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Rensselaer

BIOSAFETY PROGRAM

Rensselaer

Foreword

Rensselaer Polytechnic Institute has adopted this Biosafety Plan to protect our employees working with or near biohazardous materials from exposure to potentially infectious materials, as well as to protect the community and environment from the accidental release of biohazardous materials. Work with biohazardous materials can be performed safely, provided that proper training in Biosafety fundamentals is completed by laboratory users, facility design is appropriate for the work being performed and the work practices outlined in this document are followed. All researchers and effected employees involved in biological research at Rensselaer are expected to comply with this plan.

Section 1. Scope and General Summary

Rensselaer's Biosafety Plan encompasses all work performed involving microbial agents, recombinant DNA, human cell lines and other tissue, and the use of laboratory animals. Researchers are required to submit a registration form outlining their research projects to the Institute Biosafety Committee (IBC) before beginning work, and/or procuring all biohazardous materials to ensure that the work will be performed according to applicable regulations and guidelines. The Office of Environmental Health and Safety will work closely with the IBC to ensure that all personnel are trained as required and that proper waste disposal procedures are followed. The application of the policies and procedures contained in this document will ensure the safety of Rensselaer employees and the protection of the surrounding community and environment.

The Biotechnology and Interdisciplinary Studies Center is designed to facilitate the use and storage of organisms classified by the Centers for Disease Control and Prevention (CDC) as Biosafety Level 1 and Level 2. **The following activities are currently not allowed at Rensselaer:**

- Work with materials classified by the Centers for Disease Control and Prevention as Biological Safety Level 3
- Work with materials classified by the Centers for Disease Control and Prevention as Biological Safety Level 3
- Work with materials classified by the National Institutes of Health as Risk Group 3
- Work with materials classified by the National Institutes of Health as Risk Group 4
- "Select Agents" as per Appendix A of 42 CFR Part 72
- Research involving the use of live animals

See Section 3 for more information on Biosafety Levels, and Appendices 3 and 4 for lists of permitted and prohibited organisms.

Section 2. Applicable Regulatory Requirements and Guidelines

This program contains the practices and procedures required to comply with following guidelines and regulations:

For work with all microbial agents and recombinant DNA:

- *Biosafety in Microbiological and Biomedical Laboratories*, 5th Edition (Centers for Disease Control and Prevention & the National Institutes of Health, 1999) (BMBL)
- *NIH Guidelines for Research Involving Recombinant DNA Molecules* (the National Institutes of Health, 2001) (NIH Guidelines)

NOTE: The above guidelines must be followed to ensure continuation of federal grant funds. Copies of the guidelines are available online at http://bmbl.od.nih.gov/ and

http://www.nih.gov/od/orda/toc.htm; researchers are encouraged to download and read the complete guidelines before initiating grant proposals or work.

For work with human blood, fluids, and tissue:

- Rensselaer's *Bloodborne Pathogen Exposure Control Plan*, based upon 29 CFR 1910.1030: Occupational Exposure to Bloodborne Pathogens (OSHA)

For handling and disposal of biohazardous waste/regulated medical waste:

- Title 15, Article 27 of the Environmental Conservation Law; Chapter 438, Laws of 1993, New York State Department of Environmental Conservation, 10 NYCRR Part 70, New York State Department of Health

For packaging and shipment of biohazardous materials:

- 42 CFR Part 72: Interstate Shipment of Etiologic Agents and sections of 49 CFR 171-180, Department of Transportation Hazardous Materials

For use of animals in research:

- The Animal Welfare Act, U.S. Code, Title 7, Sections 2131 to 2156 and 9 CFR Chapter 1, Subchapter A, Parts 1, 2, and 3
- *Institutional Animal Care and Use Committee Guidebook*, Office of Laboratory Animal Welfare, NIH (2002)*

* Note-At the current time, live animal research is prohibited at Rensselaer.

Section 3. Definitions

Aerosol: airborne droplets of liquid containing viable microorganisms

Aseptic technique: the practices used to avoid infection of the worker and contamination of a live culture or equipment used with live cultures with other microorganisms present in the environment. Techniques include sterilization of the work area, use of sterile materials and instruments, and use of flame or air flow (such as in a biological safety cabinet) to prevent airborne microorganisms from contacting the materials.

Bacteriophage: ("bacteria-eater) a virus parasitic in bacteria; used in recombinant DNA research.

Biohazardous Materials: All infectious organisms (bacteria, chlamydia, fungi, parasites, prions, rickettsias, viruses, etc.) that may cause disease in healthy humans, or cause significant environmental or agricultural impact. Additionally, materials and products associated with work with human or primate tissues, fluids, cells, or cell culture, recombinant DNA (including releases to the environment), transgenic plants or animals, human gene therapy, and animals known to be vectors of zoonotic diseases.

Biological Safety Cabinet (BSC): Biological safety cabinets are effective and commonly used devices that provide containment of infectious splashes or aerosols generated in many microbiological procedures. Properly maintained BSCs, when used in conjunction with good microbiological techniques, provide an effective containment system for the safe manipulation of infectious materials. It must be noted that the HEPA filtration used in BSCs, while effective in trapping particulates such as infectious agents, are not capable of capturing volatile chemicals or gases. Only BSCs that are properly rated for chemical use should be used when working with volatile chemicals. Also, ducting to the outside may be required depending upon the concentration and specific chemical(s) in use. Chemical-intensive work not involving infectious agents must always be conducted in a chemical fume hood.

There are three classes of BSCs; each type provides personnel and environmental protection using HEPA filtration. The various BSC classes are described below, and are summarized and compared in Table 3.1.

Class I Biological Safety Cabinet:

The Class I biological safety cabinet is a negative-pressure, ventilated cabinet operated with a minimum face velocity of at least 75 linear feet per minute. All of the air from the cabinet is exhausted through a HEPA filter either into the laboratory or to the outside.

The Class I BSC is designed for general microbiological research and low- and moderate-risk agents. This BSC is not appropriate for handling materials that are vulnerable to airborne contamination, since the inward flow of unfiltered air from the laboratory can carry microbial contaminants into the cabinet. Class I BSCs are currently being manufactured in limited amounts; Class II BSCs are generally preferred, since they provide the research materials with protection from contamination.

Class II Biological Safety Cabinet:

The Class II Biological Safety Cabinet is the most commonly used, and is the type recommended for work with infectious materials at Rensselaer. These cabinets provide protection to personnel, the environment, and the research materials by utilizing three types of air flow – inward air flow at 75-100 linear feet per minute protects personnel, HEPA-filtered downward vertical laminar flow protects the product from outside contamination, and HEPA-filtered exhaust air prevents infectious particles from exiting the cabinet. Class II BSCs are further classified as listed below:

Class II A: Type A cabinets re-circulate air within the cabinet, making them suitable for microbiological research in the absence of volatile or toxic chemicals and radionuclides. Type A cabinets are usually exhausted into the laboratory, but may be exhausted to the outdoors via a thimble connection to the building exhaust system. An unducted Class II Type A cabinet cannot be used for work involving volatile or toxic chemicals, since the build-up of chemical vapors in the cabinet by recirculated air and in the laboratory from exhaust air can create health and safety hazards.

Class II B: Type B cabinets are hard-ducted (thimble connection) to the building exhaust system. Face velocities in Class II B cabinets should be 100 linear feet per minute, which allows for work with volatile or toxic chemicals and radionuclides.

Class III Biological Safety Cabinet:

Class III biological safety cabinets is are totally enclosed, gas-tight, ventilated cabinets. They are suitable for work that requires Biosafety Level 3 or 4 containment.

Biologicals: serum, antigens, antitoxins, vaccines, or other materials or preparations isolated or synthesized from living organisms.

Biosafety Levels: designations describing the combinations of laboratory practices, safety equipment, and facilities required to safely conduct research on infectious agents. There are four biosafety levels; the level recommended for work with a particular infectious agent depends upon many factors including its virulence and means of infection. Descriptions of each biosafety level are listed below:

> **Biosafety Level 1**: The least stringent level; recommended for work performed on defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans and of minimal potential hazard to the environment under normal use conditions. This level requires a basic level of containment that relies on standard microbiological techniques with no specialized primary or secondary barriers, other than a sink for hand washing - these agents can be handled safely in the laboratory without special apparatus or equipment. Biosafety Level 1 agents are recommended for use in undergraduate teaching laboratories.
>
> **Biosafety Level 2**: This level is recommended for work involving moderate-risk agents that are present in the community and associated with human disease. The primary hazards when working with these agents are accidental percutaneous or mucous membrane exposures and ingestion. Extreme caution must be taken with contaminated sharps, and personnel must avoid practices that may lead to ingestion of infectious substances. These agents may be used safely on the open bench, provided the potential for producing splashes or aerosols is low. Work may also be performed in a biosafety cabinet; biosafety cabinets must be used when there is a potential risk of producing splashes or aerosols. Biosafety Level 2 is also recommended for work involving human blood, body fluids, tissues, or human cell lines where the presence of an infections agent may be unknown. Additional precautions for working with human-derived materials may be found in Rensselaer's ***Bloodborne Pathogen Exposure Control Plan***. In addition to hand washing sinks, waste decontamination facilities must be available for Biosafety Level 2 work.
>
> **Definitions for Biosafety Levels 3 and 4 are provided as reference material, however work at Biosafety levels 3 and 4 is NOT PERMITTED AT RENSSELAER.**
>
> **Biosafety Level 3**: This level is recommended for work involving indigenous or exotic agents that may cause serious and potentially lethal infection, with a potential for respiratory transmission. All laboratory manipulations must be performed in a biosafety cabinet or other enclosed equipment, there must be controlled access to the laboratory,

and there are ventilation requirements to minimize the release of aerosols from the laboratory. **The use and storage of Biosafety Level 3 agents is not permitted at Rensselaer.**

Biosafety Level 4: This level is recommended for work involving dangerous and exotic agents that pose a high individual risk of life-threatening disease for which there is no available vaccine or therapy. Agents with a close or identical antigenic relationship to Biosafety Level 4 agents should also be handled at this level. The laboratory worker must be completely isolated from the infectious materials, which is accomplished by using a Class III biosafety cabinet or a full-body air-supplied positive pressure suit. Biosafety Level 4 facilities must be a separate building or a completely isolated zone with complex, specialized ventilation requirements and waste management systems. **The use and storage of Biosafety Level 4 agents is not permitted at Rensselaer.**

Biotechnology: The use of microorganisms, such as bacteria or yeasts, or biological substances, such as enzymes, to perform specific industrial or manufacturing processes. Applications include the production of certain drugs, synthetic hormones, and bulk foodstuffs as well as the bioconversion of organic waste and the use of genetically altered bacteria in the cleanup of oil spills.

