Biosafety Protocol

STEVENS INSTITUTE OF TECHNOLOGY

Prepared and Approved by:
Institutional Biosafety Committee

Rev: 02/2010

This document describes the Biosafety Protocol for Stevens Institute of Technology. This plan conforms to regulations set forth by the Code of Federal Regulations, Title 29 (Labor), Chapter XVIII (Office of Safety and Health Administration), Part 1910, Subpart I, Sections .132 (29 CFR 1910.132) " Personal Protective Equipment", Subpart Z, Sections .1030 (29 CFR 1910.1030) "Bloodborne Pathogens" and .1450 (29 CFR 1910.1450) "Occupational Exposures in the Laboratory", Title 32 (National Defense), Parts 626 and 627, (Biological Defense Safety Program; "Administrative and Work Practices Controls" and "Technical Safety Requirements", respectively)(32 CFR 626 and 32 CFR 627), Title 40 (Protection and Environment), Part 725 (40 CFR 725) "Reporting Requirements and Review process for Microorganisms" and Title 49 (Transportation), Chapter I, Part 173, Sections .1-.133 and the New Jersey *Community Right to Know Act*, the *Federal Emergency Planning and Community Right to Know Act of 1986 (EPCRA)* and the New Jersey Administrative Code Title 7, Chapter 26 (G), 7:26A.

In order to comply with these standards, Stevens Institute has established this Biosafety Plan to ensure that employees and students are informed about and protected from any health hazards that are associated with biohazradous materials they may be exposed to in the laboratory or other teaching or research environments.

This plan is made readily available to the entire Stevens community in hard copy and internally on the World Wide Web ("the Web"). A review and evaluation of this Biosafety Plan is conducted annually and is updated as necessary.

Questions regarding this plan should be directed to:

Tsan-Liang Su, Research Associate Professor, Committee Chair, CES, x5697, t1su@stevens.edu

(name, title, campus address, phone and email)

The individuals responsible for implementation of this plan are members of the Institutional Biosafety Committee (IBC), a division of the Stevens Safety Committee. Members of the IBC are:

(name, title, campus address, phone and email)

Phil Leopold, Dept. Director and Professor, CCBBME, x8957, phil.leopold@stevens.edu

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Contact Information

All Emergencies

On Campus Phone	**x5105**
Or, dial	**201-216-5105**

(May also use red phones located in corridors on campus or follow instructions posted near conventional phones)

Biological Safety Officer	
Frank Cannavale	x8291
Institutional Biosafety Committee	
Chair: Tsan-Liang Su	x5697
Risk & Safety Oversight Task Force Committee	
Chair: Keith Sheppard, Professor, Material Science & Engineering	x5260
Tony Blazini, Associate Director, Residence Life	x5130
Frank Cannavale, Technical Assistant, Dept. of Chem. & Chem Biology	x8291
Jane Cannavale, Safety Officer	x5276
James Collins, Deputy Chief of Campus Police	x5106
Norman Forster, Director of Maintenance, Physical Plant	x5278
Timothy Griffin, Chief, Campus Security	x5107
Mark Samolewicz, Vice President, Human Resources	x5122
Chuck Shaw, Director of Risk Management, Office of Finance	x8568
Tsan-Liang Su, Research Associate Professor, Center for Environ. Sys.	x5697
Safety and Environmental Officer	
Jane Cannavale	x5276
Biohazard Waste and Chemical Waste Disposal Information	
Frank Cannavale, Technical Assistant	x8291
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Custodial Services	
Norman Forster, Director of Maintenance, Physical Plant	x5278
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Tsan-Liang Su, Research Associate Professor, Center for Environ. Sys.	x5697
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Foreword

This manual (*Biosafety Protocol)* has been developed as part of Stevens Institute of Technology's Biosafety program. The manual sets forth a Biosafety Protocol, which provides a complete program of administration controls, medical surveillance, vaccination and containment strategies for reducing risk posed by biohazardous agents. This program has been established so that the Stevens campus presents an environment that is suitable for high quality research and teaching while maintaining a safe work place. This is accomplished by protecting personnel and students from exposure to infectious agents, preventing environmental contamination, and complying with applicable federal, state and local requirements. The Biosafety program ensures this by providing Institute-wide safety guidelines, polices, and procedures for the use and manipulation of biohazards. Although implementation of these procedures is the responsibility of the Principal Investigator (PI) or course Instructor, its success depends largely on the combined efforts of laboratory supervisors, personnel and students. Therefore, planning for implementation of biological safety must be part of every laboratory activity in which biohazardous materials are used.

It is important to remember that although this manual provides assistance in the evaluation, containment and control of biohazards, it is imperative that any individuals involved receive the proper supervision and training.

THE MOST IMPORTANT ELEMENT IN MAINTAINING A SAFE WORK ENVIRONMENT **IS STRICT ADHERENCE TO GOOD MICROBIOLOGICAL AND LABORATORY PRACTICES AND TECHNIQUES**. ALL INDIVIDUALS MUST BE AWARE OF POTENTIAL RISKS AND MUST BE TRAINED AND PROFICIENT IN THE PRACTICES AND TECHNIQUESS REQUIRED FOR HANDLING BIOHAZRDOUS MATERIAL. **IT IS THE RESPONSIBILITY OF THE PRINCIPAL INVESTIGATOR OR COURSE INSTRUCTOR TO PROVIDE AND/OR ARRANGE FOR APPROPRIATE TRAINING OF ALL PERSONNELL.**

NOTE: as NIH guidelines are continually updated and amended, the reader is directed to the NIH website, www.niehs.nih.gov/odhsb/manguide/man.htm
and the Code of Federal Regulations website, www.access.gpo.gov/nara/cfr/cfr-table-search.html for any updates made to the NIH guidelines, upon which this Protocol is based.

OVERVIEW

The following section presents a brief overview of the information presented in this Biosafety Protocol (***the Protocol***), the reference documents cited within it, the *NIH Guidelines* and *Biosafety in Microbiological and Biomedical Laboratories*. It is intended as a summary only; the reader is urged to obtain full, detailed information pertaining to each subject from within the text of this Protocol from the pertinent reference documents cited or from the *NIH Guidelines*. Use this information as a checklist for compliance rules, regulations, responsibilities and procedures. Where relevant, topics are accompanied by a reference to the sections within this *Protocol.*

What are Biological hazards and Biosafety?

A *Biohazard* is an agent of biological origin that has the capacity (or is perceived) to produce deleterious affects on humans; e.g. recombinant DNA molecules, their hosts and sources, bloodborne pathogens, etiological agents, genetically engineered organisms, cell cultures and tissues.
Biosafety is a program of administrative controls, medical surveillance and containment strategies for reducing risks of exposure to biohazards. (***for details, see Section I, Introduction to Biosafety)*.**

What activities are addressed in this Protocol?

This Protocol details guidelines which must be adhered to if any of the following activities are conducted (see also ***Section I, I):***

- transfer of drug resistance trait into microbes which may compromise the use of the drug to control disease agents in humans, veterinary medicine or agriculture
- formation of recombinant DNA molecules which biosynthesize lethal toxin molecules **(*Section I,E)***
- transfer of recombinant DNA or RNA into human subjects ***(Section I,E)***
- use of Risk Group 2, 3 or 4 agents or restricted agents ***(Section I,G2 & Appendix E)***
- cloning of DNA from Risk Group 2, 3 or 4 agents into nonpathogenic prokaryotic or lower eukaryotic organisms agents ***(Section I,G2 & Appendix E)***
- use of infectious DNA or RNA viruses in tissue culture systems ***(Section I,H)***
- experiments involving whole animals or plants whose genome has been altered through the introduction of recombinant DNA molecules agents ***(Section I,H)***
- experiments involving more than 10 liters of culture of any kind ***(NIH Guidelines Appendix K)***
- research that is conducted at or is sponsored by an institution that receives any support for recombinant DNA research from the NIH
- research directly supported by NIH funds

Who must adhere to these guidelines?

All individuals who are involved in experiments which include any of the above activities or come in contact with any aspect of these activities or facilities, instrumentation, containment or storage systems and waste products generated from these activities. Individuals include

- Principal Investigators, laboratory supervisors
- Instructors of educational laboratories
- Teaching assistants, other laboratory aides and laboratory staff
- Graduate and undergraduate students working in research laboratories or attending educational laboratory courses
- Custodial and maintenance staff performing work-related activities in research or educational facilities

What are the responsibilities of the members of the Stevens Community regarding Biological Safety?

The Institution (Stevens) must ensure that any recombinant DNA or other types of biohazard research funded by the NIH is in compliance with the *NIH Guidelines* as summarized in this Protocol.

The Institution's responsibilities include;

- Establish and implement policies that provide safe conduct of biohazard research and ensure compliance with these guidelines
- Establish an Institutional Biosafety Committee (IBC) to carry out policies
- Appoint a Biological Safety Officer (a member of the IBC) if the institution conducts rDNA research at Biosafety Level (BL) 3 or higher or engages in large scale (greater than 10 liters) research (***see Appendices B & C and NIH Guidelines Appendix K***)
- Appoint an individual with expertise in plants or animal containment to the IBC if rDNA research involving plants or animals is conducted ***(Section I,H & Appendix C and NIH Guidelines Appendix P &Q)***
- Ensure that the IBC has adequate expertise and training if activities involving transfer of rDNA into humans are conducted ***(NIH Guidelines Appendix M)***
- Ensure appropriate training of IBC, PI, laboratory and maintenance staff regarding biological safety and implementation of these guidelines
- Establish and maintain a health surveillance program for personnel involved in large scale research and activities involving organisms of Biosafety Level 3 or 4 (see ***Laboratory Safety Monograph*** for components of such a program; available from IBC or the ORDA, NIH)
- Report any significant problems, violations or significant research-related accidents within 30 days to the NIH, unless report filed by PI or IBC

(What are the responsibilities of the members of the Stevens Community regarding Biological Safety? con't)

Institutional Biosafety Committee (IBC) is similarly responsible for ensuring that all biohazardous activities conducted at or sponsored by the institution is in compliance with these guidelines. Compliance is ensured by the review, approval and oversight of all projects involving these activities.

Who's on the IBC? A minimum of five members; two members, not affiliated with Stevens, who represent the interests of the surrounding community with respect to health and protection of environment; one member who represents the Stevens laboratory technical staff. The members should have collective experience and expertise in rDNA technology, biological and general safety, and physical containment techniques and procedures. No member can review or approve their own work. In addition, a consultant with knowledge of institutional commitments and policy, applicable laws, standards of professional practice, community attitudes and the environment should be available. IBC must have a rotating Chair, a Biological Safety Officer, and plant, animal and human gene therapy experts, if applicable.

The IBC responsibilities include;

- Establish Biosafety policies and the Biosafety Protocol to insure safe conduct of work involving biohazardous materials and revise and update the Biosafety Protocol as necessary

- File annual report with the NIH/ORDA including a roster of IBC members, along with biographical sketches

- Conduct open regular meetings and make minutes available

- Review and approve all proposed projects/course material involving biohazardous materials for compliance with this Protocol. Review to include assessment of containment levels, facilities, procedures, practices, training and expertise and should ensure compliance with surveillance requirements, data and adverse event reporting ***(Section II & Appendix F)***

(IBC Responsibilities con't)

- Review and approve all purchases or transfer of materials (gifts, donations collaboration reagents, etc) associated with biohazard activities and notify PI/Instructor of results ***(Section II & Appendix F)***

- Conduct periodic (yearly, until biohazard activities increase at Stevens) reviews of ongoing activities to ensure compliance ***(Appendix H, Form 1)***

- Adopt emergency plans for accidental spills and personnel containment from biohazard activities ***(Section IV)***

- Report significant violation and biohazard activity-related accidents to the NIH

- Provide technical consultation to PI and instructors

- Establish and monitor procedures for the disposal, storage and transport of biohazardous materials and update when necessary ***(Section III)***

- Provide basic biosafety training sessions for the Stevens' community and consultation when requested

- Establish guidelines for the use, handling and disposal of animals ***(Section I, Appendix C & NIH Guideline Appendix Q)***

- Keep records of committee activities, applicable laws and regulations, proposals, maintenance and training records, and accident reports.

