed settings are also desirable for field settings. If for any reason a Steven's employee requires handwashing facilities when none are available (for example when collecting field samples) the employee will utilize antiseptic towelettes or germicidal hand cream followed by the application of a moisturizing lotion. Clean cloths or paper towels may also be used. The employee will then wash their hands with soap and water at their first opportunity.

Hepatitis B Vaccination
Although the potential for occupational exposure to HBV is much higher than HIV, HBV infection is preventable by vaccination. A safe and effective vaccine to prevent HBV has been available since 1982. The original vaccine was plasma derived; made from the pooled sera of positive carriers. Currently, the vaccine most often used for protection against HBV is a genetically engineered yeast based vaccine called Recombivax. Vaccines produced through recombinant DNA technology are termed subunit vaccines. There is no risk of infection with subunit vaccines. Typically, the hepatitis B vaccine protects 90% of those who receive it for approximately 7 years.

The Hepatitis B vaccine is available to all Stevens' employees who may be reasonably anticipated to have contact with blood or other potentially infectious materials in the laboratory setting. The Director of the Environmental Health and Safety Department will ensure that all eligible Stevens' employees are offered the hepatitis B vaccine at no cost to the employee.

The vaccine is to be given after eligible MRL employees receive initial BBP training (described below) and sign the "Hepatitis B Vaccine Consent Form" but no later than one month from the consent date. The vaccine will be offered to new eligible Stevens' employees within 10 days of the new assignment of duties with occupational exposure.

An eligible Stevens' employee may decline the vaccine by signing the "Hepatitis B Vaccine Declination Form" An eligible Stevens' employee who initially declined the vaccination may change their mind at any time and request the vaccination by signing the "Hepatitis B Vaccine Consent Form".

Work Practice Controls
Work practice controls refer to practices and procedures which reduce or eliminate the chance of occupational exposure to bloodborne pathogens. Examples of work practice controls include:

- Always wear the appropriate personal protective equipment for the task being performed.

- Always perform laboratory manipulations of potentially infectious materials with the potential to create aerosol droplets in a properly functioning and certified biological safety cabinet.

- When potentially infectious materials must be manipulated outside of a biological safety cabinet, always use Universal Precautions.

- Follow Biosafety Level 2 (BL-2) safeguards when handling blood, human body fluids, and human-derived materials (including human cell lines) in the laboratory.

- Wash hands promptly after removal of gloves, after performing laboratory procedures, prior to exiting a laboratory, and prior to eating and or drinking.

- Do not eat, drink, smoke, apply lip balm or makeup, and handle contact lenses in areas where occupational exposure to blood or other potentially infectious material may occur.

- Never store food or drink in refrigerators, freezers, cabinets, or on shelves, countertops, and benchtops, where blood and other potentially infectious materials may be (or have been) present.

- Observe hazard warning signs and labels applied to all biological safety cabinets, laminar flow hoods, incubators, and other pieces of laboratory equipment where blood and other potentially infectious materials may be (or have been).

- Ensure that all laboratory equipment that was used to store or manipulate blood or other potentially infectious material is appropriately surface decontaminated prior to servicing.

- Promptly decontaminate any work surface or equipment following exposure to blood or other potentially infectious material.

Handling of Sharps

Contaminated sharps such as needles, scalpel blades, broken test tubes, Pasteur pipettes, and other sharp instruments present the greatest risk of transmission of bloodborne pathogens in the laboratory. Disposable syringes, scalpel blades, and other sharp items should be deposited into an appropriate leak-proof, puncture-resistant, and labeled sharps container immediately after use. Disposable needles should never be recapped, bent, broken, sheared, or removed from disposable syringes.

Sharp items should be deposited directly into the sharps container immediately following use. Full sharps containers should be disposed in the laboratory's Regulated Medical Waste box.

Sharps containers should be located in all work locations where it is reasonably anticipated that sharps may be used. Sharps containers should only be filled to within one inch of the top of the container. Sharps containers should never be overfilled. Never attempt to force additional material into a full container.