Bloodborne Pathogen: a microorganism present in blood, certain body fluids, & body tissue that can cause disease. Diseases include but are not limited to: Hepatitis B (HBV), Syphilis, Malaria, and HIV (Human Immunodeficiency Virus).

Body Fluids: body fluids such as blood, saliva, amniotic fluid and any other fluid visibly contaminated with blood.

Cell Culture: the culturing of cells *in vitro*. (an experimental situation outside the organism)

Cell Line: a culture of cells resulting from the creation of a cell suspension of fresh tissue, which is then passaged (grown for more than one generation) or split.

Containment: safe methods for reducing or eliminating the exposure of laboratory workers and the outside environment and population to infectious agents. There are three elements of containment: laboratory practices and techniques, safety equipment, and facility design.

Contaminated: the presence or the reasonably anticipated presence of potentially infectious materials, including pathogens or blood.

Etiologic Agent: a viable microorganism or its toxin that causes or may cause human disease. This term is used by the Department of Transportation.

Eukaryote: A single-celled or multicellular organism whose cells contain a distinct membrane-bound nucleus. This classification includes the "higher" orders of organisms, including plants and animals.

Exposure Incident: means a specific eye, mouth, or other mucous membrane, non-intact skin, or parenteral contact with blood or other potentially infectious materials that results from the performance of job duties.

Infectious agent: Any organisms such as a virus or bacteria capable of causing disease or an adverse health impact in humans, and any material that may potentially contain such organisms, such as raw sewage and human body fluids or tissues.

Institute Biosafety Committee (IBC): a committee composed of representative faculty, staff, and administrators familiar with biotechnology research convened to oversee the Institute Biosafety program and to review and approve proposed biotechnology research. If institute research falls under the *NIH Guidelines for Research Involving Recombinant DNA Molecules*, the IBC must be established according to the NIH Guidelines.

Institutional Review Board: An independent review board that ensures the ethical treatment of human test subjects in clinical trials. IRBs have the authority to approve, require modifications to, or disapprove the proposed study protocols and consent forms for research that involves human subjects. IRBs must review and approve or disapprove the investigator for the research and monitor the progress of ongoing research.

Interstate shipment: the shipment of materials from state to state (or possession to state, etc.) within the United States or its possessions. Interstate shipping regulations for biological materials listed in Section 10 of this document are to be used for any shipment of biological materials, including those within the state of New York.

In vitro: biological experimentation, growth, or production of biologicals in an artificial environment outside the living organism

In vivo: biological experimentation, growth, or production of biologicals within a living organism

LD_{50}: Lethal Dose 50 – a term used to describe the toxicity of a poison, toxin, or other material; the dose having 50% probability of causing death.

Microorganism: any microscopic form of life, including bacteria, yeasts, and protozoan.

Non-infectious material: material incapable of causing disease or a negative health impact on humans. Examples include Biosafety Level 1 agents such as *Bacillus subtilis* and Simian virus 40. Human-derived materials, such as blood and cell lines, are never considered non-infectious.

Pathogen: any disease-producing microorganism.

Plasmid: A circular, double-stranded unit of DNA that replicates within a cell independently of the chromosomal DNA. Plasmids are most often found in bacteria and are used in recombinant DNA research to transfer genes between cells.

Principal Investigator (PI): the primary initiator and director of research activities for a particular laboratory or other assigned space; usually a faculty member.

Prokaryote: a unicellular organism having cells lacking membrane-bound nuclei; bacteria, blue-green algae, actinomycetes and mycoplasma belong to this classification.

Recombinant DNA (rDNA): molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, and the molecules that result from that replication.

Recombinant DNA Advisory Committee (RAC): the public advisory committee that advises the Department of health and Human Services and the NIH Director concerning recombinant DNA research.

Regulated Medical Waste: waste capable of transmitting disease to humans, including that which was generated in the diagnosis, treatment, or immunization of human beings or animals, in research pertaining thereto, or in production and testing of biologicals. Regulated medical waste categories include sharps, cultures and stocks, human blood, blood products, and human pathological wastes, and animal wastes.

Risk Groups: classifications of microbial agents used in the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (2001). There are four risk groups:

> **Risk Group 1**: agents not associated with disease in healthy adult humans
> **Risk Group 2**: agents associated with human disease that is rarely serious and for which preventative or therapeutic interventions are often available.
> **Risk Group 3**: agents associated with serious or lethal human disease for which preventative or therapeutic interventions may be available.
> **Risk Group 4**: agents that are likely to cause serious or lethal human disease for which preventative or therapeutic interventions are not usually available.
>
> **Work with agents classified as Risk Group 3 or 4 is not permitted at Rensselaer.**

Select Agent: a microorganism (virus, bacterium, fungus, rickettsia) or toxin listed in Appendix A of 42 CFR Part 72. The term also includes genetically modified microorganisms or genetic elements from organisms on Appendix A of 42 CFR Part 72, shown to produce or encode for a factor associated with a disease, and genetically modified microorganisms or genetic elements that contain nucleic acid sequences coding for any of the toxins on Appendix A of 42 CFR Part 72, or their toxic subunits. **Most select agents require Biosafety Level 3 or higher facilities and practices, and are not permitted at Rensselaer**. However, a few are permitted for work in Biosafety Level 2 facilities. Work with select agents cannot be performed without additional registration with the federal government, personnel restrictions, and other procedures.

Sharps: any discarded item that had been in contact with infectious agents and capable of causing a cut or laceration to the skin, including razor blades, glass microscope slides, and labware such as culture tubes. Discarded used or unused needles, hypodermic needles, and complete syringes are always considered sharps, even if not exposed to any infectious agents.

Standard Microbiological Practices: The techniques and procedures used to prevent contamination of media and supplies, and infection of personnel, when performing microbiological or biotechnological work.

Tissue culture: the growing of cells, tissues, and organs *in vitro*. Applications of tissue culture include virus isolation and identification, research in cancer and virology, and investigation of toxic effects of pharmaceuticals.

Universal Precautions (Standard Precautions): an approach to infection control where **all** human blood and certain body fluids are treated as if known to be infectious for HIV, Hepatitis B virus, and other bloodborne pathogens. Universal precautions may include the use of engineering controls, personal protective equipment, and work practice controls to prevent the spread of infectious disease. Research involving human-derived materials must be performed at Biosafety Level 2.

Vaccine: A preparation of a weakened or killed pathogen or of a portion of the pathogen's structure that upon administration stimulates antibody production or cellular immunity against the pathogen.

Vector: A bacteriophage, plasmid, or other agent that transfers genetic material from one cell to another

Virus: Any of various simple submicroscopic parasites of plants, animals, and bacteria that often cause disease and that consist essentially of a core of RNA or DNA surrounded by a protein coat. Unable to replicate without a host cell, viruses are typically not considered living organisms.

Zoonotic Diseases: diseases of animals that can be transmitted to humans.

Section 4. Responsibilities

4.1 Office of Environmental Health and Safety

The Office of Environmental Health and Safety will:

- 4.1.1 Coordinate the Institute Biosafety Committee and schedule meetings for proposal reviews.
- 4.1.2 Work with the IBC to review research proposals and approve those proposals that meet the applicable requirements for safe use of biohazardous materials and/or rDNA.
- 4.1.3 Oversee Rensselaer's ***Bloodborne Pathogen Program*** and provide bloodborne pathogen training to laboratory personnel as needed.
- 4.1.4 Oversee Rensselaer's ***Biosafety Program*** and provide Biosafety training to laboratory personnel as needed
- 4.1.5 Advise personnel on proper waste packaging procedures.
- 4.1.6 Advise personnel on proper sample packaging and shipping procedures
- 4.1.7 Oversee institute waste pick-up and transport to treatment facilities.
- 4.1.8 Conduct routine laboratory EHS audits and advise the laboratory Principle Investigators of deficiencies and areas in which improvements are needed

4.2 Institute Biosafety Committee

The Institute Biosafety Committee will:

- 4.2.1 Oversee Rensselaer's Biosafety Plan, providing technical guidance including an annual review.
- 4.2.2 Review research proposals and approve those proposals that meet the applicable requirements for safe use of biohazardous materials and/or rDNA.

4.3 Principal Investigators

Principal Investigators will:

- 4.3.1 Complete biological work registration and approval forms for any proposed research involving biological agents, rDNA, human cell lines or other human tissue, and/or laboratory animals.
- 4.3.2 Incorporate biosafety procedures into standard operating procedures in a biosafety manual adopted or prepared specifically for the laboratory. This Biosafety Plan may be used as a starting point by individual laboratories, but laboratory-specific hazards must be identified and practices addressing them must be incorporated into the laboratory-specific manual. An example of a laboratory specific format in included in Appendix 6.

4.3.3 Ensure that laboratory personnel are knowledgeable in aseptic technique and other standard microbiological practices, specific protocols, and other work practices.

4.3.4 Ensure that all laboratory personnel attend bloodborne pathogen training if blood or other human products are used in the laboratory, and other EHS training as required.

4.3.5 Ensure that laboratory personnel receive any necessary medical surveillance and/or immunizations. If the employee declines to receive a vaccine, a declination form must be completed and sent to the Office of Environmental Health and Safety.

4.3.6 Ensure that laboratory personnel comply with this document and any other applicable regulations and guidelines.

4.3.7 Ensure that biological safety cabinets are certified as needed and that personal protective equipment is provided and used.

4.3.8 Maintain a log of all biological material received and sent out.

4.3.9 Immediately report any laboratory spills, accidents, containment failures, or violations of biosafety practices that result in the release of biohazardous materials and/or the exposure of laboratory personnel or the public to infectious agents to the Office of Environmental Health and Safety and Public Safety.

4.3.10 Follow all applicable requirements of the NIH Guidelines for Research Involving Recombinant DNA Research when conducting non-exempt rDNA research.