(What are the responsibilities of the members of the Stevens Community regarding Biological Safety? con't)

Biological Safety Officer (BSO) is required if the institution engages in large-scale research activities involving viable organisms containing rDNA of level BL-3 or BL-4.

The BSO responsibilities include;

- Periodic (yearly, until Stevens' biohazard activities increase) inspections of laboratories and facilities to ensure compliance ***(Appendix H, Form 1)***
- Report to the IBC any significant problems, violations and research-related accidents
- Determining emergency plans for handling accidental spills, personnel contamination and investigation of laboratory accidents involving biohazards
- Provide advice on laboratory/facility security
- Provide technical advice to PI and IBC

(See ***Laboratory Safety Monograph,*** available from the IBC or the ORDA and NIH, for more details regarding BSO)

(What are the responsibilities of the members of the Stevens Community regarding Biological Safety? con't)

Principal Investigator, Course Instructor or Facility Supervisor has the primary responsibility for complying with these guidelines.

The Principal Investigator's responsibilities include;

- Be adequately trained in good microbiological techniques and Stevens Biosafety training

- Determine the types of biohazardous agents that will be encountered in the laboratory or research facility and assess the biological characteristics of agents used and/or created ***(see Section I and Appendix H, Form 1)***

- Plan and implement the appropriate biosafety protocol to insure the safe use of these biohazards, including the required level of physical and biological containment ***(Sections I & I, DI and Appendix H, Forms 2, 3)***

- Before performing research or educational activities, submit for review and approval a description of the appropriate biohazards protocols that will be employed in the research or teaching facility ***(Section II,D and Appendix H, Forms 2, 2a & 3)***

- Submit a yearly description, with quarterly updates, to the IBC of all work involving biohazardous materials, including appropriate biosafety protocols utilized ***(Section II,D and Forms 2, 2a & 3)***

- Maintain communication with the IBC and notify it of any changes in research activities that would alter their approved Biohazard protocol ***(Appendix H, Forms 2, 2a, 3)***

- Comply with protocols specific to their research activities, the Stevens' Biosafety Protocol, the *NIH Guidelines* and any applicable federal and local laws

(PI responsibilities, con't)

- Assure that personnel working with biohazardous materials are appropriately trained so they are aware of the hazards and are proficient in the practices and techniques required for safe handling ***(Appendix H, Form 7)***

- Inform staff/student/TA of any advisable precautionary medical practices and relate when it may be inadvisable for pregnant or immune compromised individuals to work with biohazardous materials. Decline access when deemed necessary ***(Appendix H, Form 4)***

- Ensure the integrity of physical and biological containment systems ***(Section II)***

- Assure required hazard warning signs are posted and biohazard containers are labeled to educate all visitors, including custodial and maintenance staff

- Assure that biohazardous waste is treated and disposed of properly ***(Section III)***

- Comply with shipping requirements for biohazard molecules ***(Section II, F)***

- Immediately report any biohazard accident to the BSO and IBC ***(Appendix H, Forms 5 & 6)***

- Assure that all biohazardous materials are properly disposed of and all relevant biohazardous information is fully disclosed to IBC before closing or leaving a laboratory facility ***(Appendix H , Form 8)***

Laboratory Personnel, Teaching Assistants and Students, although under the supervision of PI, instructor or lab supervisor, also bear specific responsibilities including;

- Receive biosafety training from either the Stevens' IBC or laboratory or course supervisor
- Comply with all written and oral rules pertaining to the work being conducted
- Report any biohazardous accidents immediately to supervisor
- Use PPE as directed by protocol or supervisor, or insure appropriate PPE use ***(Section II,B)***

LIST OF ABBREVIATIONS

NIH Guidelines- Guidelines for Research Involving Recombinant DNA Molecules

The Protocol- Stevens Biosafety protocol

BL- Biosafety Level

BSC- Biological Safety Cabinets

BSE- Bovine Spongiform Encephalopathy

CDC- Centers for Disease Control

HPS- Hantavirus Pulmonary Syndrome

HEPA- High Efficiency Particulate Air

IBC- Institute Biosafety Committee

NIH- National Institutes of Health

ORDA- Office of Recombinant DNA activities

PI-Principal Investigator

PPE- Personal Protective Equipment

rDNA- Recombinant DNA

RG- Risk Group

RMW- Regulated Medical Waste

TA- Teaching Assistant

TCE- Transmissible Spongiform Encephalopathy

USDHHS- U.S. department of Health and Human Services

UV- Ultraviolet

SECTION I

Introduction to Biosafety

Individuals who work in scientific laboratories or similar type research facilities are exposed to many kinds of hazards. In fact, this can be said of most workplaces; in some the hazards are well-recognized (fire, for example) and the appropriate precautions are obvious. However, some hazards are not so readily apparent and may call for precautions not ordinarily encountered elsewhere. The purpose of this manual is to address the **biohazardous agents** that may be encountered in the occupational and educational settings present at Stevens; namely research and teaching laboratories and research facilities. The intent is to provide basic information on biohazards and the control of biohazard exposures that can be applied within these settings. This manual does not address radiation or chemical hazards. For information regarding protocols designed to handle these materials the reader is directed to the Radiation Safety Officer or the Stevens Chemical Hygiene Plan, respectively.

(A) Biosafety Containment

Biological safety or biosafety, is the application of knowledge, techniques and equipment to prevent personal, laboratory and environmental exposure to potentially infectious agents, or biohazards. Biosafety protocols utilize containment or barrier strategies to accomplish this. The three elements of containment include laboratory practice and technique, safety equipment (**primary barrier**), and facility design (**secondary barrier**).

- **Primary Barriers (safety equipment)** provide protection of personnel and the immediate laboratory environment through good microbiological technique (laboratory practice) and the use of appropriate safety equipment.

- **Secondary Barriers (facilities)** provide protection of the environment external to the laboratory through a combination of facility design and operational controls.

(B) Biosafety Levels

The level of containment or barrier required protecting the individual and/or the environment from exposure to the biohazardous agent is defined by a rating termed the **Biosafety Level (BL),** of which four currently exist. Biosafety Level 1 (BL-1) is the least restrictive while Biosafety Level 4 (BL-4) requires a special containment facility, which is currently not available at Stevens. Most research and teaching involves Biosafety Levels 1-3; therefore this manual will only focus on these three levels. Fo0r information pertaining to BL4, the reader should consult the IBC, Biosafety *in Microbiological and Biomedical Laboratories* and *Biosafety Reference Manual* (see References Cited) The following is a summary of the Biosafety Levels 1-through 3.

- **Biosafety Level 1 (BL-1):** includes agents not known to cause disease in healthy adults. It requires standard microbiological practices with no safety equipment (primary barrier) and only an open bench-top sink (secondary barrier). BL-1 practices, safety equipment, and facilities are appropriate for undergraduate teaching laboratories and other work using defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans (e.g. *Bacillus subtilis, Naegleria gruberi*).
  NOTE: Many agents not normally associated with disease in humans are opportunistic pathogens and may cause infection in the young, the aged and the immune compromised individual. In addition, vaccine strains that have undergone multiple *in vivo* passages should **not** be considered a virulent.

- **Biosafety Level 2 (BL-2):** agents associated with human disease through inoculation, ingestion or mucous membrane exposure hazards. It requires BL-1 practices plus limited access, biohazard warning signs, "Sharps" precautions ***(see Section IIIA)*** and a biosafety manual. Primary barriers include Biological Safety Cabinets (***BSC; see Section IIB)*** or other physical containment devices and Personal Protective Equipment ***(PPE; see Section II, B2)*** as needed, and an autoclave as a secondary barrier. BL-2 practices, safety equipment and facilities are applicable to clinical, diagnostic, teaching and research facilities where work involves a broad spectrum of indigenous, moderate-risk agents present in the community and associated with human disease of varying severity (e.g. Hepatitis B, *Salmonella* spp. And *Toxoplasma* spp.). With good microbiological techniques, these agents can be used safely on the bench-top if the aerosol potential is kept low; however procedures with high aerosol potential must be conducted in primary containment equipment. ***This level of biosafety practice is consistent with the concept of Universal Precautions, which requires the treatment of all materials as if they are infectious.***

- **Biosafety Level 3 (BL-3):** indigenous or exotic agents with potential for aerosol infection and associated with serious or lethal disease in humans. It requires BL-2 practice plus controlled access, decontamination of all waste and lab clothing, the use of primary barriers, such as BSC and PPEs ***(Section IIB)*** for all agent manipulations, and physical separation from access corridors and non-recirculated or negative airflow into laboratory. BL-3 practices, safety equipment and facilities are applicable to clinical, diagnostic, teaching, research and production facilities which work with agents associated with serious or lethal disease which pose auto-inoculation or ingestion exposure risks. Examples include *Mycobacterium tuberculosis* and *Coxiella burnetti.*

Practical Description of BL-1, BL-2 and BL-3 Biosafety Practices

To aid the reader in determining what safety precautions should be employed in their laboratory situation, a simplified summary of the appropriate guidelines follows, including specific recommendations for PPE and other barriers. These guidelines apply to biomedical and microbiological research and teaching laboratories, including laboratories working with recombinant DNA.

BL-1 and BL-2 Laboratories

Standard microbiological practices in these laboratories requires the wearing of laboratory coats, gowns or uniforms to prevent contamination or the soiling of street clothes, and the removal of these before leaving the laboratory area. These practices prohibit eating, drinking, smoking, applying cosmetics and storing food in the work area. Most research, teaching and diagnostic laboratories operate under BL-1 and BL-2 safety precautions.

BL-3 Laboratories

These laboratories rely heavily on primary and secondary barriers and engineering controls. The wearing of laboratory coats or gowns is required with a decontamination step prior to laundering necessary. In addition, entry must be through a controlled access with no individuals under the age of 16 permitted. No plants or animals not included in the research are allowed.

These Biosafety Level requirements are described in greater detail in **Table 1**. For in-depth descriptions of Biosafety Levels as put forth by the USDHHS, CDC and NIH, see **Appendix B** for an excerpt from HHS Publication No. 93-8395, *Biosafety in Microbiological and Biomedical Laboratories.*

Insert Table 1 Here

(B1) Biosafety Levels for Animal Work

Biosafety levels are also defined for activities with experimental animals. As Stevens does not support animal experimentation, these practices will not be described here. For more information see ***Appendix C, Table C1***, and the CDC publication *Biosafety in Microbiological and Biomedical Laboratories*.

(B2) Biosafety Levels for Recombinant DNA Work

Containment for large-scale recombinant DNA experiments or production (greater than 10 Liters) is described in the National Institutes of Health (NIH) *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. Four levels of containment are described in Appendix K of that publication. See ***Reference list, Appendix A*** for Internet Address of this publication. A hard copy of this publication is also available. Contact the Biosafety Officer or the IBC for its location.

(C) Biohazard Definition

For the purpose of this manual, a biohazard is "an agent of biological origin that has the capacity to produce deleterious effects on humans" (Heinsohn, 1995). In general, a biohazard presents a risk or potential risk to the health of both humans and animals, either through direct infection or indirectly through damage to the environment. These agents include both a variety of microorganisms and multicellular organisms and the toxins and allergens derived from them (see next section). It is important to note that the **presence of any of these organisms or their biologically derived substances in the workplace does not necessarily represent a hazard.** The hazard potential depends on complex relationships among the agents, hosts and environment, which must all be considered when assessing and dealing with risk potential.