Wherever possible the use of sharps should be eliminated or minimized from protocols involving blood, human body fluids, and human-derived materials (including human cell lines) in the laboratory. When sharp use is essential great care must be used to handle sharps carefully to reduce the chance of occupational exposure.

Needle Stick and Mucous Membrane Exposure Policy

A needle stick may be defined as a skin puncture with a needle or other sharp object that has been used to inject a patient, draw blood from a patient, or penetrate a patient's skin or mucous membrane. Alternatively, a needle stick may be defined as a skin puncture with a needle or other sharp object that has been used to manipulate blood or other potentially infectious material in the laboratory or other setting. Needle sticks with an unused sterile needle or needles used to draw up medications are not considered needle sticks in the context of the Bloodborne Pathogen Standard, however, needle sticks of this type should be reported to the employee's supervisor. A mucous membrane exposure may be defined as a splash, spray, or aerosolization of blood or other potentially infectious material that comes into direct contact with an employee's eyes, nose, or mouth or penetrates an employee's open wound or sore.

In the event that a Stevens' employee sustains:

- a needle stick, cut, or puncture wound involving a piece of potentially contaminated laboratory equipment;
- a splash of blood or other potentially infectious material to the face;
- contact with blood or other potentially infectious material to an open wound, sore, or non-intact skin the following procedures shall be followed:

Employee

- Incidents or injuries that require medical attention must be addressed immediately. In extreme situations Stevens' employees may be required to dial 911 or report to a local hospital emergency room for medical attention.

- Immediately clean the exposed area. The skin should be thoroughly washed with soap and running water. Vigorous scrubbing should be avoided as this may damage the skin and increase the chance of disease transmission. Exposed mucous membranes should be thoroughly rinsed with copious amounts of running water.

- Immediately after cleaning the exposed area, the affected employee will contact their laboratory manager (usually the Principal Investigator) and report the incident. All information concerning the exposure incident, including the name of the source patient, if applicable, should be reported.

- At their earliest opportunity, the affected employee will complete a Stevens Institute Injury and Incident Report Form. Information will be reported in a complete and honest manner. All information concerning the exposure incident, including the name of the source patient (if applicable), the location where the exposure occurred, the nature of the exposure, should be included in the incident report. This information will assist Stevens' administrators to determine the root cause(s) of the incident and determine if additional safeguards or procedures need to be established.

- Following an exposure incident where a Stevens' employee is required to receive medical attention at a local hospital emergency room or other medical provider, Stevens' management may require the employee to report to a specified health care provider for medical evaluation and follow-up.

Laboratory Supervisor

- Assure that injured employee receives appropriate emergency medical attention.
- Assure proper protocol is followed while maintaining appropriate medical confidentiality.
- Alert Stevens' management of the incident as well as the need for source patient/individual counseling, if applicable.
- Assure that injured employee promptly presents to the specified Stevens' health care provider for medical evaluation and follow up.
- Provide a description of the exposed employee's duties as they relate to the exposure incident to the specified Stevens' health care provider. The BBP Standard also requires that a copy of the BBP Standard is provided to the health care provider performing the evaluation. The Stevens' Director of Environmental Health and Safety, or other administrative official, may have already provided the BBP Standard to the specified health care provider.
- Document the route(s) of exposure and circumstances under which the exposure occurred and provide that information to the specified Stevens' health care provider.
- Complete the Stevens' Injury and Incident Report Form and submit to Stevens' management within 24 hours of the incident.
- Since much of the same information is required by the specified health care provider and Stevens' management, it is prudent that a copy of the completed Injury and Incident Report Form accompany the affected employee when they present to the specified health care provider for medical evaluation and follow-up.

Specified Health Care Provider

- Assure confidentiality of all medical information.
- Inspect contact site of exposed employee and ensures that proper immediate care is/was provided.
- If applicable, counsel source patient/individual and obtains informed consent for HIV antibody testing and authorization for the use of confidential HIV related information.
- Provide post-test counseling for exposed employee and source patient/individual, if applicable.
- Provide the exposed employee with a confidential medical evaluation and follow-up that includes:
    - Documentation of source individual's HIV and HBV status as determined by serological testing, if applicable.
    - Review of all medical records, including vaccination status, relevant to the appropriate treatment of the exposed employee.
    - Collection and testing of the exposed employee's blood for serological status.