4.4 Employees working with Biohazardous Materials and/or rDNA

Employees working with biohazardous materials and/or rDNA will:

4.4.1 Comply with this document and any other applicable regulations and guidelines.

4.4.2 Attend all required training in a timely manor

4.4.3 Be familiar with aseptic technique and other standard microbiological practices, specific protocols, and other work practices.

4.4.4 Receive any necessary medical surveillance and/or immunizations. If the employee declines to receive a vaccine, a declination form must be completed and sent to the Office of Environmental Health and Safety.

4.4.5 Wear personal protective equipment as required.

4.4.6 Immediately report any laboratory spills, accidents, containment failures, or violations of biosafety practices that result in the release of biohazardous materials and/or the exposure of laboratory personnel or the public to infectious agents to their PI.

4.4.7 Inform their PI if there is a change in immune status, such as pregnancy, chemotherapy, or other immunocompromising state or treatment.

4.4.8 NOT permit unauthorized personnel to enter the laboratory.

4.5 Environmental & Site Services and Physical Plant Employees/Outside Contractors

When entering laboratories where biohazardous work is being conducted, Environmental & Site Services and Physical Plant employees, as well as outside contractors will:

4.5.1 Announce their presence and explain their need to enter the facility before entering.

4.5.2 NOT enter unoccupied laboratories in which work with bio hazardous materials is on-going unless the PI has authorized the entrance and all applicable hazards have been identified

4.5.3 NOT enter if Biosafety Level 2 work is being performed – researchers must stop work and decontaminate the work area before access is given unless the PI has authorized the entrance and all applicable hazards have been identified and deemed as not being affected by the work

4.5.4 NOT touch any equipment or materials other than those required by job duties.

4.5.5 Wear proper personal protective equipment for the specific hazards identified in the laboratory at all times.

4.5.6 Wash hands before leaving the laboratory.

Section 5. Work Registration and Approval Process

5.1 Scope

The following research must be registered and/or approved by the IBC before initiation of work:

- 5.1.1 Work with bacterial, fungal, parasitic, viral, or other potentially infectious agents
- 5.1.2 Work with toxins
- 5.1.3 Work with human blood and/or tissue, including human cell lines
- 5.1.4 Work with rDNA, including work specifically exempt in the NIH Guidelines for Research Involving Recombinant DNA Molecules
- 5.1.5 Work with noninfectious bacterial, fungal, parasitic or other agents (Biosafety Level 1 research; registration required only)

5.2 Process

The first step in performing Biosafety Level 1 and 2 research at Rensselaer is obtaining approval from the IBC. See Appendix 1 for the Biological Research Registration and Approval Form. Copies of the forms are available at the Office of Environmental Health and Safety. Forms must be completed and sent to the IBC, care of the Office of Environmental Health and Safety, before research is initiated and/or biological materials are ordered or arrive on campus. Approvals must be renewed annually; any proposed changes in protocols in the interim must be reported to the IBC using an Approved Biological Research Revision Form (see Appendix 2). The Biological Work Registration and Approval Form requests information on the agents and protocols being used, employee qualifications and training, location of the proposed research and the availability of safety equipment, and the quantity of waste expected to be generated. The information will be reviewed to determine if applicable regulations and guidelines are met – if not, the form will be returned to the PI for revision. Only those proposals that meet all applicable regulations and guidelines will be approved. **Work with agents requiring Biosafety Level 3 or 4 practices and facilities are not permitted at Rensselaer. See Appendix 4 for a listing of prohibited agents.**

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Section 6. Facility Requirements

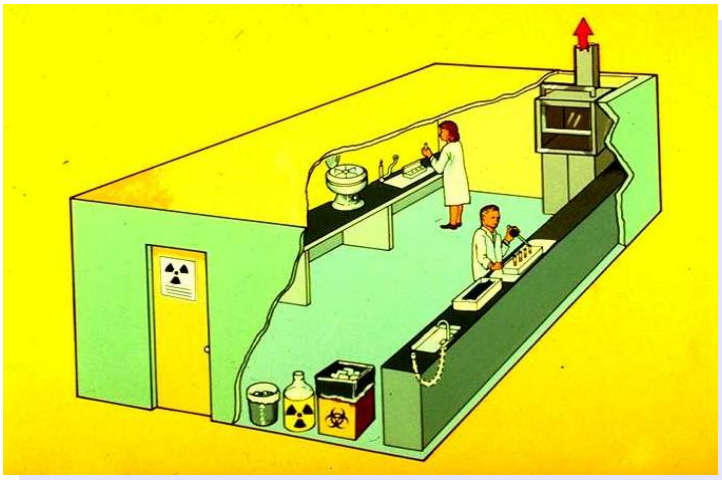
The CDC/NIH document *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) lists facility design requirements for research involving viable microorganisms. Work involving viable microorganisms may be conducted only in laboratories that meet the following requirements:

6.1 Facility Requirements for work involving Biosafety Level 1 and 2 agents:

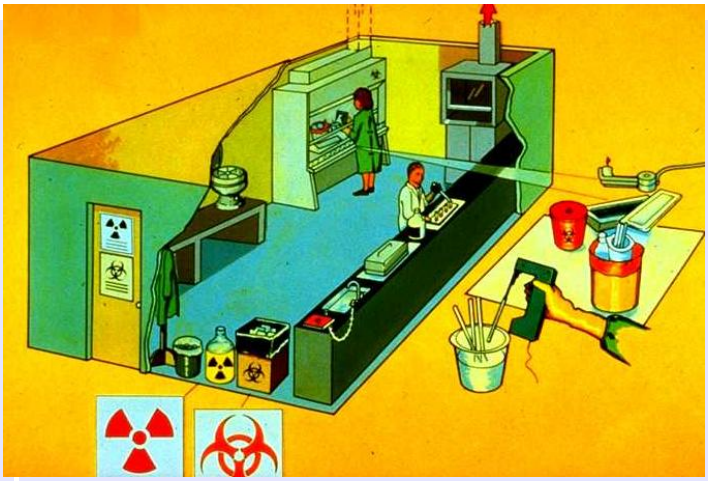
6.1.1 Laboratories must have lockable doors for access control.
6.1.2 Each laboratory must contain a sink for hand washing.
6.1.3 The laboratory must be designed so that is can be easily cleaned. Carpets, rugs, and cloth furniture are not appropriate. Spaces between benches, cabinets, and equipment must be accessible for cleaning.
6.1.4 Bench tops must be impervious to water and resistant to moderate heat and organic solvents, acids, alkalis, and chemicals used to decontaminate the work surface and equipment.
6.1.5 If the laboratory has windows that open to the outside, the windows must be fitted with fly screens.
6.1.6 Eyewash stations must be readily available.
6.1.7 Illumination must be adequate for all activities, avoiding reflections and glare that may impede vision.
6.1.8 Biosafety Level 2 laboratories should be located away from public areas of the building.

Included below are schematic representations of BSL-1 AND BSL-2 Facilities.

BSL-1 Facility



BSL-2 Facility



Information adopted from Biosafety in Microbiological and Biomedical laboratories-3rd Edition, JY Richmond & RW McKinney

Section 7. Work Practices

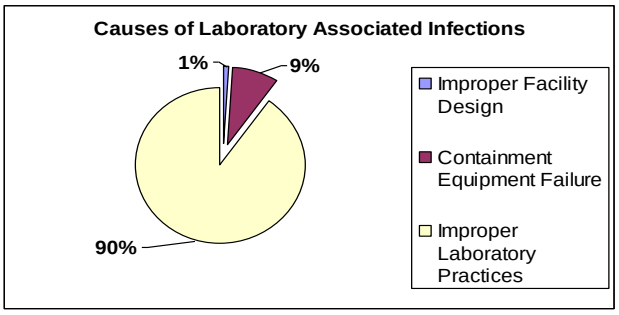
7.1 General Lab Safety

Biological laboratory personnel must attend "Biosafety" training offered by the Office of Environmental Health and Safety prior to initiating work with biological materials in the laboratory. Additionally, laboratory personnel who work with human blood or other potentially infectious human materials must attend "Bloodborne Pathogens" training. These EHS courses are offered on a routine basis and a course schedule can be obtained through the Office of Environmental Health and Safety. These courses address hazard communication, chemical use and storage, hazardous waste handling, compressed gas safety, biological safety practices and containment levels and exposure control as well as other general laboratory safety issues. If radioactive materials are used in the lab, personnel must attend "Radiation Safety" training. The following general lab safety requirements apply to biology laboratories:

7.1.1 Proper personal protective equipment must be worn at all times. See Section 7.4 of this document for more information.
7.1.2 Shorts and sandals are inappropriate clothing for laboratory work. Wear long pants and closed-toed shoes at all times.
7.1.3 Aisles and exits must be kept clear at all times.
7.1.4 There must be unrestricted access to fire extinguishers, eye washes, and safety showers at all times.
7.1.5 Hazardous waste labeling and storage procedures must be followed at all times.
7.1.6 Chemicals, reagents, and samples must be labeled as to their contents.
7.1.7 Chemicals must be stored properly; incompatible materials must be segregated.
7.1.8 Equipment must be in good repair and used properly.

7.2 Standard Microbiological Practices

The importance of utilizing safe microbial practices in the biology/biotechnology laboratory cannot be overstated. Of the three types of protection provided in the biology/biotechnology laboratory (safe laboratory practices, containment equipment (Primary Barriers) and facility design) safe laboratory practices has, by far, the greatest impact. Consider the following data:



* Richard Gilpin-Laboratory Practices and Techniques

In addition, according to information provided in the Centers for Disease Control and Prevention and National Institutes of Health *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) **only 16% of all reported infections are associated with a specific documented accident.**

All personnel working with microorganisms must be trained and proficient in standard microbiological techniques, including culturing and handling microorganisms, appropriate sterilization and decontamination procedures (including proper autoclave use), and contamination prevention.(See Section 7.5) Working with biohazardous materials requires strict adherence to standard microbiological techniques at all times. Persons working in laboratories containing biohazardous materials must be aware of potential hazards and the practices and procedures needed to minimize or eliminate risks. Biosafety level 2 work must be directed by competent scientists and performed by persons trained in handling pathogenic agents. Standard microbiological practices recommended for Biosafety Level 1 and 2 laboratories by the CDC/NIH document *Biosafety in Microbiological and Biomedical Laboratories* are listed below.