(D) Categories of Biohazards

The following is a list of biohazard agents or potentially infectious material:

- Human, animal and plant pathogens including bacteria, fungi, viruses, parasites and prions (see below for more detail).
- All human blood, blood products, tissues and certain body fluids
- Cultured cells (all human and certain animal) and potentially infectious material that these cells may contain
- Allergens
- Toxins (bacterial, fungal, plant, etc)
- Clinical specimens
- Infected animals and animal tissues
- Certain recombinant products, including recombinant DNA

(E) Recombinant DNA (rDNA):

Definition of rDNA molecules: as defined by the *NIH Guidelines*, recombinant DNA molecules (rDNA) are molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cells or molecules that result from the replication of rDNA molecules.

Experiments involving the generation of rDNA may require registration and approval by the IBC, **in addition** to the NIH. The NIH *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* is the definitive reference for rDNA research in the U.S For an overview of laboratory practices appropriate for handling rDNA and the associated Biosafety Level ratings, see **Appendix D1**. If the experimental protocol is not covered by the *NIH Guidelines*, contact the Biosafety Officer or the IBC for further information. For specific questions about particular host-vector system not covered in the *NIH Guidelines*, call the Office of Recombinant DNA Activities, NIH or visit the Federal Register web site (**Appendix A**).

There are some rDNA molecules that are exempt from the *NIH Guidelines* and are not required to be registered with the ORDA (see **Appendix D2**) ; **however registration with the IBC is still required (see the following paragraph).**

As a condition of funding for recombinant DNA research, Stevens Institute must ensure that research conducted at or sponsored by Stevens must comply with the NIH *Guidelines*. However, it is the standard practice at Stevens to comply with the most current *Guidelines*, irrespective of funding source. **Therefore, all experiments involving recombinant DNA must be registered to the IBC by the PI or Course Instructor by submitting Form 3, *Registration Document for Recombinant DNA Research*** (see **Appendix H**). This form provides information regarding

- Host strain
- Source of DNA/RNA
- Recombinant vector
- Transgene introduction
- The use of large-scale fermentation of recombinant organisms
- Release of these organisms into the environment
- Containment conditions
- Use of transgenic plants or animals

(F) Human Gene Therapy and Transgenic Animals

All protocols involving human gene therapy or the creation of transgenic animals or plants must be approved locally by the IBC prior to submission to outside agencies. Consult the *NIH Guidelines* (Appendices L, M, P, Q) for further details.

(G) Human and Animal Pathogens

(G1) Introduction to Microorganisms/Infectious Agents

A detailed understanding of the dynamics of microorganisms within various settings is required to effectively realize and control their potential hazard. Microorganisms or microbes include the viruses, bacteria, some fungi and the recently identified prions. Although there is a large array of these organisms, only a small number of them are pathogenic to humans. Each organism requires a specific set of parameters for efficient growth, metabolism, development and reproduction. It is important to note that in the past 50 years there has been a change in the agents responsible for laboratory associated infections with a shift from bacterial associated illness to viral illness.

Viruses

A submicroscopic, subcellular agent consisting of a nucleic acid core (either DNA or RNA) surrounded by a protective protein coat sometimes surrounded by a lipoprotein membrane. Since viruses are incapable of generating energy or conducting biosynthetic mechanisms without a host organism, they are considered a "host-dependent" living organism. They are obligate intracellular parasites. When presented with the appropriate host organism, the virus invades the host cell and commandeers it to perform all functions necessary for viral replication.

- Size: 0.02- 0.3 microns (comparable to large protein macromolecule)
- Classification*:* based on shape, protein coat composition, presence or absence of a lipoprotein membrane, type of nucleic acid and host specificity

  Examples: *Bacteriophage* (or phage) are viruses that parasitize bacteria.

  Retroviruses are animal viruses that have an RNA molecule as their primary nucleic acid.

Bacteria (Prokaryotes)

A cellular, simple organism which lacks a nucleus and membrane bound organelles. They have an outer cell wall composed of peptidoglycan, sometimes surrounded by a slime sheath which provides protection from host defenses. They reproduce asexually by fission. The bacterial chromosome is comprised of a single circular strand of DNA. In addition, they may incorporate DNA molecules from another bacteria through the process of conjugation or by the uptake of free DNA released by dead bacteria in their environment. Their nutritional modes vary greatly and the can live under extreme conditions. When faced with unfavorable conditions, bacteria can form an **endospore**; a dehydrated bacterial cell encased in heavy protective spore coats which allows it to survive under extremely harsh conditions. Frequently described in terms of colony-forming units (CFU) where colonies result from the growth of an individual bacteria cell.

- Size: 0.5 – 1.0 microns by 2.5 micron
- ❖ Classification: based on shape, or composition of the cell wall as determined by ability for bacteria to uptake an aniline dye (Gram staining); either Gram-positive (GPB) or Gram-negative (GNB). Also classified by their nutritional characteristics.

Fungi

Mostly multicellular eukaryotes, with varied structures that share a common mode of nutrition. May reproduce sexually through spore formation **(**a nonmotile, reproductive cell that can grow directly into a new organism) which provides considerable resistance to various environmental conditions and will develop under favorable conditions. May also reproduce asexually through budding. A number of subtypes include:

- **Yeast (single-celled)**
  Size: 3.0 –5.0 microns diameter ovals

- **Filamentous fungi**
  Size: 2.0- 10.0 micron diameter fibers

- Classification: based on reproductive mode and the type of spore producing structure.

Prions
A protein particle that lack any type of nucleic acid. They are believed to reproduce by structurally converting other proteins normally found in cells. Examples of characterized prions include the agents which cause the animal disease Scrapie and the Bovine Spongiform Encephalopathy (BSE) which causes “mad cow disease”.

Parasites
An organism that lives off of and obtains nutrition from another organism called a host. This is a broad category of organism that includes the viruses (e.g. HIV), bacteria (e.g. streptococcus), protists and fungi.

Bloodborne Pathogens
An additional means of classification of some biohazard agents is the term bloodborne pathogens. These agents include the hepatitis viruses, human immunodefiency virus (HIV), malaria, and syphilis. These are commonly transmitted through body fluids, either human or other animal.

It is important to remember that hazardous biological agents described above have the ability to replicate, which is in sharp contrast to hazardous chemical agents. Therefore there is no such thing as a safe dose of biohazardous agents- there is <u>NO</u> safe level.

(G2) Classification of Pathogenic Agents into Risk Groups

Risk assessment is ultimately a subjective process. There are several systems for classifying human and animal pathogens according to the hazard they present to the individual and the community. In general, however, the pathogenicity of the organism, its mode of transmission and host range, and effective preventive measures and/or treatment are criteria considered when establishing classification groups. The most current classification is found in the NIH *Guidelines for Research Involving Recombinant DNA Molecules*. In this classification scheme, biohazard agents are placed into four Risk Groups (RG), where Risk Group 1 (RG-1) contains agents of low or no hazard and Risk Group 4 (RG-4) contains highly infectious agents.

- **Risk Group 1 (RG-1)** contains agents that are not associated with disease in healthy adult humans (e.g. *Escherichia coli* K-12, *Saccharomyces cerevisiae*).

- **Risk Group 2 (RG-2)** contains agents associated with human disease which are rarely serious and for which preventive or therapeutic interventions are available.

- **Risk Group 3 (RG-3)** contains agents 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)** contains agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual and community risk)

A comprehensive list of Risk Group agents and their Biosafety Level definitions can be found in **Appendix E, Tables E1 through E4.** It is important to realize that none of these lists are inclusive. Any unlisted agent needs to be subjected to a risk assessment based on the known and potential properties of the agent and its relationship to agents that are listed.

In addition, a listing of organisms and toxins **which are restricted by the CDC** due to their infectious nature are also provided in **Appendix E**. Use of any of these organisms must be registered with the IBC and the CDC.

Determining the RG of a biological agent is a part of the biosafety risk assessment and is important for assigning the correct Biosafety Level for containment. In general, RG-2 agents are handled at BL-2 and RG-3 are handled at BL-3. However, large quantities may alter containment conditions. For more information see the NIH *Guidelines for Research Involving Recombinant DNA Molecules, Biosafety in Microbiological and Biomedical Laboratories* or contact the Biosafety Officer or the IBC.

(H) Other Potentially Hazardous Biological Materials

(H1) Human Blood, Blood Products, Body Fluids and Tissues

Biosafety Level 2 practices and procedures (or Universal Precautions), must be followed when handling these materials because of the infectious agents they may contain. If a highly infectious agent might be encountered in the human blood or body products, (e.g. Tuberculosis, Ebola Virus), utilize BL-3 precautions

(H2) Animal Use

The use of animals in research requires compliance with the "Animal Welfare Act" and any state and local regulations. As Stevens does not support animal experimentation, the biohazards associated with this type of work will not be discussed here.

(H3) Tissue Culture/Cell Lines

When cell cultures are known to contain a biohazard agent, the cell line can be classified as the same level as that recommended for the agent. Cell lines of human origin should be handled at Biosafety Level 2. Non-primate or normal primate origin cell lines, which do not harbor primate viruses, nor are contaminated with bacteria, mycoplasma, or fungi, may be handled at Biosafety Level 1. If cells lines are from tumor or lymohoid tissue, or are transformed by an oncogenic virus they should be handled at Biosafety Level 2.

Note: Recent product recalls for bovine serum have raised awareness of the potential Bovine Spongiform Encephalopathy (BSE) or Transmissible Spongiform Encephalopathy (TCE) contamination of those sera. For more information on the testing or purity of bovine serum, contact the supplier.

(H4) Tuberculosis

Work with *Mycobacterium tuberculosis or M. bovis* cultures must performed at BL-3 and requires the approval of the IBC. For more information about working safely with *Mycobacterium sp*. in the laboratory, see the CDC publicatio*n Guidelines for Preventing the Transmission of Tuberculosis in Health-Care Facilities.*

(H5) Wild Rodents

If course subject matter or research activities require individuals to work/study outdoors where wild rodents may be encountered, precautions against contracting hantavirus pulmonary syndrome (HPS) should be taken. Generally, the likelihood of exposure through inhalation is low. **All outdoor excursions and exposure risks should be planned with the IBC or the BSO well in advance of the activity.**

(I) Experiments Covered by this Protocol

The guidelines described in this *Protocol* apply to the following activities:

(I1) Experiments involving the transfer of a drug resistance trait to micro-organisms that are not known to acquire the trait naturally if such acquisition would compromise the use of the drug to control disease agents in humans, veterinary medicine or agriculture.

(I2) Formation of rDNA molecules containing genes for the biosynthesis of toxin molecules lethal for vertebrates at a lethal dose (LD) LD_{50} of less than 100 nanograms per kilogram body weight

(I3) Experiments involving the transfer of rDNA or rRNA into human subjects

(I4) Experiments using Risk Group 2, 3 or 4 agents or restricted agents as host or vector systems

(I5) Experiments in which DNA from Risk Group 2, 3 or 4 agents or restricted agents is cloned into nonpathogenic prokaryotic or lower eukaryotic organisms.

(I6) Experiments involving whole animals or whole plants whose genome has been altered by the stable introduction of rDNA (transgenic) or who are used to test rDNA-modified organisms.