- Provide post-exposure prophylaxis, when necessary, as recommended by the U.S. Public Health Service.

- Advise employee with respect to medical risks, treatment options, vaccination status, and results of medical evaluation and serological testing.

- Provide Stevens management with a written opinion. Stevens' management is responsible to provide the affected employee with a copy of the written opinion within 15 days of the completion of the evaluation.

- The written opinion shall be limited to whether the HBV vaccine is indicated for the affected employee and if the employee has received the HBV vaccination.

- Information in the written opinion concerning post-exposure evaluation and follow-up shall be limited to a statement that the employee has been informed of the results of the evaluation. Also, that the employee has been informed of any medical conditions resulting from exposure to blood or other potentially infectious material that require further evaluation or treatment. All other finding or diagnoses shall remain confidential and not be included in the written report.

- Provide the unit supervisor with documentation that the exposed employee has been evaluated and that the appropriate treatment and follow up has been offered.

Training

Stevens' employees who are reasonably anticipated to come into contact with blood or other potentially infectious material will participate in a training program provided at no cost to the employee and conducted during normal working hours. The purpose of the training is to alert employees of the potential hazards posed by bloodborne pathogens and to assist employees in eliminating or minimizing occupational exposure to bloodborne pathogens in their work environment. Training will be offered to eligible Stevens' employees initially and upon assignment to new duties in which exposure to blood or other potentially infectious material may be reasonably anticipated. Refresher training will be offered to all eligible employees on an annual basis. At the end of a Bloodborne Pathogen training session an employee will be able to:

- Obtain a copy of the Stevens Biosafety Guide including the Exposure Control Plan and the BBP Standard's regulatory text.

- Define bloodborne pathogen, and cite examples.

- Understand modes of transmission of bloodborne pathogens as well as basic symptoms of bloodborne diseases.

- Identify tasks and situations that may involve exposure to blood or other potentially infectious material.

- Take measures to eliminate, minimize, or reduce exposure to blood or other potentially infectious material by using appropriate administrative and work practice controls and personal protective equipment.

- Recognize the benefits of the Hepatitis B vaccination for employees who have potential exposure to blood and other potentially infectious materials. Additionally, employees will know how to obtain the HBV vaccination, understand information regarding its safety, efficacy, method of administration, and that it is offered at no cost.

- Take appropriate measures in response to an exposure incident or other contact involving blood or other potentially infectious materials. Additionally, employees will understand the post-exposure medical evaluation and follow-up required after an exposure incident.

- Recognize the biohazard symbol as well as other signs and labels pertinent to this standard and understand their appropriate use.

Recordkeeping
The Bloodborne Pathogen Standard requires that employers maintain medical records and training records for all eligible employees.

Medical Records
Stevens Institute will establish and maintain a medical record for each eligible employee. The Administrative Safety Officer for the duration of the employee's employment plus 30 years will maintain medical records in a confidential manner. Medical records will not be disclosed or reported without the employee's written permission to any person within or outside of Stevens Institute. However, medical records may be made available, upon request, to the Assistant Secretary of Labor, U.S. Department of Labor. Medical records will include at least the following:

- Employee's name, social security number, and job title.

- The employee's HBV vaccination status including the dates of all vaccinations and all medical records relative to the employee's ability to receive the vaccine.

- Results of medical examinations, medical testing, and post-exposure evaluation and follow-up.

- The employer's copy of the healthcare professional's written opinion limited to the information described above.

- A copy of the information provided to the healthcare professional.

Training Records
Stevens will maintain training records relative to the training requirements of the Bloodborne Pathogen Standard. Training records will be maintained for three years from the date the training occurred. Training records may be made available, upon request, to the Assistant Secretary of Labor, U.S. Department of Labor, or an authorized representative. Training records will include:

- The employee's name social security number, and job title.

- Dates and summaries of the training sessions.

- Names and qualifications of persons conducting training.