7.2.1 Biosafety Level 1 and 2 Standard Microbiological Practices:

7.2.1.1 Access to the laboratory should be limited or restricted in consultation with the PI when experiments or work with cultures and specimens is in progress. (Not including emergency situations)

7.2.1.2 Persons must wash their hands after they handle viable material, after removing gloves, and before leaving the laboratory.

7.2.1.3 Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in work areas.

7.2.1.4 Mouth pipetting is prohibited; mechanical pipetting devices must be used.

7.2.1.5 Policies for the safe handling of sharps must be instituted and followed. Sharps must be placed in puncture resistant containers. Needles should never be re-sheathed.

7.2.1.6 All procedures must be performed carefully to minimize the creation of splashes or aerosols.

7.2.1.7 Work surfaces must be decontaminated at least twice a day (before and after work) and after any spill of viable material. (See Table 7.1)

7.2.1.8 All cultures, stocks, and other regulated wastes must be decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and closed for transport from the laboratory.

7.2.1.9 An insect and rodent control program must be in effect.

7.2.1.10 Laboratory coats or gowns are recommended for Biosafety Level 1 work and required for Biosafety Level 2 work.

7.2.2 Biosafety Level 2 Special Practices:

7.2.2.1 Persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, such as persons who are immunocompromised or immunosuppressed, should not be permitted in the laboratory. **It is the responsibility of the laboratory worker to disclose to the PI such conditions or health related issues**. The PI and the Office of Environmental Health and Safety have the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

7.2.2.2 The PI must establish policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (when applicable, such as immunization) may enter the laboratory.

7.2.2.3 A biohazard sign must be posted on the entrance to the laboratory. Additional information, such as the agent(s) in use, biosafety level, the PI's name and telephone number, PPE requirements, required immunizations, and special procedures for exiting the laboratory should be posted as applicable.

7.2.2.4 Appropriate immunizations or tests for the agents handled or potentially present in the laboratory must be made available to all laboratory personnel.

7.2.2.5 When appropriate, considering the agents handled, baseline serum samples for laboratory and other at-risk personnel should be collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled.

7.2.2.6 A high degree of precaution must always be taken with any contaminated sharp items, including needles, syringes, slides, pipettes, capillary tubes, and razors.

7.2.2.6.1 Sharp instruments should be restricted for use only where there is not alternative.

7.2.2.6.2 Only needle-locking syringes or disposable syringe-needle units (i.e. the needle is integral to the syringe) should be used for the injection or aspiration of infectious materials.

7.2.2.6.3 Used needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

7.2.2.6.4 Broken glassware must not be handled directly by hand – remove with a brush and dustpan, forceps, or tongs. Contaminated glassware must not be disposed in the lab glass containers – dispose in a labeled sharps container. Any tools used to pick up contaminated glassware must be decontaminated with an appropriate disinfectant.

7.2.2.7 Cultures, tissues, or other potentially infectious wastes must be placed in a container with a cover that will prevent leakage during collection, handling, processing, storage, transport, or shipping.

7.2.2.8 Laboratory equipment and work surfaces must be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after spills, splashes, or other contamination.

7.2.2.9 When vacuum lines are used, they must be connected to a trap flask containing appropriate disinfectant and protected with a HEPA filter to prevent contamination of the lines and the pump.

7.2.2.10 Animals not involved in the work being performed are not permitted in the lab.

7.2.2.11 Properly maintained and certified Class II biological safety cabinets or other appropriate physical containment devices must be used whenever procedures with a potential for creating infectious aerosols or splashes are being conducted. Examples of such procedures include:

- centrifuging
- grinding
- blending
- vigorous shaking or mixing
- sonication
- opening containers of infectious materials whose internal pressures may be different from ambient pressures
- inoculating animals intranasally
- harvesting infected tissues from animals or embryonate eggs

Biological safety cabinets must also be used whenever high concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.

7.2.3 Work with Toxins of Biological Origin:

7.2.3.1 Each laboratory should develop a toxin-specific chemical hygiene plan that identifies the hazards encountered and lists the policies and practices used to minimize risks.

7.2.3.2 All laboratory personnel should have access to the chemical hygiene plan, and be adequately trained in working with the specific toxin.

7.2.3.3 An inventory control system should be in place – toxin receiving, shipping, production, and destruction should be noted in a log.

7.2.3.4 Toxins should be stored in locked storage areas, cabinets, or freezers when not in use.

7.2.3.5 Access to areas containing toxin should be restricted.

7.2.3.6 Manipulations of dry forms of toxins, including the preparation of stock solutions, should be conducted in a chemical fume hood, glove box, or a

BSC capable of protecting against chemical fumes (Class II Type B1 or higher). HEPA and/or charcoal filtration of exhaust air may be required.

7.2.3.7 The user should verify inward air flow of the hood or BSC before initiating work.

7.2.3.8 When toxins are in use, the room should be posted with a "Toxins in Use – Authorized Personnel Only" sign. Only personnel whose presence is required should be permitted in the room when toxins are in use. High-risk operations should be conducted with two knowledgeable persons present

7.2.3.9 Before containers are removed from the hood, BSC, or glove box, the exterior of the closed primary container should be decontaminated and placed in a clean secondary container. Toxins should be transported only in leak/spill-proof secondary containers.

7.2.3.10 Wear proper personal protective equipment when working with toxins, including gloves, eye protection, lab coats, long pants, and closed-toed shoes.

7.2.3.11 When vacuum lines are used with systems containing toxins, they must be protected with a HEPA filter to prevent entry of toxin into the lines.

7.2.3.12 Solid or liquid waste contaminated with toxin is hazardous waste. Materials contaminated with toxins as well as infectious or viable agents are considered combination waste – see Section 9.3 for information on combination wastes.

7.3 Biological Safety Cabinets (BSCs)

The biological safety cabinet is the principal primary barrier used to provide containment of infectious splashes or aerosols generated in many microbiological procedures. BSCs are to be used in Biosafety Level 2 work when aerosol-generating procedures are performed, and/or when high concentrations or large volumes of infectious agents are used. Biological safety cabinets should be installed so that fluctuations of the room supply and exhaust air do not cause the BSC to operate outside appropriate operating parameters for containment. BSCs must be located away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment. It must be noted that the HEPA filtration used in BSCs, while effective in trapping particulates such as infectious agents, are not capable of capturing volatile chemicals or gases. Only BSCs that are properly rated for chemical use should be used when working with volatile chemicals. Also, ducting to the outside may be required depending upon the concentration and specific chemical(s) in use. Chemical-intensive work not involving infectious agents must always be conducted in a chemical fume hood.

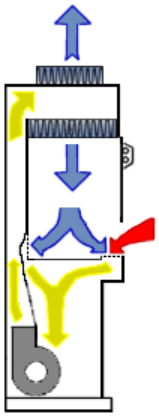
There are three classes of BSCs; each type provides personnel and environmental protection using HEPA filtration. The various BSC classes are described below, and are summarized and compared in Table 7.1.

Class I Biological Safety Cabinet:

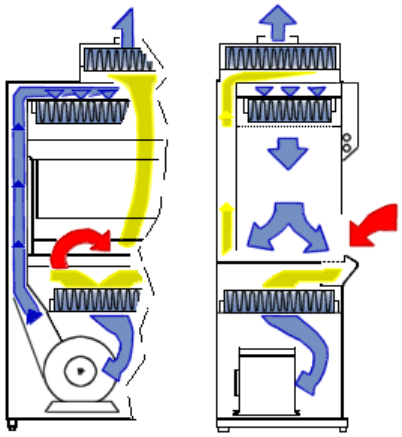
The Class I biological safety cabinet is a negative-pressure, ventilated cabinet operated with a minimum face velocity of at least 75 linear feet per minute. All of the air from the cabinet is exhausted through a HEPA filter either into the laboratory or to the outside. The Class I BSC is designed for general microbiological research and low- and moderate-risk agents. This BSC is not appropriate for handling materials that are vulnerable to airborne contamination, since the inward flow of unfiltered air from the laboratory can carry microbial contaminants into the cabinet. Class I BSCs are currently being manufactured in limited amounts; Class II BSCs are generally preferred, since they provide the research materials with protection from contamination.

Class II Biological Safety Cabinet:

The Class II Biological Safety Cabinet is the most commonly used, and is the type recommended for work with infectious materials at Rensselaer. These cabinets provide protection to personnel, the environment, and the research materials by utilizing three types of air flow – inward air flow at 75-100 linear feet per minute protects personnel, HEPA-filtered downward vertical laminar flow protects the product from outside contamination, and HEPA-filtered exhaust air prevents infectious particles from exiting the cabinet. Class II BSCs are further classified as listed below:



Class II A: Type A cabinets re-circulate air within the cabinet, making them suitable for microbiological research in the absence of volatile or toxic chemicals and radionuclides. Type A cabinets are usually exhausted into the laboratory, but may be exhausted to the outdoors via a thimble connection to the building exhaust system. An unducted Class II Type A cabinet cannot be used for work involving volatile or toxic chemicals, since the build-up of chemical vapors in the cabinet by re-circulated air and in the laboratory from exhaust air can create health and safety hazards.



Class II B: Type B cabinets are hard-ducted (thimble connection) to the building exhaust system. Face velocities in Class II B cabinets should be 100 linear feet per minute, which allows for work with volatile or toxic chemicals and radionuclides. Type B cabinets are further designated as either Type B1 or B2. **Type B1** cabinets maintain an average inflow velocity of 100ft/min (0.5 m/s) through the work access opening, have HEPA filtered down flow air, exhaust most of the contaminated down flow air through a dedicated duct exhausted to the atmosphere after passing through a HEPA filter and have all of the biologically contaminated ducts and plenums under negative pressure or surrounded by negative pressure ducts and plenums. **Type B2** (often referred to as "total exhaust") cabinets maintain an average inflow velocity of 100ft/min (0.5 m/s) through the work access opening, have HEPA filtered downward air drawn from the laboratory or outside air, exhaust all inflow and down flow air to the atmosphere after filtration through a HEPA filter without recirculation in the cabinet or return to the laboratory and have all contaminated ducts and plenums under negative pressure or surrounded by directly exhausted negative pressure ducts and plenums.