(I7) Experiments involving more than 10 liters of culture (see Appendix K of the *NIH Guidelines)*

(see the *NIH Guidelines*, Section III, for more details regarding the above activities

All of these activities must be reported to and approved by the IBC using Form 3, "Registration for Recombinant DNA Research" and Form 2, "Annual Protocol for Use of Biohazardous Materials". In addition, activities (I1) and (I2) require that Form 3 also be submitted to the NIH for approval (see *Section II, D*)

Section II

Control Measures

(For more details regarding containment practices *see Biosafety in Microbiological and Biomedical Laboratories)*

The term biosafety describes a complete program of administrative controls, medical surveillance and containment strategies for reducing the risk of potential exposure to infectious agents or other biologically derived materials. Effective application of a biosafety protocol involves four elements:

- Laboratory Practice and Technique
- Safety Equipment
- Facility Design

  } Engineering Controls

- Administrative Controls

(A) Laboratory Practice and Technique

The most important element of containment is adherence to good laboratory practices. Individuals working with biohazards must be proficient in the practices and techniques required for the safe handling of those materials. When standard laboratory practices are not sufficient to control the hazards, additional measures may be required. It is the responsibility of the PI or the course Instructor or supervisor to select additional practices (see ***Section II, A2***) and ensure the correct application of these practices. Regardless of the specific biohazard agent(s) encountered, however, there are standard laboratory safety guidelines which must always be followed to insure the safety of all those working in the facility.

(A1) General Laboratory Safety Practices

The following is a list of some general safety considerations and is presented solely as a reminder and guideline for working in a laboratory/research setting. Details pertaining to some of these safety rules and guideline may be found elsewhere (e.g. Stevens Chemical Hygiene Plan). For purposes of this Protocol, the laboratory or research facility is the area where any biohazard exposure may occur. This includes the location of activities/experiments, storage of biohazards, transfer locales and waste storage.

General Rules

- No running, jumping or horseplay in laboratory/research areas
- No one shall work alone at any time when performing a task that is considered exceptionally hazardous by a supervisor or a safety officer
- Spills shall be cleaned immediately (see ***Section III***). Water spills are included, since they pose a slip potential and potential flooding and equipment damage (particularly to the floor below).
- Exercise caution with step stools
- Make certain the laboratory is left clean after work is performed.
- All animals (including pets) should be excluded from the Institute.

Personal Hygiene

- Wash promptly whenever a material has contacted the skin. Know what you are working with and have all the necessary cleaning material on hand and available.

- No open toe shoes (sandals included) should be worn.

- Clothing should provide protection from splashes and spills and should be easily removed in case of an accident. Lab coats with snaps, rather than buttons, should be worn as additional protection and should be removed before leaving the laboratory or facility. Lab coats should not be worn outside of the laboratory (e.g. bathroom, lounge, computer facility).

- Hands should be washed with soap and water and any other appropriate cleaning/decontamination procedure should be performed before leaving the laboratory/research facility.

- Be aware of means of inhalation and ingestion (see ***Section II, A2***).

- Never pipette by mouth.

- Do not drink, eat, smoke or apply cosmetics in the laboratory /research facility.

- Do not use ice from lab ice machines for beverages.

- Do not bring food, beverages, tobacco products or cosmetics into the laboratory/research facility since cross contamination may occur.

General Housekeeping

- Keep work area as clean as the work permits.

- Each individual is responsible for maintaining the cleanliness of his/her area.

- Return all reagents, equipment and samples to their proper place after use. Place contaminated glassware in the proper cleaning location and do not allow it to accumulate.

- Stored equipment should not project beyond shelf/bench limit and should not block access to fire extinguishers, safety equipment or other emergency items.

- All working surfaces and floors should be cleaned regularly by standard cleaning methods (commercial grade cleansers used; see **Contact List,** for Stevens Custodial Services).

- All containers should be labeled clearly with the identity of the contents and the type of hazard it presents. If a container contains hazardous waste of any type (biohazard, chemical or radioactive) **it is extremely important that custodial services are made aware of the contents so that they do not dispose of it with the regular trash**. For more information on biohazardous waste disposal, see Section III of this Protocol. For details on chemical and or radioactive waste disposal, see the Stevens Chemical Hygiene Plan or contact the Radiation Safety Officer.

Safety Practices with Glassware

Glassware breakage is a common cause of injury and contamination. Therefore it is important to inspect all glassware before use. Use only glassware in good condition. Do not use chipped broken or otherwise compromised glassware. All broken glassware requires special handling and should be disposed of in a manner that prevents injury to all individuals who may come in contact with it, including custodial staff. Therefore, broken glassware should be discarded in designated containers that should read "BROKEN GLASS".

- ***Handling broken glassware***

  Use hand protection for picking up and disposing of broken glassware. This may include gloves, dustpan and broom, forceps or tongs. Additional eye protection, such as safety goggles, should also be worn if concern about further breakage or shattering exists.

- ***Disposal of glassware***

  "BROKEN GLASSWARE" containers should be rigid and puncture proof and should not be overloaded. Only uncontaminated glassware should be placed directly into these containers. Acceptable disposal containers for broken glassware include:
  - reinforced cardboard box (seams taped or lined with plastic)
  - plastic buckets
  - metal cans

❖ ***Glassware contaminated with biological material***

This includes but is not limited to glassware in contact with biohazardous agents such as
- cover slips and/or slides
- test tubes
- beakers
- pipettes
- petri dishes
- tissue culture dishes or flasks
- cryogenic storage containers

Dispose of in clearly marked, rigid, puncture proof containers, known as "Biological Sharps" containers. For further information see **Biohazard Waste Disposal, Section III.**

- ***Glassware with chemical or radioactive contamination***

  Dispose of according to the Stevens Chemical Hygiene Plan or in accordance with special instructions issued by the Radiation Safety Officer.

Centrifuge Safety

Most laboratories and research facilities have some type of centrifuge. It is very important that users of these instruments be fully instructed in their safe and correct use. Errors not only result in sample loss and damaged equipment but also may present a serious safety and health threat. The following is a checklist for general centrifuge safety. It is presented as a guideline only. Individual users should be trained and become fully acquainted with the particular centrifuge(s) they will be using by the laboratory or research supervisor or the course instructor.

Before using the centrifuge perform the following checklist:

- ✓ which type of centrifuge is required
- ✓ which rotor is required
- ✓ inspect rotor for rough spots, scratches, pitting, discoloration; read rotor users manual for further information
- ✓ what is the correct tube size and is an adapter required
- ✓ inspect tubes for pitting, scratches, chips
- ✓ what speed (rpm or *g force*) is needed and what length of time
- ✓ ensure the rotor over-speed rings are intact (if applicable)
- ✓ ensure correct tube fit and rotor positioning and seating
- ✓ sign the users log associated with the centrifuge

Although it is important to prevent sample leak regardless of sample type, the following list will aid in the prevention of a biological contamination if a biological agent is being used:

- can material form an aerosol? are centrifuge tubes covered
- should the rotor be loaded/unloaded in a biological safety cabinet?
- before starting the instrument, ensure that the interior of the centrifuge chamber, the spindle and the exterior of the tubes are dry
- do not overfill the tubes
- are the tubes balanced?
- is the centrifuge lid closed properly?
- verify that the centrifuge has obtained proper speed, is running normally and no imbalance has occurred

After the centrifuge run has finished:

- has the rotor completely stopped
- open the lid and CHECK FOR SPILLS; if a spill occurred close the lid and following the instructions in the ***Section IV, A2***.
- if run was without incident remove rotor and samples
- any liquid within the chamber or on the should be removed and the areas dried
- avoid scratching any surface of the centrifuge, rotor or tubes
- rinse off rotor, adapters or buckets with deionized water and store properly

(A2) Biohazard Laboratory Safety Practices

As described above, when standard laboratory practices are not sufficient to control the hazards encountered, additional measures may be required. It is the responsibility of the PI or the course instructor or supervisor to select the additional practices. Supervisors, in conjunction with the IBC, must conduct a hazard assessment, certified in writing (see **Appendix H**, **Form 1, *Laboratory Inspection Report***) to determine if hazards present in the laboratory or research setting necessitate the use of PPE (see ***Section II, B***). The most important element of containment is strict adherence to standard microbiological practices and techniques. Biohazard laboratories are special, often unique, environments that may pose infectious disease risks. The purpose of containment is to reduce exposure of individuals and the environment to potential hazardous agents.

Understanding Routes of Exposure

Occupational infection can be a serious concern in certain work environments. Different work setting present different threats. Historically, anthrax is a hazard for industrial, agricultural and veterinary workers who process or handle animals, animal hides, hair, bone and fluids. In the working laboratory, however, the infectious agents may not be so readily identifiable. For example, in 1987, five molecular biologists working in the Pasteur Institute developed cancer as a result of working with tumor viruses, oncogenes and mutagens. Another case of laboratory exposure involved a worker who received an accidental injection of a human carcinoma cell line. A 1976 published report of laboratory-associated infections reveled that only 18% of the infections were caused by identifiable accidents, with the remainder resulting from unknown or unrecognized causes. The commonly held view is that infections occur in individuals working in the health care industry. However it was found that 59% of the infectious reported occurred in people engaged in research activities. It should be evident from these examples that working with biohazardous agents poses serious hazards. Prevention of contamination requires specialized control measures. Therefore, adherence to a Biosafety Protocol through good microbiological practice is essential**. IT IS IMPORTANT THAT THESE PRACTICES BY PERFORMED CONSISTENLY EVEN IF A BIOHAZARD IS NOT READILY APPARENT.**

Means of exposure to pathogenic agents include:

- ***Ingestion:*** usually results from poor personal hygiene and poor laboratory practice, including eating, drinking, smoking, mouth pipetting, placing contaminated fingers or objects in the mouth

- ***Inoculation*** through the skin, usually a result of an accidental injection with a contaminated needle or sharp laboratory glassware/disposable, or instruments or bites/stings from animals or insects. May also occur through a skin cut or scratch.

- ***Inhalation/Mucous Membranes*** accounts for the majority of all laboratory infection. It generally results from performing aerosol generating procedures such as centrifugation, sonication, homogenization, mixing, pipetting. **Table 2** provides data which shows the numbers of viable particles generated by some standard laboratory operations.

- ***Eye Exposure*** results from splashes to the eye or transfer of agents to the eye through contaminated fingers/hands

Infectious dose is the number of organisms necessary to initiate an infection in the host. It varies with agent, route of exposure, the virulence of the organism and the immune status of the host.

Table 2

General Control Measures to Prevent Exposure

The following preventive measures should be applied where appropriate to control against:

- ***Ingestion***
  -Wear gloves (see ***Section II, B2***)
  -Wash hands thoroughly
    -Never eat, drink, smoke, apply cosmetics, contact lenses in the biohazard environment
  -Don't chew writing utensils, nails, etc.
- ***Inhalation/Mucous Membranes***
  -Perform procedures in contained, controlled environment (e.g. hoods, covered centrifuge rotor, sonication chamber, covered test tube, biological safety cabinets- see ***Section II, B3***)
  -Wear face masks (see ***Section II, B2***)
- ***Inoculation***
  -Dispose of sharps correctly (see ***Section III, B2***)
- ***Eye Exposure***
  -Wear safety goggles (see ***Section II, B2***)
  -Avoid touching eyes with hands

Other Recommended Laboratory Practices

♦ *Pipettes and Pipetting Aids*

Pipettes are used for volumetric measurements and the transfer of fluids. Contamination can also result from use of a finger to control pipetting.

- Never mouth pipette- use mechanical pipet aids
- Work within a BSC if possible
- Always use cotton-plugged, disposable pipettes
- Do not forcibly discharge biohazardous materials from a pipette; use "to deliver" type
- Discard contaminated pipettes and Pasteur pipettes in "Sharps" container (see **Section III, B2**)

♦ *Syringes and Needles*

These are dangerous instruments and should be restricted to procedures for which there is no alternative. Do not use in place of a pipette.