Transfer of Records
In the event that Stevens ceases to do business, and there is no successor employer to receive and retain the above described medical and training records, in the prescribed time period, Stevens may be required to transmit the records to an appropriate government agency. In this event Stevens will notify the NIOSH Director, or designated representative, at least three months prior to cessation of company activities and transmit the training records if required by the Director to do so.

Stevens Institute of Technology
Laboratory Specific Exposure Control Plan (ECP) Summary

The Stevens Institute of Technology's Exposure Control Plan (ECP) has been developed in compliance with 29 CFR 1910.1030; the OSHA Bloodborne Pathogens Standard. The ECP is intended to establish policies, practices, and procedures that minimize or eliminate occupational exposure to potentially infectious materials among Stevens's employees. Acknowledging that the OSHA Bloodborne Pathogens standard (and the written ECP required by the standard) are generic in nature, Stevens has developed the following Laboratory Specific ECP to document specific policies and procedures of each laboratory group.

Please enter the required information in the spaces below. The Laboratory Specific ECP should be updated on an annual basis or whenever required (e.g., new hire, new laboratory protocol).

Laboratory Group/Department:

Supervisor/Director/Principal Investigator:

Location: Date:

Eligible Employee Listing: In the space below please list the names, titles, and assigned duties of all eligible employees in this laboratory/work location. Eligible employees include those who may be reasonably anticipated to come into contact with human-derived materials including human blood or other potentially infectious materials (OPIM) as a result of performing their job duties.

Employee Name	Title	Assigned Duties

Engineering Controls: In the space below please list all engineering controls in use in this laboratory/work location that serve to eliminate or minimize occupational exposure to human-derived materials including human blood or OPIM. Engineering controls are mechanical devices serve to create a barrier between a worker and a potential hazard.

Engineering Control	Nature of Potential Hazard

Work Practice Controls: In the space below please list the work practice controls in use in this laboratory/work location that serve to eliminate or minimize occupational exposure to human blood or OPIM. Work practice controls are policies and procedures put into place to help eliminate or minimize occupational exposure to human blood or OPIM. Please use general categories (e.g., proper control of sharps, universal precautions, adhering to aerosol minimization techniques) rather than listing each individual work rule except those work rules that may be unique to this particular setting.

Work Practice Control	Nature of Potential Hazard

Personal Protective Equipment: In the space below, please list all personal protective equipment (PPE) issued to employees in this laboratory/work location that serve to protect workers from occupational exposure to human blood and OPIM. Please be specific when describing the type of PPE (e.g., disposable non-sterile nitrile exam gloves, cloth lab coat, disposable Tyvek lab coat, safety glasses with side shields).

Personal Protective Equipment	Nature of Potential Hazard

Cleaning, Disinfection, and Sterilization: In the space below, please list and describe the frequency and method of cleaning, disinfection, and sterilization in this laboratory/work location.

Work Surfaces:

Item (e.g., bench, hood)	Disinfectant	Frequency	Method

Personal Protective Equipment:

Item (e.g., safety glasses, reusable gloves)	Disinfectant	Frequency	Method

Waste Materials:

Item (e.g., sharp containers, medical waste)	Disinfectant	Frequency	Method

Reusable Instruments, Equipment, Other:

Item	Disinfectant	Frequency	Method

Emergency Response: In the space below please list and note the location of all emergency response equipment on hand in this laboratory/work location (and in adjacent locations that may be used) to safely and effectively clean and decontaminate a spill of human-derived materials including human blood or OPIM.

Emergency Equipment	Location	Intended Use

Emergency Contact Information: In the space below please list the applicable emergency contacts for this laboratory/work location.

Emergency Contact	Daytime Phone Number(s)	Off-Hour Phone Number(s)
Lab/Department Director		
Stevens Safety Officer		
Police		
Fire		
Ambulance		
Other		

Appendices

Appendix I	Registration Document for Biohazards
Appendix II	Classification of Human Etiological Agents on the Basis of Hazard
Appendix III	Characteristics of Common Laboratory Disinfectants (Excerpted from the World Health Organization's Laboratory Biosafety Manual, Third Edition, Geneva, 2004)
Appendix IV	Biological Waste Management