Class III Biological Safety Cabinet:

Class III biological safety cabinets are totally enclosed, gas-tight, ventilated cabinets. They are suitable for work that requires Biosafety Level 3 or 4 containment. **Work with agents requiring Biosafety Level 3 or 4 practices and facilities are not permitted at Rensselaer. See Appendix 4 for a listing of prohibited agents.**

Table 7.1 Summary of Biological Safety Cabinet Types:

Type	Face Velocity (lfpm)	Air flow pattern	Radionuclides, toxic chemicals	Biosafety Levels	Product Protection
Class I	75	In at front; rear and top through HEPA filter	No	2,3	No
Class II Type A	75	70% recirculated through HEPA; exhaust through HEPA	No	2,3	Yes
Class II Type B1	100	30% recirculated through HEPA; exhaust via HEPA and hard ducted	Yes (low levels/volatility)	2,3	Yes
Class II Type B2	100	No recirculation; total exhaust via HEPA and hard ducted	Yes	2,3	Yes
Class II Type B3	100	Same as Class IIA, but under negative pressure to room and exhaust air is ducted	Yes	2,3	Yes
Class III	N/A	Supply air inlets and exhaust through 2 HEPA filters	Yes	3,4	Yes

Source: Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, 1999 (CDC/NIH

Proper work practices for biological safety cabinet use include the following:

7.3.1 Turn the cabinet on for at least 10 minutes prior to use.
7.3.2 Before and after use, disinfect the work surface with a suitable disinfectant.
7.3.3 Place items in the cabinet so that they can be used efficiently without disruption of air flow. Keep clean and contaminated materials and equipment separate.
7.3.4 Do not use the cabinet for storage of unnecessary items.
7.3.5 Adjust the height of the stool so that the worker's face is above the front opening.
7.3.6 Minimize the frequency of moving hands in and out of the cabinet. Do not use a standing pipette jar placed outside the cabinet; place a tray containing a suitable disinfectant in the hood for contaminated pipettes.
7.3.7 Work at a moderate pace; rapid movements can cause air flow disruption.
7.3.8 Do not cover the grillwork or vents; doing so will disturb the air flow.
7.3.9 Do not tape waste bags or other materials to the sides of the cabinet.
7.3.10 Do not use an open flame in the BSC, since they disrupt air flow and produce filter-damaging heat. Flaming flasks is unnecessary in the cabinet's sterile environment. If items such as loops must be sterilized, use a small electric sterilization furnace or sterile single-use loops for BSC work.
7.3.11 Inform other personnel when the cabinet will be in use; limit unnecessary traffic past the cabinet when in use.

7.3.12 Some BSCs contain ultraviolet light settings. Never use the UV light when working in the BSC. Because UV light cannot penetrate clusters of cells, it should not be used as a substitute for chemical disinfection of the work surface.

7.3.13 BSCs should be positioned in the laboratory away from sources of airflow disruptions such as doorways and windows.

7.3.1.1 Certifications of Biosafety Cabinets

BSCs must be tested and certified *in situ:*

1. At the time of installation
2. Any time the BSC is moved
3. After filter changes or other maintenance activities
4. And at least annually thereafter.

BSC certification is the responsibility of the PI and is typically provided by an outside vendor. The Office of Environmental Health and Safety will assist in the coordination of Biosafety Cabinet testing and will provide a schedule to all affected PIs for annual testing. BSCs may need to be decontaminated using paraformaldehyde gas, or other appropriate method, before maintenance work requiring disassembly, such as filter replacement, is performed. Decontamination must also be performed before the cabinet is moved to another laboratory. Gas decontamination is provided by an outside vendor and must be scheduled through the Office of Environmental Health and Safety. For additional information regarding BSC certification and/or decontamination, please contact Will Fahey-Training and Program Development Specialist at extension 2318 or by electronic mail at faheyw@rpi.edu. **Biological Safety Cabinets that have not been tested and certified according to the schedule outlined above should not be used.**

7.4 Personal Protective Equipment

Personal protective equipment (PPE), such as gloves, safety glasses or goggles, lab coats and/or other protective clothing, and face shields play an important role in protecting personnel from biohazardous materials. The following PPE requirements apply to biohazardous areas:

7.4.1 It is Rensselaer policy that chemical splash goggles be worn whenever there is a potential splash hazard (chemical or biohazardous). Goggles and a face shield must be worn when infectious or other hazardous materials must be manipulated outside a biological safety cabinet and splashes or sprays are possible. Properly rated safety glasses with side shields must be worn at all times in laboratories at Rensselaer.



7.4.2 Gloves should be worn and changed as needed. Gloves should be worn if the skin on the hands is broken or a rash is present, and whenever it is likely that the hands will come into contact with biological and/or infectious material or contaminated surfaces or equipment. Gloves should be removed when overtly contaminated, when work is completed, and when the integrity of the glove is compromised. Care should be taken to insure that the gloves chosen for a specific application are suitable, properly fitting, and will provide adequate protection. In order to make an informed decision regarding the style and type of glove that will provide adequate protection for a given procedure, the following information must be taken into consideration:

1. Specific biological/chemical(s) material(s)to be used-and their properties
2. Degree of biological/chemical contact/concentration
3. Dynamics of the procedure i.e. Puncture hazards, thermal hazard etc.
4. Amount of hand and/or arm to be protected
5. Physical dexterity requirements of the procedure

Specific performance data, which is supplied by the glove's manufacturer, must be reviewed. Some common information that is important to note regarding specific gloves includes:

- Degradation: the rate at which the physical properties of the gloves change due to contact with a biological/chemical
- Breakthrough: a measure of the time it takes for a biological/chemical to degrade a glove to the point of pass through
- Permeation: the measure of a materials breakthrough on a molecular level

The Office of Environmental Health and Safety should be used as a resource when selecting gloves.

7.4.3 Lab coats are <u>required</u> for Biosafety Level 2 work. Laboratory coats with rear closures are preferable. Soiled lab coats must not be taken out of the building for any purpose, including laundering, by laboratory personnel. Contaminated Laundry should be disposed of as Regulated Medical Waste or a Laundry service must be arranged with a contractor specializing in laboratory coat laundry.

7.4.4 Non-disposable PPE must be promptly removed and decontaminated with an appropriate disinfectant or disposed of as Regulated Medical Waste. Contaminated disposable PPE should be disposed of as Regulated Medical Waste.

7.4.5 PPE must not be worn in non-laboratory areas such as offices and cafeterias.

7.4.6 Gloves must be removed and hands must be washed before answering the phone or handling doorknobs or other general use equipment such as computers. Disposable PPE such as gloves may not be washed or reused.

7.4.7 Chemical splash goggles, thermal resistant gloves, and a lab coat must be worn when cultures are accessed from or replaced into liquid nitrogen storage.

7.4.8 Chemical splash goggles, thermal resistant gloves, and a lab coat must be worn when inserting or removing items from the autoclave or benchtop sterilizers.

7.5 Decontamination and Disinfection

To ensure that acceptable levels of microbial loads are achieved/maintained on equipment, media, and other items used in microbiological research, various types of decontamination and disinfection are available. The process utilized and associated materials are dependent upon the biological agent(s) and equipment within the laboratory. The following terminology must be understood when evaluating proper procedures:

Decontamination: The destruction or removal of microorganisms to some lower level, not necessarily zero.

Disinfection (Liquid Sanitization): The process of reduction in the number of microorganisms in or on an inanimate matrix, by the action of an agent on their structure or metabolism to a level judged to be appropriate for a defined purpose.

Cleaning: Removal of contamination by physical means from a surface to render it visibly clean. Wiping should be towards you, in straight horizontal lines, each time overlapping the previous by 10%-25%. Wiping should be from top to bottom, from back to front, and from cleanest to dirtiest.

Sanitization: Reduction of microbial load on an inanimate surface to an acceptable level.

Sterilization: Complete destruction of all living or viable organisms, with a probability of one in a million that even one organism survives the process.

The following several pages outline techniques and materials designed to ensure that acceptable levels of microbial loads are achieved/maintained on equipment, media, and other items used in microbiological research. The Office of Environmental Health and Safety should be consulted for additional information.

7.5.1 Autoclaving and other steam sterilization

Autoclaves use pressurized steam to sterilize and/or decontaminate materials. The heat from a steam process is designed to cause significant damage to the cellular metabolic functions, causing death to the cells/molecule. Laboratory-grade "pressure cookers" perform the same function on a smaller scale and are referred to as benchtop sterilizers. This form of decontamination is used during sterile media or reagent preparation and instrument and other equipment sterilization. Sterilization is defined as a 10^{-6} reduction in the number of specific microorganisms being considered. Autoclaving may also be performed on solid and liquid wastes to decrease the biological load before waste pick-up.

Safe work practices when using autoclaves or benchtop sterilizers include the following:

1. The machine becomes hot when running; do not touch the sides or door/cap with bare hands.
2. When placing or removing items into the sterilizer, safety goggles, a lab coat, and thermal resistant gloves must be worn. Extra care must be taken when removing hot items from the sterilizer.
3. Items to be autoclaved must be placed in heat-resistant plastic or metal tubs before being placed into the autoclave.
4. Do not overload the tub – items should not be touching to allow for proper heat transfer and the tub must be light enough to be safely lifted into the autoclave.
5. Do not carry tubs of materials to and from the autoclave room; use a cart for transport.
6. To prevent pressure buildup within screw-capped containers, do not cap tightly before sterilizing.
7. Safety features do not permit the opening of the autoclave before the entire run (including a cool-down period) has been completed. Do not override this safety feature by shutting down the autoclave.
8. Stand to the side of the sterilizer when opening after running; steam may escape when the door/cap is opened.
9. Sterilizers must not be operated by untrained individuals.
10. Autoclave tape or another indicator must be used with every load to ensure that proper temperature and time was achieved.
11. Autoclaves should be tested monthly using *Bacillus sterothermophilus* spore strips to ensure proper function.
12. If the sterilizer is in need of service or repair, place a sign on it indicating that is it not to be used.

The processing time to effectively decontaminate laboratory wastes is at least 60 minutes (unless a shorter interval has proven effective when tested with biological indicators). A minimum of at least 90 minutes is recommended for decontaminating waste in low sided polypropylene containers with bags half filled and loosely gathered. If bags are tightly closed, a processing time of 120 minutes is recommended.