- Use disposable needle locking syringe units whenever possible
- Work in BSC if possible
- Wear gloves
- Fill syringe to minimize air bubbles
- Do not use syringe to mix fluids
- Do not bend or clip needle

- Dispose of in appropriate “Sharps” container (see **Section III, B2**)

♦ ***Cryostats***

Frozen sections of either human or animal tissues may pose a threat if infected with a biohazard agent. Do not apply freezing propellants under pressure to these tissue samples.

- Decontaminate the cryostat by wiping with 70% ethanol
- Defrost cryostat frequently and disinfect with hospital grade disinfectant
- Handle microtome knives with extreme care. Wear stainless steel gloves or use tools to change blades

♦ ***Blenders, Ultrasonic Disrupters, Grinders, Homogenizers and Lyophilizers***

The use of any of these devices results in considerable aerosol production. Therefore these activities should be performed in a BSC.

♦ ***Ampoules***

These should be opened in a BSC to protect against splashing and the production of aerosols.

♦ ***Loops, Sterilizers and Bunsen Burners***

Sterilization of inoculating loops or needles in open flames generates small-particle aerosols. Disposable plastic loops and needles should be used for culture work.

♦ ***General Housekeeping***

Dry sweeping and dusting may lead to the formation of aerosols. A wet/dry vacuum must not be used in the biohazard laboratory due to the production of aerosols unless it is equipped with a HEPA filter exhaust.

(A3) Transport of Biological Materials On and Off Campus

All biological materials should be transported in a way that maintains the integrity of the material during normal transportation conditions, as well as prevents any accidental release and endangerment of the public and the environment.

- All materials need to be packaged in a sealed, leak-proof primary container which is securely positioned in a secondary and closable container labeled with a clearly visible biohazard symbol on the outside.

- A list of contents, as well as emergency information (e.g. contact phone number), should accompany material.

- Only Stevens' vehicles should be used for on-campus transport; the use of personal vehicles is prohibited.

- Off-campus transportation is regulated by national and international transportation rules. See the OSHA Laboratory Standard (29 CFR 1910) and the Department of Transportation Standards (29 CFR 173) for detailed information (available through the Code of Federal Regulations website, ***Appendix A***) or contact the IBC.

(A4) Decontamination Procedures

Decontamination is a term used to describe a process or treatment that renders a surface or instrument safe to handle. It can be defined as the reduction of microorganisms to an acceptable level. A decontamination procedure can range from sterilization to simple cleaning with soap and water. In order to select the proper method and tools, it is important to consider the type of biohazard, concentration and risk potential, and the physical and chemical hazard to the environment and personnel.

Forms of Decontamination

- ***Disinfection*** eliminates all pathogenic non-sporeforming microorganisms but not necessarily all microbial forms on inanimate objects. Effectiveness is influenced by the kinds and numbers or organisms, the amount of organic matter, the object being disinfected, the chemical disinfectant, the exposure time, temperature and concentration.

- ***Sterilization*** is the use of physical or chemical procedures to destroy all microbial life, including bacterial endospores.

- ***Antisepsis*** is the application of a liquid antimicrobial to skin or living tissue to inhibit or destroy microorganisms (e.g. swabbing injection site).

- **Physical and Chemical Means of Decontamination**

Heat: can be applied in wet or dry form

Wet Heat is the most dependable and has the advantage of better heat transfer into the material resulting in shorter exposure times and lower temperatures. Steam sterilization with an autoclave uses pressurized steam (15 PSI) at 121-132^{o}C (250-270^{o}F) for 30 minutes. This will kill bacterial endospores ***(Section II,G1).***

Dry Heat is less efficient and requires longer time and/or higher temperatures; 160-170^{o}C for a period of 2-4 hours is equivalent to the above described wet heat conditions.

Incineration has a great advantage in that it reduces the volume of material prior to final disposal. Stevens Institute has no on-site incinerator.

Autoclave Use

As autoclaves may vary, all autoclaves on the Stevens' campus must have detailed instructions on their use clearly posted.

- Biohazardous material should not be placed in autoclaves overnight in anticipation of next day autoclaving
- Autoclaves should be operated only by trained personnel and should not be left unattended.
- Precaution should be taken to prevent the removal of material from the autoclave before it is sterilized- use of autoclave indicator bags or tape is recommended in addition to good communication practices.
- Strong oxidizing material must not be autoclaved with organic materials, such as paper, cloth or oil.

Liquid Disinfection is the most practical for surface decontamination and at sufficient concentration, for the decontamination of liquid wastes. Liquid disinfectants are available under a wide array of trade names and vary in their effectiveness dependent upon the agent involved and their chemical composition. Properties of common disinfectants can be found in **Tables 3a, 3b, 3c.**

General Considerations for Liquid Disinfection

- Nature of surface; the more porous and rough, the longer the exposure time.
- Number of microorganisms; the higher the concentration, the longer the exposure time.
- Resistance of microorganisms; microbes vary in their resistance to disinfectant and heat. From least to greater resistant are: lipid or medium sized viruses, vegetative bacteria, fungi, nonlipid or small viruses, mycobacteria, bacterial spores.
- Presence of organic material, such as blood, body fluids and tissue, can hamper certain disinfectants.
- Duration of exposure and temperature.

Some General Types of Disinfectants

- *Alcohols*, such as ethyl or isopropyl in concentration of 70% to 90% are good general use disinfectants. **NOTE:** They have limited exposure time due to rapid evaporation. Concentrations above 90% are less effective.

- *Formalin* is 37% formaldehyde in water. Dilution to 5% results in an effective disinfectant**. NOTE:** Formaldehyde is a human carcinogen and may create respiratory problems at low concentrations.

- *Glutaraldehyde* is more effective than formalin against bacteria, fungi and viruses. **NOTE**: vapors are irritating to eyes, nasal passages and upper respiratory tract. Wear PPE when using.

- *Phenol and Derivatives* are used from 5-10% dilutions. They effectively kill bacteria, including *Mycobacterium tuberculosis*, fungi and lipid viruses. **NOTE**: Phenol may be toxic; Use appropriate PPE when handling.

- *Quaternary Ammonium Compounds* are cationic detergents with strong surface activity. They are good for general use disinfectants against gram positive bacteria and lipid viruses. They are easily deactivated by organic materials, anionic detergents and salts of metal found in water. They are relatively non-toxic.

- *Chlorine and Iodine (Halogens*); chlorine containing solutions have broad-spectrum activity. Sodium hypochlorite is the most common chlorine disinfectant. Common household bleach (5% chlorine) can be diluted at 1/10 or 1/100 with water to yield a satisfactory disinfectant. It is best to use freshly diluted solutions. They may be deactivated by excess organic materials. **NOTE:** They have strong odors are very corrosive; always use PPE when handling. Iodine is similar and is relatively nontoxic to humans.

Insert Table 3a

Insert Table 3b

Insert Table 3c

Vapors and Gases possess germicidal properties. The most commonly used are

- *formaldehyde*, to decontaminate spaces or biological containment equipment. **NOTE**: Toxic and a suspected human carcinogen; use considerable caution.
- *ethylene oxide*, used in gas sterilizers. **NOTE**: a human carcinogen.

Radiation, specifically gamma and x-ray, are mainly used for sterilization of prepackaged medical devices. Ultraviolet (UV) radiation may be used to inactivate viruses, mycoplasm, bacteria and fungi and is effective in the destruction of airborne microbes. UV lamps are used often for space decontamination in BSC, tissue culture facilities and clean rooms. UV lamps used for this purpose should be interlocked with the general room or cabinet illumination so that turning on the lights extinguishes the UV.

Engineering Controls

(The following is a list of controls pertinent in all laboratory settings. For additional information regarding these controls, see the Stevens Chemical Hygiene Plan)

(B) Safety Equipment (Primary Barriers)

(B1) General Safety Equipment

- ***Fire extinguishers*** are monitored and maintained on campus by Vigilance Fire and Technical. All laboratory personnel should be trained regarding potential fire hazards associated with their work and how to respond to them. For further information regarding fire extinguishers, see the information posted on all extinguishers or contact the Safety Officer.

- ***Safety showers*** are available if protective measures fail and an individual receives a body exposure. All laboratory personnel should familiarize themselves with the location and operation of safety showers.

- ***Eyewash faucets*** are available if protective measures fail and an individual receives an eye exposure. All laboratory personnel should familiarize themselves with the location and operation of eyewash faucets.

- ***First aid kits*** should be located in conspicuous areas and clearly marked. They are maintained by the laboratory PI, supervisor or the course instructor. They should be used in response to minor injuries **but should not replace the option of obtaining medical treatment or consultation** (see **Contact List**).

- ***Explosion proof refrigerators and freezers*** should only be used in the laboratory or research setting. They should not be used for the storage of food or beverages. It is the responsibility of the individual academic departments to ensure that the appropriate type cooling units are used.

- ***Ventilation hoods*** (fume hoods) keep toxic or irritating vapors and fumes out of the general working area and therefore should be used for work that involves hazardous or noxious materials which are toxic, odiferous, volatile or harmful. When not in use the sash of the hood should be kept closed. Only items necessary for working in the hood should be in the hood. **Do not work with infectious/pathogenic material in fume hoods; these materials should be manipulated in *Biological Safety Cabinets* only** (see next). Always assure the hood is operational before initiating an experiment or procedure. Fume hoods are inspected yearly by D.P. Technologies. For further information, contact the Safety Officer.

- ***Biological Safety Cabinet (BSC)*** is used as primary containment devices for working with pathogenic or infectious agents. It is important that a licensed technician (see ***Appendix A***) certify them at least annually to verify performance capability. Individuals should be properly trained to work within a biological safety cabinet. These cabinets are not chemical fume hoods and should not be used as such. For more detailed information concerning the appropriate techniques for working in a *biological safety cabinet* see ***Section II, B3*** & ***Appendix F*** and *Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets* ***(Appendix A)***).

- ***Safety Shields*** are used for protection against possible explosion, implosion, splash or radioactive exposure. An individual should be aware of the range and limitation of the safely shields protection capabilities.

(B2) Personal Protective Equipment

A variety of personal protective equipment (PPE) is available and commonly used in laboratory and research facilities. This equipment requires proper management and use to perform properly. Laboratory/research facility supervisors should determine the need for such equipment, monitor its effectiveness, train individuals and monitor and enforce the proper use of this equipment. Supervisors, in conjunction with the Stevens Safety Committee or the IBC, must conduct a hazard assessment, certified in writing to determine if hazards present in the laboratory or research setting necessitate the use of PPE (see ***Appendix H, Form 1***). The equipment must be in working condition and all affected workers must be properly trained to use the equipment.

The following is a general list of PPE that is provided as a guideline and reminder. Only those that pertain directly to Biohazard protection will be described in detail. .For more information about PPE, see the Stevens Chemical Hygiene Plan, Section VII.

- ***Eye protection*** should be utilized as a means of injury protection and contamination protection from biohazard agents contained in aerosols or liquids. Both individuals working continually in designated areas and those who may be in the area temporarily, such as maintenance, clerical and visitors should wear it. All eye protection should comply with standards set forth in American National Standard for Occupational and Educational Eye and Face Protection, Z 87.1-1968. The type of protection depends on the hazard present. For most situations containing biohazardous agents, safety glasses with side shields are adequate. If ultraviolet light is emitted in the area, the safety glasses should be rated UV protective (see manufacturer's information for more detail). It is not recommended that contact lenses be worn in the laboratory setting, since among other reasons, the lenses can prevent tears from removing irritants.

- ***Lab coats*** are best worn over clothing and should be worn at all times in the laboratory area. They should be removed before leaving the facility. When biohazardous materials are present, lab coats should be fully closed, preferably with snaps.

- ***Gloves*** may be worn to protect the hands from contamination. The type of glove varies with the type of hazard present. Proper selection of the glove is essential to its performance as a barrier. Properties to consider are permeability, thickness and chemical susceptibility. See information provided by glove manufacturers for more information. When working with biohazardous agents, latex gloves are adequate. Inspect gloves thoroughly before use for discoloration, punctures and tears. Gloves may be inflated with air and submersed in water to detect small leaks. When donning gloves, the user should rinse them before touching reagent bottles or other laboratory fixtures. Gloves should be removed before leaving the work area, at the completion of the task that required them and should never be worn when using light switch, telephone, doorknob, etc. They should be removed

by pulling the cuff over the hand and disposed of in the proper location (see ***Biohazardous Waste, Section III***).

- ***Respirator*** use should be minimized and replaced with engineering controls such as fume hoods. If respirators are required, a respirator program should be established in accordance with OSHA rules as indicated in the Code of Federal Regulations, Title 29, 1910 section .134. Refer to the Stevens Chemical Hygiene and the Safety Officer for more information.

- ***Face masks***, surgical grade may be utilized to reduce inhalation risks of certain biohazard agents by providing a limited aerosol barrier.

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- ***Lab coats*** are best worn over clothing and should be worn at all times in the laboratory area. They should be removed before leaving the facility. When

biohazardous materials are present, lab coats should be fully closed, preferably with snaps.

- ***Gloves*** may be worn to protect the hands from contamination. The type of glove varies with the type of hazard present. Proper selection of the glove is essential to its performance as a barrier. Properties to consider are permeability, thickness and chemical susceptibility. See information provided by glove manufacturers for more information. When working with biohazardous agents, latex gloves are adequate. Inspect gloves thoroughly before use for discoloration, punctures and tears. Gloves may be inflated with air and submersed in water to detect small leaks. When donning gloves, the user should rinse them before touching reagent bottles or other laboratory fixtures. Gloves should be removed before leaving the work area, at the completion of the task that required them and should never be worn when using light switch, telephone, doorknob, etc. They should be removed by pulling the cuff over the hand and disposed of in the proper location (see ***Biohazardous Waste, Section III***).

- ***Respirator*** use should be minimized and replaced with engineering controls such as fume hoods. If respirators are required, a respirator program should be established in accordance with OSHA rules as indicated in the Code of Federal Regulations, Title 29, 1910 section .134. Refer to the Stevens Chemical Hygiene and the Safety Officer for more information.

- ***Face masks***, surgical grade may be utilized to reduce inhalation risks of certain biohazard agents by providing a limited aerosol barrier.

(B2) Personal Protective Equipment

A variety of personal protective equipment (PPE) is available and commonly used in laboratory and research facilities. This equipment requires proper management and use to perform properly. Laboratory/research facility supervisors should determine the need for such equipment, monitor its effectiveness, train individuals and monitor and enforce the proper use of this equipment. Supervisors, in conjunction with the Stevens Safety Committee or the IBC, must conduct a hazard assessment, certified in writing to determine if hazards present in the laboratory or research setting necessitate the use of PPE (see ***Appendix H, Form 1***). The equipment must be in working condition and all affected workers must be properly trained to use the equipment.

The following is a general list of PPE that is provided as a guideline and reminder. Only those that pertain directly to Biohazard protection will be described in detail.

- ***Eye protection*** should be utilized as a means of injury protection and contamination protection from biohazard agents contained in aerosols or liquids. Both individuals working continually in designated areas and those who may be in the area temporarily, such as maintenance, clerical and visitors should wear it. All eye protection should comply with standards set forth in American National Standard for Occupational and Educational Eye and Face Protection, Z 87.1-1968. The type of protection depends on the hazard present. For most situations containing biohazardous agents, safety glasses with side shields are adequate. If ultraviolet light is emitted in the area, the safety glasses should be rated UV protective (see manufacturer's information for more detail). It is not recommended that contact lenses be worn in the laboratory setting, since among other reasons, the lenses can prevent tears from removing irritants.

- ***Lab coats*** are best worn over clothing and should be worn at all times in the laboratory area. They should be removed before leaving the facility. When biohazardous materials are present, lab coats should be fully closed, preferably with snaps.

- ***Gloves*** may be worn to protect the hands from contamination. Boxes of gloves should be available in every laboratory. The type of glove varies with the type of hazard present. Proper selection of the glove is essential to its performance as a barrier. Properties to consider are permeability, thickness and chemical susceptibility. See information provided by glove manufacturers for more information. When working with biohazardous agents, latex gloves are adequate. Inspect gloves thoroughly before use for discoloration, punctures and tears. Gloves may be inflated with air and submersed in water to detect small leaks. When donning gloves, the user should rinse them before touching

reagent bottles or other laboratory fixtures. Gloves should be removed before leaving the work area and at the completion of the task that required them. They should never be worn when using a light switch, telephone, doorknob, etc. They should be removed by pulling the cuff over the hand and disposed of in the proper location (see ***Biohazardous Waste, Section III***).

- ***Respirator*** use should be minimized and replaced with engineering controls such as fume hoods. If respirators are required, a respirator program should be established in accordance with OSHA rules as indicated in the Code of Federal Regulations, Title 29, 1910 section .134. Refer to the Stevens Chemical Hygiene Plan and the Safety Officer for more information.

- ***Face masks***, surgical grade may be utilized to reduce inhalation risks of certain biohazard agents by providing a limited aerosol barrier.

(B3) Biohazard-Specific Safety Equipment

Safety equipment used in the handling of biohazards include many of those addressed in **Section II, B1 and B2 (i.e. gloves, lab coats, face shields, safety centrifuge cups, etc**). The following is a list of some additional standard equipment found in a facility utilizing biohazardous materials. Many of these Primary Barriers are used in combination with each other when handling biohazards. Each laboratory/research facility is a unique environment and requires biosafety programs tailored to meet specific needs. This list is not meant to be inclusive but to serve as a guideline. See **Appendix A** for further information.

Biohazard Warning Signs and Posting

Each laboratory must have a room sign that provides safety information to visitors and service personnel. Room signs must contain designations for all laboratory hazards in use (e.g. carcinogens, toxic agents, biohazards). All areas or equipment in which RG-2 or 3 agents are handled or stored or where BL-2 or 3 procedures are practiced are required to prominently post a biohazard sign/label (see **Figure 1**). This includes posting on the main entrance door, and on equipment like refrigerators, incubators and transport and disposal containers.

Figure 1

Biological Safety Cabinets

The Biological Safety Cabinet (BSC) is designed to provide personnel, environmental and sample protection when appropriate practices and procedures are followed. BSCs contain aerosols through the use of laminar airflow and high efficiency particulate air (HEPA) filtration. Three types of BSCs, designated Class I, II and III are used. BSC must not be confused with "clean benches"; horizontal flow cabinets which direct air towards the operator (see ***Appendix F, Figure F1a & F1b***). These instruments (clean benches) should never been used for handling infectious material. Personnel should be trained in the correct use and maintenance of BSC to ensure protection (*see* ***Appendix F, Tables F1 & F2***). All BSCs used for RG-2 or 3 and rDNA work must be inspected annually and certified by trained and accredited service personnel **(*Appendix A*)** according to the National Sanitation Foundation Standard 49. For further information, see the CDC/NIH publication *Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets.*

- ***Class I BSC***
  This is a ventilated cabinet for personnel protection with an unrecirculated inward airflow away from the operator. This unit is fitted with a HEPA filter to protect the environment from discharged agents. Class I is suitable for working with low to moderate risk agents where there is a need for containment but not sample protection (i.e. not sterile) (see ***Appendix F, Figure F2***)

- ***Class II BSC***
  This is a ventilated cabinet for personnel, sample and environment protection that provides inward airflow and HEPA-filtered supply and exhaust air. There are four designed depending on how much air is recirculated and/or exhausted and if it is hard-ducted to the ventilation system. Class II may be used with low to moderate risk biohazard agents (see ***Appendix F, Figures F3a, b, c***)

- ***Class III BSC***
  This is a totally enclosed ventilated cabinet that is gas-tight and maintained under negative pressure. The air supply is HEPA-filtered and the exhaust has two HEPA filters. This is also known as a "glove box". It is used with high-risk biohazard agents (see ***Appendix F, Figure F4***).

Safe and Effective Use of BSC

Before Use

- ✓ Make sure BSC is certified; check gauges regularly
- ✓ Understand the use of the BSC (be trained)
- ✓ Do not disrupt airflow pattern by rapidly moving arms, leaving lab doors open or keeping a Bunsen burner lit.
- ✓ Minimize storage of materials in and around BSC
- ✓ Plan work before beginning work in BSC

Operation

- Wash hands thoroughly
- Wipe surface with 70% alcohol or other suitable disinfectant (see ***Section II, A4).*** Wipe off each item before placing inside cabinet.
- Do not block front air intake grill or rear exhaust grille.
- Work form clean to dirty (see ***Appendix F, Figure F5***)
- Keep all pipettes horizontal not vertical (upright)
- Do not use flame- it will disrupt airflow and may damage HEPA filter
- Move arms slowly
- Protect house vacuum from biohazard contamination by setting up a trap system (see ***Appendix F, Figure F6***).
- Clean up spills immediately- then wait 10 minutes before resuming work.
- When work is complete, remove all material and wipe surface with 70% alcohol
- Wash hands thoroughly with soap before leaving lab

(C) Laboratory Facilities (Secondary Barriers)

Architectural and engineering features of the laboratory or research facility can form a secondary barrier to protect personnel and the environment from exposure to biohazardous agents in areas outside of the laboratory. Some examples include:

- Materials and methods of construction that facilitates cleaning and prevent accumulate contamination.
- Protection of utility distribution systems (water supply, house vacuum)
- Treatment of liquid and air effluents to remove contamination
- Air pressure gradients to suppress outward airflow from the laboratory

Three classifications of research facilities have been established by the National Cancer Institute on the basis of contamination control features:

- ***Basic Laboratory*** provides a general space where work is done with low risk biohazardous agents. These include BL-1 and BL-2 facilities. Most work is performed on the open bench or in BSC of Class I or II. These areas should be separate from areas known for general contamination (animal rooms) and also separate from public and office areas.

- ***Containment Laboratory*** has special engineering features that allow the handling of moderate to high-risk biohazards pertaining to a BL3 facility. Unique features to this laboratory include provisions for controlled access from areas open to the public and a specialized ventilation system. In addition, these labs must be either an entire building or a single module within a building.

- ***Maximum Containment Laboratory*** has special engineering and containment features that allow safe conduct of activities involving extreme hazards pertaining to a BL4 facility. These laboratories are usually separate buildings with secondary barriers such as a sealed entrance, double-door autoclave, biowaste treatment center and separate ventilation systems.

It is the responsibility of the PI or Instructor to determine the level of containment in the laboratory (see *Section II, D*). For more information regarding secondary barriers, refer to *Biosafety, A Reference Manual* and *Biosafety in Microbiological and Biomedical Laboratories (*see ***Appendix A****).*

Administrative Controls

(D) Registration of Biohazardous Activities with the IBC

The first level of control that must be exercised before conducting any activities involving biohazards is the registration with the IBC of the protocols that will be employed.

These protocols must address;

- PI and other individuals involved in the activities
- Rooms used for activities including storage and waste locations
- Biosafety Level, Risk Agent Group
- Host strain
- Source of rDNA/RNA
- Recombinant vector
- Use of large-scale fermentation of recombinant organism
- Description of pathogenicity
- Duration of activities
- Immunization and/or any medical surveillance aspects
- Containment conditions, including storage and transfer
- Decontamination/Disposal procedures

The PI must register with the IBC by using Form 2 (***Annual Protocol for Use of Biohazardous Materials***) and Form 3 (***Registration Documents for Recombinant DNA Research***) if rDNA molecules will be employed (see ***Appendix H***). Once the protocol has been reviewed and approved by the IBC, the PI must re-file Form 2 (and Form 3 if applicable) annually. In addition, quarterly updates must be filed using Form 2a (***Quarterly Updates on Biohazard Use***) **and** any time a change in biohazard activity occurs.