A minimum decontamination processing time of 60 minutes is recommended for materials in metal pans with the lid removed. The United States Environmental Protection Agency (EPA) has reported that; "Infectious Wastes from departments of health care facilities may be rendered non-infectious by subjecting the waste to autoclave temperatures of 121C(250F) and 15 minutes of pre-vacuum of 15 psi for the following dwell times when proper containers are used".

Item	Dwell Time
Trash	60 Minutes
Glassware	60 Minutes
Liquids	60 Minutes/gallon
Animal Carcasses*	**Do Not Autoclave***
Animal Bedding	120 minutes

Questions related to specific materials and/or dwell times should be directed to the *Office of Environmental Health and Safety*.

* Note-At the current time, live animal research is prohibited at Rensselaer

7.5.2 Flame Sterilization

Use of the open flame of a Bunsen or alcohol burner is a common technique for work bench sterilization of items such as loops and the lips of flasks containing sterile media or cultures. Flame sterilization can be avoided by using sterile, disposal plastic loops and other equipment. This practice is recommended instead of flame sterilization.

The following safe work practices apply to use of an open flame:

1. Keep the burner lit only when necessary.
2. When using a Bunsen burner, turn on the gas immediately before lighting the burner. Do not allow the gas to flow without being lit.
3. Do not hang papers or other flammable materials above the work bench where open flames are used. Do not store flammable materials, such as paper towels and flammable liquids, near the burner.
4. Disinfect the work surface and allow to dry before lighting the burner - do not use alcohol or other flammable disinfectants near an open flame.
5. Keep long hair tied back when using an open flame.
6. Do not wear flowing clothing, especially long, wide sleeves, when working with an open flame.
7. Stand away from the flame at all times. Do not place the head or arms directly above the flame.
8. When finished with a Bunsen burner, ensure that the gas is completely off before exiting the room.
9. Use of an open flame in a biological safety cabinet is unnecessary and prohibited.
10. Consider the use of sterile disposable loops and other tools, or a benchtop electric sterilization furnace, instead of using an open flame for sterilization.

7.5.3 Chemical Disinfection (Liquid)

7.5.3.1 Choosing the right disinfectant

Chemical disinfection is used to sterilize/decontaminate work surfaces, equipment, and other items that cannot be steam sterilized. There are variety of materials used to disinfect; Table 7.1 summarizes their properties and uses. When choosing a disinfectant, the following factors should be considered:

1. The nature of the biological agent
2. The type of surface to be disinfected
3. The amount of contact time required to inactivate the agent when using the selected agent.
4. The volume of disinfectant that will be required to inactivate the agent.
5. The toxicity of the chemical disinfectant.
6. Whether the disinfectant will chemically react with the materials to be disinfected.

7.5.3(a) Chemical Disinfection (Gas)

Occasionally, such as in the case of a decontamination project involving a Biosafety cabinet prior to the movement of the BSC, gases may be used for chemical disinfection or sterilization. Gases commonly used for decontamination include, Ethylene Oxide, Hydrogen Peroxide, Chlorine Dioxide and Formaldehyde. No process involving gas disinfection is allowed at Rensselaer without the written approval of the *Office of Environmental Health and Safety*.

Table 7.1 Summary and Comparison of Liquid Disinfectants

Disinfectant Type	Recommended Use	Deactivation Process	Advantages	Disadvantages	Comments & Hazards	Examples
70% Isopropyl Alcohol solution	Cleaning instruments, surfaces	Changes protein structure of microorganism;	Fairly inexpensive	Not active when organic matter is present, not active against certain types of viruses, evaporates quickly	Flammable Eye Irritant Toxic	N/A
Bleach (Sodium hypochlorite)	Cleaning instruments, surfaces; disinfectant of choice for blood and other human-derived material spills	Oxidizes the cell membrane	Inexpensive; effective against a broad range of organisms	Not active when organic matter is present, activity degrades over time (solutions must be made fresh; solutions will last longer in an opaque bottle), needs at least 20 minutes to deactivate microorganisms; disinfections should be followed by a rinse with sterile distilled water to protect equipment	Corrosive Eye Irritant Toxic	Clorox
Glutaraldehyde	Bactericidal - Good Fungicidal - Good Tuberculocidal - Excellent Virucidal - Good Sporicidal - Good	Coagulates cellular proteins	Non-staining, relatively noncorrosive; useable on plastics, rubber, lenses, stainless steel and other items that can't be autoclaved	Not stable in solution , has to be in an alkaline solution; inactivated by organic material	Eye, skin and respiratory irritant Sensitizer Toxic	Calgocide 14 Cidex Vespore
Iodophors (Iodine with carrier)	Disinfecting some semicritical medical equipment; used in the food industry Bactericidal - Very Good Fungicidal - Excellent Virucidal – Excellent Sporicidal - Some	Binds with cellular components	Kills a broad range of organisms; highly reactive; kills immediately; not affected by hard water; may be used on food prep surfaces; tuberculocidal, with extended contact time	may stain plastics or corrode metal ; may stain skin/laundry; odor; some organic and inorganic substances neutralize effect	use only EPA registered hard surface iodophor disinfectants; Skin and eye irritant Corrosive Toxic	Bactergent Hy-Sine Ioprep Providone Wescodyne
Phenolic Compounds	Bactericidal - Excellent Fungicidal - Excellent Tuberculodial - Excellent Virucidal - Excellent	Disrupts cell walls, precipitates cell proteins; low concentrations inactivate essential enzyme systems	Nonspecific bactericidal and fungicidal action	Unpleasant odor; some areas have disposal restrictions; effectiveness reduced by alkaline pH, natural soap or organic material, not sporicidal	Skin and eye irritant Sensitizer Corrosive Toxic	Hil-Phene Lph Metar Vesphene
Quaternary Ammonium compounds (QUATS)	Ordinary housekeeping (e.g. floors, furniture, walls) Bactericidal - Excellent Fungicidal - Good Virucidal - Good	Affects proteins and cell membrane of microorganism	Contains a detergent to help loosen soil , rapid action; colorless, odorless, non-toxic, less corrosive, highly stable, may be used on food prep surfaces	Does not eliminate spores, TB bacteria, some viruses; ffectiveness influenced by hard water; soap interferes with action	Select from EPA list of hospital disinfectants Skin and eye irritant Toxic	Coverage 258 End-Bac Hi Tor

7.5.3 Disinfectant Use

When using a chemical disinfectant it must be noted that the disinfectant is a potentially toxic chemical that could be corrosive, flammable or carcinogenic. The following safe work practices apply to disinfectant use:

7.5.3.1 Consult the MSDS before use and wear proper PPE.
7.5.3.2 Use a chemical fume hood when diluting or pouring disinfectants.
7.5.3.3 Allow sufficient contact time after applying the disinfectant to ensure inactivation of the biological agent or contamination.
7.5.3.4 Avoid using concentrated or undiluted disinfectants to prevent excessive exposure and damage to the items being disinfected.
7.5.3.5 Label all disinfectant bottles with their name and concentration.
7.5.3.6 Paper towels and other cleaning materials used with disinfectants to sterilize work surfaces become hazardous waste – do not dispose in the regular trash. Use a labeled hazardous waste jar to dispose of disinfectant-soaked materials.
7.5.3.7 The EPA defines antimicrobials as pesticides. Be sure to follow all label requirements and instructions when using these products.

7.6 Tissue Culture, including Cell Culture

An inherent risk of tissue culture is that the growth media or cell lines may be infected with latent viruses or other agents. Because of this risk, cultures must be handled at the containment level of likely contaminating agents. To avoid unnecessary risk of exposure to infectious agents, researchers should use only well-characterized, certified cell lines and guaranteed contamination-free media. The safe work practices addressed in Sections 7.1 to 7.5 of this document also apply to tissue culture. Additional work practices are listed below:

7.6.1 All tissue culture work should be performed in a BSC.
7.6.2 The following tissue cultures **must be handled at Biosafety Level 2**:
7.6.2.1.1 All cell lines of human origin
7.6.2.1.2 All cell lines of primate origin
7.6.2.1.3 Any cell lines derived from lymphoid or tumor tissue
7.6.2.1.4 All cell lines exposed to or transformed by any oncogenic virus
7.6.2.1.5 All cell lines new to the laboratory, until proven free of latent infectious agents
7.6.2.1.6 All mycoplasma-containing cell lines

7.6.3 When vacuum lines are used, they must be protected with a HEPA filter to prevent contamination of the lines and building services.

7.6.4 Tissue culture waste is biohazardous, and is regulated medical waste if any components are human-derived. Consult Section 9 of this document for waste disposal procedures.

7.7 Use of Human-derived Materials

Due to the potential hazards (exposure to bloodborne pathogens, self-inoculation with tumorigenic cells) of working with human-derived materials, such as blood and other body fluids, cell, and tissues, all work involving such materials must be performed using Biosafety Level 2 practices and facilities. All laboratory personnel must participate in **Bloodborne Pathogen** training, including annual refreshers, and work under the policies and guidelines established in Rensselaer's *Bloodborne Pathogens Exposure Control Plan*. Associated Hepatitis-B vaccines, as required by the Occupational Safety and Health Administration's (OSHA) Bloodborne Pathogens standard (29 CFR 1910.1030) will be offered to employees with occupational exposure and will be the financial responsibility of the Principal Investigator.

In New York State, human-derived material waste is considered regulated medical waste, and must be treated and disposed as such. See Section 9 of this document for more information on waste handling and disposal.

NOTE: Human-derived materials for research must be obtained from reputable suppliers. Under no circumstances should anyone at Rensselaer manipulate cells or other materials derived from themselves, family members, or members of the Rensselaer community.

7.8 Recombinant DNA Research

7.8.1 Applicability of the *NIH Guidelines for Research Involving Recombinant DNA Molecules*

The major reference document for conducting rDNA research safety is the *NIH Guidelines for Research Involving Recombinant DNA Molecules*. These guidelines were written to specify policies and procedures for the safe conduct of experiments involving rDNA. The NIH guidelines outline the six categories of rDNA experiments and the approvals required before beginning such research, as well as safety information on containment, certified host-vector systems, and transgenic plant and animal research. Researchers conducting rDNA work at institutions receiving funding from the NIH for rDNA work, even if the funding does not impact the individual's specific project, must follow all applicable NIH Guidelines. Failure to follow the guidelines can result in loss of NIH funding for the Institute and suspension of research. **It is the policy of Rensselaer to adhere to the requirements and suggestions contained within the *NIH Guidelines for Research Involving Recombinant DNA Molecules* document as a prudent practice, regardless of the funding group/agency.**

The six categories of rDNA experiments covered by the NIH guidelines are listed below:

1. **Experiments that require IBC approval, the NIH Recombinant DNA Advisory committee (RAC) and the NIH Director before initiation**
    a. The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if such acquisition may compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture.

2. **Experiments that require NIH Office of Biotechnology Activities and IBC approval before initiation**
    a. The cloning of toxin molecules with an LD_{50} of less than 100 nanograms per kilogram of body weight.

3. **Experiments that require IBC, Institutional Review Board, and RAC review before research participant enrollment**
    a. Deliberate transfer of rDNA or DNA or RNA derived from rDNA into one or more human research participants.

NOTE: Due to facility design, research involving human subjects is not permitted at Rensselaer.

4. **Experiments that require IBC approval before initiation**
    a. Use of Risk Group 2, 3, or 4, or restricted agents (such as exotic plant or animal pathogens), as host-vector systems.
    b. Cloning Risk Group 2, 3, or 4, or restricted agent, DNA into nonpathogenic prokaryotic or lower eukaryotic host-vector systems
    c. Use if infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper viruses in tissue culture systems.
    d. Experimentation on whole animals
    e. Experimentation on whole plants involving the use of pathogenic plant microorganisms/insects, or recombinant plants with potentially hazardous properties
    f. Use of more than 10 liters of culture (large-scale experiments)

Work with agents classified as Risk Group 3 or 4 is not permitted at Rensselaer

5. **Experiments that require IBC notice simultaneous with initiation**
    a. Experiments not conducted in categories 1-4 or 6 are considered in category 5. These experiments may be conducted in Biosafety Level 1 containment. (Example: all components derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes).
    b. Formation of rDNA molecules containing no more that two-thirds of the genome of any eukaryotic virus
    c. Experimentation on whole plants involving the use of non-pathogenic plant microorganisms, or recombinant plants with non-hazardous properties.
    d. Experimentation on transgenic rodents

6. **Exempt Experiments: The following rDNA molecules are exempt from the NIH guidelines. The guidelines should be followed as a best practice and experiments must be registered with the IBC.**
    a. rDNA not in organisms or viruses
    b. rDNA that consists entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.
    c. rDNA that consists entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host or a closely related strain of the same species, or then transferred to another host by well established physiological means.
    d. rDNA that consists entirely of DNA from a eukaryotic host including its chloroplasts, mitochondria or plasmids (but excluding viruses) when propagated only in that host or a closely related strain of the same species.

e. rDNA that consists entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. The current listing of such exchangers follows; rDNA work **within** each sublist is exempt from the NIH Guidelines.
   i. Genus *Escherichia*, Genus *Shigella*, Genus *Salmonella*, including *arizona*, Genus *Enterobacter*, Genus *Citrobacter*, including *levinea*, Genus *Klebsiella*, including *oxytoca*, Genus *Erwinia*, *Pseudomonas putida, Pseudomonas fluorescens, Pseudomonas mendocina, Serratia marcescens, Yersinia enterocolitica*
   ii. *Bacillus subtilis, bacillus licheniformis, Bacillus pumilus, Bacillus globigii, Bacillus niger, Bacillus nato, Bacillus amyloliquefaciens, Bacillus aterrimus*
   iii. *Streptomyces aureofaciens, Streptomyces rimosus, Streptomyces coelicolor*
   iv. *Streptomyces griseus, Streptomyces cyaneus, Streptomyces venezuelae*
   v. One-way transfer of *Streptococcus mutans* or *Streptococcus lactis* DNA into *Streptococcus sanguis*
   vi. *Streptococcus sanguis, Streptococcus mutans, streptococcus pneumoniae, Streptococcus faecalis, Streptococcus pyrogenes*

f. The following experiments, as long as they are:
   1) NOT otherwise included in Category 1 or 2 as listed in Section 7.8.1 of this document
   2) NOT involving DNA from Risk Groups 3 or 4 or restricted organisms or cells known to be infected with these agents
   3) NOT involving the deliberate introduction of genes coding for the biosynthesis of molecules that are toxic to vertebrates
   4) AND NOT involving whole plants regenerated from plant cells and tissue cultures are not raised in sterile conditions and may be contaminated by or associated with any other living organisms.
      i. RDNA molecules containing less than one-half of any eukaryotic viral genome being considered identical that are propagated and maintained in cells in tissue culture.
      ii. *Escherichia coli* K-12 Host-Vector Systems if the host does not contain conjugation proficient plasmids or generalized transducing phages **or** if lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids are used as the vectors (experiments involving the insertion of prokaryotic DNA from prokaryotes that exchange genetic information with *E. coli*

iii. may be performed with any *E. coli* K-12 vector, including conjugative plasmids).
iv. *Saccharomyces* Host-Vector Systems
v. *Bacillus subtilis* or *Bacillus licheniformis* Host-Vector Systems
vi. Extrachromosomal elements of Gram-positive Organisms
    1. See the NIH Guidelines, Appendix C-V, for a list of exempt organisms.
vii. The purchase or transfer of transgenic rodents for experiments that require Biosafety Level 1 containment.

7.8.2 Practices and Procedures

7.8.2.1 Registration and Approval

The PI must complete a *Biological Research Registration and Approval Form*, including the recombinant DNA section, (see Appendix 1) and forward it to the IBC, care of the Office of Environmental Health and Safety. The IBC will review the proposal (and forward to the NIH as necessary) for both exempt and nonexempt rDNA work. Items to be considered include the agents to be used, the potential hazards to personnel and the environment, available containment level, host-vector systems, scale of experiment, required permits (for work involving plant or animal pathogens, for example), etc. **Work falling into categories 1-4 as listed in Section 7.8.1 cannot be initiated until all approvals have been granted.**

Work with agents classified as Risk Group 3 or 4 is not permitted at Rensselaer

7.8.2.2 Work Practices

The work practice requirements listed in Sections 7.1 through 7.7 of this document apply to rDNA research, in addition to applicable requirements listed in the NIH Guidelines.

Only certified host-vector systems may be used at Rensselaer. Use of novel or other non-certified systems must be approved by the NIH director. A list of all currently certified host-vector systems is available from the Office of Biotechnology Activities, NIH.

7.8.2.3 Biosafety Level and Risk Groups

While Biosafety Level (BMBL) and Risk Group (NIH Guidelines)

designations are similar, there are some discrepancies among the agent listings – generally, the BMBL listings are more conservative. If infectious materials are to be used in rDNA research, both the BMBL listings

(Appendix 3 of this document) and the listings in the NIH Guidelines should be consulted; the work should then be assigned to the more

conservative safety level. Final approval of safety level by the IBC will be part of the registration and approval process.

7.8.2.3 Biological Containment

An additional form of containment is possible when working with rDNA. Often, the use of a highly specific vehicle (a vector, plasmid, or bacteriophage) creates natural barriers, since the vehicle is infectious for a specific host only, and cannot disseminate and survive in the environment outside of an acceptable host. This extra form of containment, when combined with physical containment facilities and standard microbiological techniques, provides for an increased level of protection for laboratory personnel and the environment.

7.8.2.4 rDNA Research involving Plants

The NIH Guidelines describes four biosafety levels for research involving rDNA in plants. These containment levels limit the possibility of release or transfer of rDNA into the environment. The various levels are recommended depending upon the biological impact expected if the rDNA containing plant material was to be released into the environment. For detailed information on the plant biosafety levels and work practices, see Appendix P of the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (2001).

7.8.2.5 rDNA Research involving Animals

The NIH Guidelines describes four biosafety levels for research involving rDNA in animals. These containment levels limit the possibility of release or transfer of rDNA into the environment. The various levels are recommended depending upon the biological impact expected if the rDNA containing animals were to be released into the environment. For detailed information on the animal biosafety levels and work practices, see Appendix Q of the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (2001). The BMBL also designates Animal Biosafety

Levels and lists recommended work practices that may apply to rDNA research involving animals.*

* Note-At the current time, live animal research is prohibited at Rensselaer

7.8.2.6 Large-Scale Experiments

Experiments involving more than 10 liters of rDNA-containing culture requires specific containment procedures to be determined by the IBC on a case-by-case basis. Safety work practice requirements for large-scale research are listed in Appendix K of the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (2001).

7.9 Research Animal Care

There is currently no animal research being conducted at Rensselaer. If animal research is proposed in the future, animal care and employee safety requirements compliant with current regulations will be issued by the Office of Environmental Health and Safety, a Laboratory Animal Safety Committee will be formed, and laboratory personnel will be trained as required. No research involving animals may be conducted at Rensselaer without written approval from the IBC and the Office of Environmental Health and Safety.*

* Note-At the current time, live animal research is prohibited at Rensselaer

7.10 Authorized Visitors Policy

The following requirements are meant to limit access to Biosafety Level 2 facilities to provide security and eliminate exposure risks to non-laboratory personnel:

7.10.1 Access to biohazardous areas for routine cleaning, maintenance, and repairs should be limited to hours when regular employees are present.

7.10.2 Non-laboratory personnel must announce their presence and explain their need to enter the facility before entering.

7.10.3 Non-laboratory personnel may not enter if Biosafety Level 2 work is being performed – researchers must stop work and decontaminate the work area before access is given.

7.10.4 Non-laboratory personnel may not touch any equipment or materials other than those required by job duties, must wear proper personal protective equipment, and must wash their hands upon leaving the laboratory.

NOTE: Laboratory employees may deposit non-hazardous trash outside the laboratory to eliminate interruptions by environmental services staff. Trash bags must not block access to exits or safety equipment and may not be placed where they may become trip hazard.

Section 8: Emergency Procedures

8.1 Spills

Rensselaer's *Hazardous Materials Contingency Plan* applies to spills involving biohazardous materials. A general summary of the plan as it applies to biohazardous materials follows; see Rensselaer's *Hazardous Materials Contingency Plan* for more detailed information.