Registration with the NIH is required with certain activities; see the *NIH Guidelines*, Section IIIB and IIIC for further information.

(E) Medical Surveillance

Specific recommendations concerning the need for either pre-assignment or periodic medical examination for individuals working with biohazardous agents must be determined on a case-by-case basis. It depends on the assessment of the biohazard and the needs of the individual and is focussed on the early detection of illness or injury. In addition, it can also identify any medical condition that may place an individual at increased risk, such as pregnancy. The NIH *Guidelines for Research Involving Recombinant DNA Molecules* impose on the IBC and the PI the responsibility "to report significant research-related illnesses...". It is the determination of the PI, supervisor or course instructor that necessitates the need for medical surveillance, dependent on the nature of the biohazardous agent encountered in the work. For more information see *Biosafety Reference Manua*l (***Appendix A***). However, some standard recommendations include:

- ***Pregnancy***
  Any individual who is pregnant and working with biohazardous agents must inform their immediate supervisor and the IBC of the pregnancy (*see* ***Appendix H, Form 4, Declaration of Pregnancy***). The IBC, in conjunction with the supervisor (e.g. PI, Instructor) will survey the normal working area for potential biohazards and will provide instruction on work practices and schedule. The pregnant individual, should in turn, inform their personal physician of workplace conditions and limitations so that proper medical surveillance may be instituted. All personnel, both male and female, planning to have children, should request the IBC and their supervisor evaluate normal working conditions for potential reproductive hazards.

- ***Vaccination***
  NIH *Guidelines for Research Involving Recombinant DNA Molecules* and OSHA *Bloodborne Pathogen Standard* requires that "if a research group is working with a pathogen for which there is an effective vaccine, the vaccine should be made available."

- ***Control Assessment***
  Periodic medical surveillance can be used to monitor the effectiveness of exposure control measures and PPE use.

In case of exposure OSHA standards requires that the Institute provide medical examination and consultation to any individual who may be exposed to or contaminated by any biohazardous agent while involved in any research or teaching situation on campus. This will be provided free of charge and at the convenience of the individual involved, or within the time frame suggested by the nature of the exposure or contamination, as determined by the PI or laboratory Instructor. In addition, a ***"Medical Consultation"*** form must be filed with the Department Head, Biosafety Officer/IBC and Stevens Student Health Service (see ***Appendix H, Form 5***)

(F) Personnel Communication and Training

Stevens Institute through the IBC and the BSO, will provide annual training to all personnel who work with or may come in contact with biohazards. This includes, but is not limited to faculty, graduate students, post-doctoral fellows, teaching assistants, undergraduates, clerical staff, laboratory technicians, custodial services and maintenance personnel. This training will be general and reflective of this Biosafety Protocol **and is mandatory!** If necessary, this training should be bilingual.

Annual training provided by Stevens will include

- New Jersey Right to Know Provisions
- Overview of the OSHA Bloodborne Pathogens Standard (Laboratory Standard), the CDC guidelines *Biosafety in Microbiological and Biomedical Laboratories*, the NIH *Guidelines for Research Involving Recombinant DNA Molec*ules and the CDC guidelines *Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets***.**
- General introduction to microorganisms and routes of exposure
- Explanation of the Biosafety Protocol, where to find hard copies and the World Wide Web address
- Use and limitations of engineering controls, laboratory practices and PPE
- Appropriate emergency actions in response to biohazardous accidents
- Review of signs and labels required
- Review of disposal guidelines
- Review of reporting/record keeping requirements
- Questions and answer with instructor

Recordkeeping of Training

Records of each training session are maintained by the Biological Safety Officer and in each department office. These include the date of the training session and session syllabus, sample copies of handouts, copies of attendance sheets and the length of the session (see ***Appendix H, Form 7***). These records should be retained for at least 5 years.

Training by Principal Investigator/Instructor

It is incumbent upon Course Instructors or PIs to provide training to address specific needs pertaining to the biohazard encountered in each individual research or teaching situation. This training must be updated with the introduction of any new biohazards into the laboratory or research environment. This training should include:

- Good laboratory and microbiological practices
- Site specific information on risks, hazards and procedures

- Laboratory- or environment-specific BL-2 or BL-3 procedures as applicable

(G) Shipping of Biohazards

Biohazard agents, including vectors that may contain them, are recognized by federal and state government as hazardous materials. These materials are routinely transported from one location to another by common land and air carriers. Regulations governing shipment can be obtained from the shipper, since they must be acquainted with the most current requirements. It is the responsibility of the PI to package the contents in such a way as to avoid leakage, withstand shocks and pressure changes and inform the shipper of the contents of the package. Some definitions:

- ***Biomedical material***s are known to or could contain infectious agents.

- ***Etiological agents*** are those viable microorganisms that cause disease in humans or animals and include bacteria, bacterial toxins, viruses, fungi, protozoan and parasites (a.k.a. infectious agents).

- ***Infectious substances*** are those which contain etiological agents.

- ***Biological product*** is a product prepared in accordance with regulations that govern the manufacture of vaccines, reagents, etc.

Transportation of biohazardous materials is subject to US Dept of Health Services and Dept of Transportation regulations, specifically 49 CFR Parts 100-199 and the NIH *Guidelines for Research Involving Recombinant DNA Molecules*. For more information about shipping biohazardous substances, refer to these documents (***Appendix A***) or contact the Biosafety Officer or the IBC.

(H) Laboratory Inspection and Maintenance

Laboratories/Research facilities are inspected on a regular basis by the Principal Investigator or Course Supervisor (in conjunction with the BSO) to assess the correct operating capabilities and status of PPE, autoclaves, BSC and other engineering controls and to ensure the practice of Standard Laboratory techniques. A report of this inspection is submitted to the IBC, using **Form 1, in Appendix H.**

(I) Record Keeping

To aid in the control of exposure by application of good laboratory practices and compliance with local and federal regulations, record keeping of many laboratory activities is required. Record keeping is required for the following:

- PI/Instructor Laboratory Inspection Report (Form 1)
- Annual Protocol for Use of Biohazardous Materials (Form 2 and 2a)
- Registration Document for Recombinant DNA Experiments (Form 3 and 3a)
  (2 versions; 3a specific for submission to NIH)
- Declaration of Pregnancy (Form 4)
- Medical Consultation (Form 5)
- Biohazard Accident Report (Form 6)
- Statement of Training and Experience for Use of Biohazardous Agents (Form 7)
- The Use of Animals (Form 8)

Template forms for these purposes are located in **Appendix H.**

Section III

Biohazard Waste

Biohazardous waste is "discarded materials that are biological agents or conditions that constitute a hazard to man or his environment". It is of primary concern to dispel the notion that laboratory wastes can be disposed of in the same manner, and with as little thought, as household waste. Therefore, selection and enforcement of safe procedures for disposal of laboratory materials are of no less importance than the consideration given to any other methodology for the accomplishment of research or teaching objectives. Stevens Institute conforms to biohazardous waste standards put forth by the State of New Jersey, which has adopted Federal Hazardous Waste Standards with some minor exceptions, and NIH *Guidelines For Research Involving Recombinant DNA Molecules* and the *CDC/NIH Biosafety in Microbiological and Biomedical Laboratories*. The State of New Jersey defines "**Regulated Medical Waste"** (RMW) as **any waste with potential for causing human disease or infection**. At Stevens Institute, the term biohazardous waste is used to describe different types of waste that ***might*** include infectious agents. **NOTE: The primary responsibility for the treatment and proper disposal of biohazardous waste rests with the PI of Instructor (See *Appendix G*)**

(A) Categories of Biohazardous Waste

- **Blood and blood products**, including body fluids, blood vials.
- **Pathological waste**, including human or animal body parts, organs, tissues, surgical specimens.
- **Laboratory waste**, such as culture and stocks of infectious agents of BSL-1, 2, 3, and biotechnology by-product.
- **Sharps**, including (but not limited to) syringes, needles, scalpel blades, glass pipettes, slides, vials, culture plates or anything that might has the potential to break or cause puncture or cuts.
- **Medical looking waste,** which is contaminated but non-infectious waste. This includes waste that does not pose a disease risk **but may be perceived as biohazardous**. Examples include rubber gloves, disposable lab gowns/aprons, face masks, syringes, bench paper.

(B) General Labeling, Packaging, Storage, and Disposal

(B1) Labeling

All containers of biohazardous waste should be readily identifiable by a universally recognized special label, the Biohazard symbol (see **Figure 1**). In addition, if waste is to be stored before final disposal, storage conditions should be designed to minimize potential for personnel exposure. Storage areas should be clearly identified and cleaned and disinfected regularly (e.g. weekly).

(B2) Packaging

The type of container used to package biohazardous waste depends on the type of waste and storage condition.

- ***Non-sharp, Non-wet Waste***: the most commonly used containers are the plastic red or orange biohazard bags. These colors are universally recognized for biohazardous materials and are tear-proof and leak-proof. (see Fischer Scientific or other scientific supplier for purchase of Biohazardous Waste, Autoclave Bags)

- ***Liquids Waste:*** placed in primary leak-proof containers enclosed in either an outer leak-proof container or an outer container with absorbent packing material.

- ***Sharps:*** placed in rigid, puncture-resistant, leak-proof container, red in color marked with the biohazard symbol; "Sharps" container (see Fischer Scientific or other scientific supplier for purchase of Biological Sharps containers)

NOTE: It is important to clearly identify biohazardous waste and equally important to indicate this to the custodial staff. A notice should be posted at the laboratory entrance prohibiting the disposal of waste labeled with the biohazard symbol. It may be necessary to place everyday trash outside of the laboratory so that there will be no error in disposal of biohazardous waste by custodial services.

(B3) Storage

Biohazardous waste must not be allowed to accumulate. Contaminated material should be inactivated (through decontaminating procedures) and disposed of on a regular basis as required. Stevens has contracted with a Biohazardous Waste Disposal company to transport and dispose of campus generated biohazardous waste. The waste company provides disposal containers that provide interim storage of treated biohazardous waste until pick-up. These containers are placed at specific locations on campus, dependent upon departmental requests. For information regarding these containers, contact the Biosafety Officer.

(B4) Disposal

All biohazardous waste generated at Stevens **must be autoclaved or otherwise decontaminated prior to its deposition in the interim waste storage containers (with the exception of animal remains; see *Section III, C3*).** Autoclaved waste is considered "treated".

Autoclave Procedure for Biohazardous Waste Decontamination

(see ***Section II, A4*** for general autoclave use)

Autoclaving is accepted as a safe and effective procedure for sterilization. To ensure proper decontamination, autoclaves should be tested yearly using heat resistant spores as an indicator of adequate sterilization conditions. In addition, a steam sterilizer integrator strip can be used to indicate pressure, moisture and time. AUTOCLAVE TAPE ALONE IS NOT A SUFFICIENT MEANS OF ASSESSMENT OF AN AUTOCLAVE.

- **Strong oxidizing materials (chemicals) must not be autoclaved with organic material Oxidizer + Organic Material + Heat = Possible Explosion**

- All biohazardous waste must be placed in a biohazard bag (double bagged) with heat sensitive autoclave tape or the heat sensitive word "Autoclaved" on the bag. These bags may be purchased from Fischer Scientific or other scientific suppliers. Contact the IBC or Biosafety Officer for more information.

- Sharps containers should be closed tightly and placed into a biohazard bag before autoclaving.

- Prior to autoclaving, a biohazard bag, or sharps container, containing waste should be kept closed to prevent airborne contamination or nuisance odors. However, when autoclaving, the bag must be open to allow steam to penetrate. To prevent spills in the autoclave, place the bag in an autoclavable tray (food service trays work well; or can purchase trays from Fischer Scientific or other suppliers)).

- Once autoclaved, place bags into the containers provided by the Waste Disposal Company. For information about obtaining these containers, contact the Biosafety Officer or the Safety Officer.

- Autoclave biohazards for 40 minutes at the standard 121^{o}C and 15 PSI.

(C) Waste Specific Procedures

(C1) BL-1 and BL-2

- ***Cell Cultures, Stocks and Related Material:*** place in biohazard bags and autoclaved. Double or triple bagging may be required to avoid rupture.

- ***Bulk Liquid Waste, Blood and Blood Products***: place in an autoclavable container, then inside a biohazard bag and autoclaved.

- ***Small Quantities of Liquid Waste***- (Non-human or animal blood products): includes microorganism cultures, treat with a 1:10 dilution of household bleach or other appropriate disinfectant (See **Section II, A4** and **Table 3**). Discard down drain.

- ***Sharps:*** placed in a rigid, closeable, puncture-proof container labeled "Sharps" and the biohazard symbol. Sharps containers are then placed in biohazard bag and autoclaved. These containers are available through Fischer Scientific.

- ***Solid Waste:*** including cloth, toweling, paper items and plastic that have been contaminated should be placed in a biohazard bag and autoclaved. Double bagging may be necessary.

(C2) BL-3

All waste including RG-2 and RG-3 agents that are handled at BL-3 should be autoclaved at point of origin. Transportation to other areas of the Stevens campus is prohibited. Therefore, if BL-3 level activities occur, a specially dedicated autoclave must be available at the research location.

(C3) Pathological Waste

Animal parts or remains, including tissue, should be placed in a sealed biohazard bag (double bagged) and stored in a freezer until the next Waste Pick-up. The freezer used for storage should be identified by the biohazard symbol. Preferably, the freezer should be dedicated solely to the storage of animal remains. **Animal remains should not be autoclaved. Although not all pathological waste is biohazardous, it is prudent to treat such waste as if it was to protect against unknown risks.**

(D) New Jersey State Guidelines

RMW is regulated by the New Jersey Tracking Act and must be tracked with a manifest and a Certificate of Destruction. The Waste Disposal Company hired by Stevens is responsible for complying with these State guidelines. The responsibility of Stevens personnel regarding biohazardous waste lies in the proper separation, treatment and disposal of the waste.

Section IV

Emergency Response

It is important to remember that the potential for an emergency situation to arise always exists in the course of normal laboratory or research activities. Although emergencies may result from a variety of events and may involve chemicals, fire or biological agents, a standard response can be enacted. Here at Stevens an emergency response plan has been implemented which includes the evacuation of the facility if such an action was deemed appropriate. The key to implementing an emergency plan is internal communication, which requires that all members of the Stevens community know how to act and react during an emergency. To accomplish this, the Institute routinely runs drills to train Stevens' personnel how to respond. These drills are part of a written Emergency Response Plan which also requires that all accidents, **REGARDLESS OF SEVERITY**, be reported and investigated. The building evacuation plans are currently prepared by the Physical Plant Department and is reviewed by the members of the Stevens Safety Committee. For additional information, see the Stevens Chemical Hygiene Plan or contact the Safety Officer.

The acronym NEAR best summarizes key elements of the emergency procedure:

NOTIFY
EVACUATE
ASSEMBLE
REPORT

IN THE EVENT OF A MEDICAL EMERGENCY

Immediately notify Security at x5325 or use the red phones located throughout the campus.

- Give information as to the nature of the emergency and the exact location
- Stay on the phone with Security until all necessary information is obtained and Security hangs up
- Security will contact local Emergency Medical Service if the nature of the emergency deems it necessary
- If a co-worker, student or visitor contacts 911 for assistance, notify Security immediately so that the EMS units can be directed/escorted to scene

If there is a person who has received first-aid/CPR training nearby, he/she should be immediately contacted to give assistance. Stay with the ill/injured person until help arrives. Do not move or transport the person unless the area is immediately dangerous.

(A) How to Respond to a Biohazard Accident

The following section describes the appropriate response to a variety of biohazard accidents. It is important to note that these are guidelines only, since every situation is different. Using the information presented here, while maintaining a calm demeanor will invariably reduce any risk associated with a biohazard accident. In addition, it is equally important that a written record be kept of the incident and the actions taken in response to it (see "Accident Reporting"). An Incident Report Form is available (see ***Appendix H, Form 6***) for this purpose.

(A1) Biohazard Spill-Kit

Laboratories working with biohazardous materials should have a basic spill-kit ready to use at all times. This kit should include:

- Disinfectant (e.g. household bleach 1:10 dilution in water, prepared fresh; see **Table 3**)
- Absorbent material (e.g. paper towels)
- Waste Container (e.g. biohazard bag, sharps container)
- PPE (e.g. labcoat, gloves, face and eye protection)
- Mechanical tools (e.g. forceps, dustpan and broom)

(A2) Biological Organism Spills

Biological spills outside a biological safety cabinet will generate aerosols that can be dispersed in the air throughout the laboratory. These spills may be minor or very serious if they involve a microorganism of Biosafety Level 3, since most of these agents may transmit disease through inhalation or ingestion of an aerosol (for definitions of Biosafety Levels see ***Section I, B*)**. Therefore occupants should leave the location of the incident immediately to reduce the risk of exposure. At least thirty minutes should elapse before reentry to the location for decontamination. This time period should be sufficient for removal of the aerosol through the exhaust ventilation system, such as a biological safety cabinet or a fume hood.

Spills on the Body

- Remove contaminated clothing and place in a biohazard bag for later decontamination
- Vigorously wash exposed area with soap and water for one minute
- Obtain medical attention, if necessary
- Report the incident to a supervisor and document with Incident Report Form (see ***Appendix H, Form 6***)

Spills Inside the Laboratory

In general, clear spill area of personnel and wait for aerosols to settle. Don a disposable gown or laboratory coat, safety goggles and gloves. Refer to the following sections for specifics in dealing with different biohazard levels.

Biosafety Level 1 Organism Spill

- Wear gloves
- Use disinfectant (e.g. household bleach) soaked in disposable towel and place over spill
- Dispose of towel in Biohazard bag
- Clean spill area with a new disinfectant soaked towel and dispose in Biohazard bag; decontaminate bag in an autoclave

Biosafety Level 2 Organism Spill

- Alert people in the area immediately
- Don protective equipment; may include laboratory coat with long sleeves, disposable gloves, disposable shoe covers, safety goggles, mask or full-face shield
- Cover spill with paper towel or absorbent materials
- Apply household bleach around the edge of the spill then pour into the spill; AVOID SPLASHING!
- Let sit for 20 minutes
- Discard soaked absorbent material into Biohazard Bag
- Clean spill area with fresh disinfectant soaked towels; discard in biohazard bag
- Decontaminate Biohazard Bags by autoclaving

Biosafety Level 3 Organism Spill

- Immediately attend injured/contaminated person and remove them from area
- Alert people in area to evacuate immediately
- Close doors to infected area
- Call Campus Emergency Response Team
- Assign an individual (from the laboratory or research facility) knowledgeable about the incident to interact with Emergency Response Team.

Spills Inside a Biological Safety Cabinet

- Wear a labcoat, safety goggles and gloves
- Allow cabinet to run during clean-up
- Soak spilled material with disposable towel and apply disinfectant with a minimum of 10 minutes contact time
- Wipe up spillage and disinfect area with soaked disposable towels
- Wipe up walls and any equipment with disinfectant soaked disposable towel
- Discard contaminated material in biohazard bag and decontaminate by autoclave
- Expose any non-disposable contaminated material to disinfectant for 10 minutes
- Remove and discard any contaminated protective equipment.
- Allow cabinet to run for 10 minutes before continuing work.

Spills Inside a Centrifuge

- Clear all personnel from area
- Wait 30 minutes to allow aerosols to settle
- Wear a labcoat, safety goggles and gloves
- Remove rotor and buckets in a BSC if available- if BL-1 spill, a standard fume hood will do.
- Thoroughly disinfect inside the centrifuge
- Remove contaminated debris, discard in biohazard bag or sharps container and decontaminate by autoclave.

Spills Outside the Laboratory (during campus transport)
Always transport biohazardous materials in an unbreakable, well-sealed primary container inside a leak-proof, closed unbreakable secondary container, labeled with the biohazard symbol (e.g. plastic cooler, bio-specimen pack).

Should a spill of an RG-2 level agent occur on campus in public, contact the IBC, Safety Officer and Stevens Security. Do not attempt a clean up without proper PPE and clean-up kit. Keep individuals away from the spill until help arrives.

(A3) Blood Spills

Universal precautions must be observed. Cleaning of blood spills should be limited to those persons who are trained for the task. Untrained individuals who encounter a spill should limit access to the spill area and immediately notify the properly trained individual. Remember that human pathogens may be transmitted through animal blood and blood products and should therefore be treated with similar precautions (see ***Universal Precautions, Section I, B, Biosafety Level 2***).

General Practices

- Only disposable absorbent material should be used to soak the spill. If a solid material like glassware is associated with the spill it should never be handled by hand, but only through mechanical means, such as a dustpan or forceps.
- Wear tear resistant disposable gloves; if tear develops, wash hands immediately and re-glove.
- Wear protective eye and face gear if splashing is a concern
- Dispose of all disposable material (gloves, absorbent material) in a double-bagged Biohazard Bag and decontaminate by autoclave.
- Disinfect the spill area with one of the following types of disinfectant;

  -EPA-registered "hospital disinfectant" chemical germicides that are effective against

  Tuberculosis or Human Immunodefiency Virus (HIV)

  -A solution of 5.25% sodium hypochlorite (household bleach) diluted 1:100 with water.

Cleaning Technique

- All blood must first be remove from spill area surface before germicidal disinfectant is applied
- Isolate area
- Wear gloves and protective apparel
- Remove blood with disposable towels in a ways that minimizes direct contact with blood
- Decontaminate area with appropriate disinfectant-soaked towel
- Double-bag (Biohazard Bag) all contaminated material and decontaminate by autoclave

(B) How to Respond to a Chemical Accident

As this plan addresses the use of biohazradous agents only, the reader is directed to the Stevens Chemical Hygiene Plan.

(C) How to Respond to a Radioactive Accident

As this plan addresses the use of biohazradous agents only, the reader is directed to the Radiation Safety Officer.

(D) Reporting an Accident

As discussed in the previous section, ALL biohazardous accidents must be reported to the IBC by the submission of an ***Incident Report Form*** (see ***Appendix H, Form 6***). Many times accidents are not reported because they are perceived as embarrassing or "careless" or because those involved believe it to be "no big deal". However, it is the responsibility of the Institutional Biosafety Committee to determine what future actions or precautions need to be taken, based on the information provided within the Incident Report and by standard Biosafety procedures**. It should be understood that the purpose of reporting and documenting accidents is not to affix blame but instead to determine the cause of an accident so that similar incidents may be prevented in the future.** Documentation of these events help ensure the safety of individuals working with biohazard agents, since taking corrective action as a result of a minor accident may prevent a major incident from occurring. It also serves as a means of preparation for complications that might only become evident at a later time. Moreover, prior knowledge of all accidents and the actions taken in response to them provides a written response protocol for future similar-type incidents and acts as an information source for updating and modifying this Biosafety Plan.

If an employee is injured during a biohazard accident, a Worker's Compensation Notice of Injury report should be filed with Stevens Personnel Department immediately.