8.1.1 Rensselaer's Hazardous Materials Contingency Plan: applications to biohazardous materials

Due to Rensselaer's policy regarding the degree of hazard permitted for biological research (i.e. Biosafety Levels 1 and 2 only), and the expected amounts of biohazardous material storage and use in laboratories, spills involving biohazardous materials are expected to be primarily incidental in nature. Incidental spills involve small quantities of material – the spill should be able to be covered by a few paper towels. Incidental releases of material may be cleaned up by the person who generated the spill if the individual is trained in the hazards and cleaning procedures of the spilled material, and certain that there is no level of personal danger to themselves or anyone else. Additionally, adequate PPE and spill response supplies (disinfectant, paper towels, autoclave bags for waste disposal, forceps or tongs for picking up contaminated glass) must be available.

If a spill is determined to be more serious, such as one that involves large quantities of biohazardous materials, contamination of ventilation equipment, chemicals, or a release into the environment, the spill may require emergency response. Personnel must call Public Safety at x6611 and activate the buildings evacuation alarm if evacuation of the building is deemed appropriate. The room or area where the spill occurred must not be entered within 30 minutes of the spill to allow for the settling of aerosols. After 30 minutes, the area may be entered only by designated clean-up personnel wearing proper PPE. <u>If you are unclear as to the severity of a particular incident, error on the side of conservancy.</u>

8.1.2 Incidental Spill Clean-up Procedures

The following procedures must be followed in case of an incidental spill:

1. Wearing gloves, a lab coat, and splash goggles, cover the spill with paper towels and gently apply disinfectant, proceeding from the outer edge of the spill to its center. Leave in place for a minimum of 20 minutes.
2. Pick up the towels and discard into a biohazard container.

3. Pick up any pieces of glass with forceps or tongs and place in a sharps container which is puncture resistant and closable. Never pick up sharps with your hands, even if you wear gloves.
4. Re-wipe the spill area with appropriate disinfectant. Wiping should be towards you, in straight horizontal lines, each time overlapping the previous by 10%-25%. Wiping should be from top to bottom, from back to front, and from cleanest to dirtiest. Dispose of paper towels in a biohazard container.
5. Wipe forceps or tongs with appropriate disinfectant if used. Dispose of paper towels in a biohazard container.
6. If lab coat or goggles become contaminated, remove. Place the lab coat in the laundry area or place in a biohazard bag for disposal and decontaminate the goggles.
7. Remove gloves and place in a biohazard container.
8. Wash hands thoroughly.
9. If the spill occurs in a biological safety cabinet, leave the blowers turned on during clean-up. Wipe down the entire cabinet, including walls, and any equipment contained in the cabinet at the time of the spill.
10. The consequences of a work area spill may be minimized by the use of plastic-backed paper liner for the benchtop. If used within a biological safety cabinet, ensure that the liner does not block the vents or grills. If a spill occurs, allow the material to soak into the liner, carefully fold the liner over the spill, and dispose in a biohazard container. Wipe down the work area with a disinfectant and allow to dry before installing a new liner.

8.2 Personnel Exposure Incidents

In the event of a specific, potential exposure incident to a biohazardous material, personnel should follow the guidelines listed below. Employee exposures or injuries of any kind must be documented using an *Occupational Injury/Illness Report*, available from departmental offices or the Office of Environmental Health and Safety. If an unaffected person is helping an exposed employee, PPE should be worn to prevent the spread of contamination. If emergency medical care is required, call Public Safety at x6611.

8.2.1 Exposure to intact skin:

- Vigorously wash contaminated skin with non-abrasive soap and water for at least 1 minute.

PREVENTION: wear gloves, long pants, closed-toed shoes, lab coats and other personal protective equipment to protect bare skin from exposures.

8.2.2 Exposure to clothing:

- Remove contaminated clothing and place in a biohazard bag. If applicable, consult the laboratory coat laundry contractor; it may agree to launder the contaminated clothing. If not, the clothing must be disposed of as biohazardous waste. Contaminated clothing must not be taken out of the laboratory for laundry by the laboratory personnel.

PREVENTION: Wear laboratory coats over street clothes.

8.2.3 Exposure to broken, cut, or damaged skin and/or puncture wounds:

- Vigorously wash contaminated skin with non-abrasive soap and water for at minimum of 5 minutes
- Contact Public Safety at x6611; immediately and seek medical attention.

PREVENTION: Wear gloves and other PPE to protect broken, cut, or damaged skin. Exercise extreme caution when handling sharps to prevent cuts and puncture wounds.

8.2.4 Exposure to the eyes

- Immediately flush eyes for at least 15 minutes with water at the closest eye wash station. Hold the eyelids away from the eyeball and rotate the eyes so that all surfaces are washed. Rinse from the nose outward to avoid contamination of the unaffected eye, if applicable.
- seek medical attention.

PREVENTION: Wear splash goggles or safety glasses at all times to protect the eyes.

8.2.4 Ingestion or inhalation

- Contact Public Safety at x6611; immediately and seek medical attention.
- Do not induce vomiting unless advised to do so by a health provider.

PREVENTION: Do not eat, drink, smoke, apply cosmetics, or store food in the laboratory. Perform all work where there is a potential for aerosol or splash production in a biological safety cabinet.

Section 9: Waste Disposal Procedures

9.1 Non-infectious Biological Waste

Solid waste generated during Biosafety Level 1 work (i.e. work involving non-infectious organisms) must be placed in a clear autoclave bag. Bags must be taped shut when ¾ full; do not overfill. Pick-up of full bags must be arranged by faxing a "Request for Hazardous Waste Pick Up" form to the *Office of Environmental Health and Safety at x2512*. (A copy of this form is included in Appendix 7) Non-infectious solid waste may be autoclaved before pick-up to reduce the biological load if desired. However, Non-infectious solid waste must be managed through the Rensselaer's Hazardous Waste program, even if it has been autoclaved. Do not dispose of this material in the municipal waste stream. Liquid non-infectious waste may be autoclaved (at 121°C for 30 minutes) or, if under 1 liter a day is generated, may be decontaminated with bleach (using 1 part bleach to 9 parts liquid waste; allow to sit for at least 20 minutes). After decontamination, liquid waste may be drain disposed if no hazardous materials were used in the media preparation or experimentation. Consult the Office of Environmental Health and Safety to ensure that media components are not listed as hazardous materials before performing drain disposal. Alternatively, liquid non-infectious waste may be contained in a closed container, placed in a clear autoclave bag, and pick-up arranged.

9.2 Regulated Medical Waste

Regulated medical waste is defined by New York State as waste capable of transmitting disease to humans, including that which was generated in the diagnosis, treatment, or immunization of human beings or animals, in research pertaining thereto, or in production and testing of biologicals. Regulated medical waste categories include:

Sharps

- Discarded used or unused needles, hypodermic needles, and complete syringes, even if not exposed to any infectious agents
- Pasteur pipettes, scalpel blades and razor blades in contact with infectious agents
- Broken glass, broken plastic Petri dishes, rigid plastic culture tubes, flasks, beakers, and other labware in contact with infectious agents
- Blood vials used in animal or human patient care, medical research, and clinical laboratories
- Broken or unbroken glass slides and their covers that have been in contact with infectious agents

NOTE: Glass and plastic material (other than syringes) that have not come in contact with infectious materials do not have to be disposed as sharps, but must be contained in a rigid-sided container. These containers do not have to be red in color or labeled as a biohazard container.

Cultures and Stocks

- Agents infectious to humans (those that require Biosafety Level 2 or higher containment), including cultures and stocks from research laboratories, and their associated biologicals (including serums, vaccines, antigens, antitoxins, cell lines, and cultures)
- Wastes from the production of biologicals
- Discarded live or attenuated vaccines and biological toxins
- Systems used to grow and maintain infectious agents *in vitro*, including but not limited to nutrient agars, gels, and broths
- Culture dishes and devices used to transfer, inoculate or mix cultures, including but not limited to plastic or glass plates, filters, stoppers, plugs, flasks and other containers, Inoculation loops and wires, contaminated pipette tips, etc.
- All human, primate, and any other mammalian cell lines, even in the absence of overt contamination.
- Materials used in the cleanup of spills of any of the above items

Human Blood, Blood Products, and Human Pathological Wastes

- Discarded waste blood and/or blood components such as serum or plasma
- Containers and/or materials containing free-flowing blood or blood components
- Materials saturated with blood or blood components
- Tissue, organs, body parts, body fluids removed during autopsy or other medical procedure
- Specimens of body fluids, their containers, and discarded material saturated with such fluids (other than urine).

NOTE: Bandages, gauze, and feminine hygiene products used to absorb menstrual flow are not considered regulated medical waste. Organs and tissues fixed for histological or cytological examinations are hazardous waste, since the fixatives used are hazardous materials.

Animal Wastes

- Carcasses, body parts, body fluids, blood, or bedding from animals known to be contaminated with infectious agents or from animals inoculated during research, production of biologicals, or pharmaceutical testing with infectious agents.

In accordance with New York State law, all regulated medical waste must be segregated and placed into properly labeled containers at the point of generation. Waste must be contained in a way that prevents its accidental release. Regulated medical waste must not be autoclaved or chemically treated and/or disposed of by Rensselaer employees – regulated medical waste must be transported to an authorized treatment facility for disposal. An exception to this requirement is combination wastes, which is addressed in section 9.3. Laboratory Workers should complete a "Request for Hazardous Waste Pick Up" 9Appendix 7) form and fax it to x2512 when waste is packaged and ready for pick-up.

Regulated medical waste should be segregated from other waste types and into the following subdivisions: sharps, liquid waste, and solid waste. Each regulated waste type must be contained in proper containers:

- Sharps: collect in approved, rigid, leak proof, puncture-resistant containers with a cap that can be secured to prevent loss of contents. Containers must be labeled with the universal biohazard sign or the word "Biohazard". Needles and syringe units must be discarded as a unit without clipping, bending, breaking, or re-sheathing.

- Liquid waste: liquids that contain infectious agents must be contained in closed containers and placed in red biohazard bags (doubled if necessary). Tape shut when filled.

- Solid waste: place in red biohazard bags. Tape shut when filled.

- Animal waste/human waste: place in red biohazard bags, tape shut, and refrigerate or freeze until pick-up.

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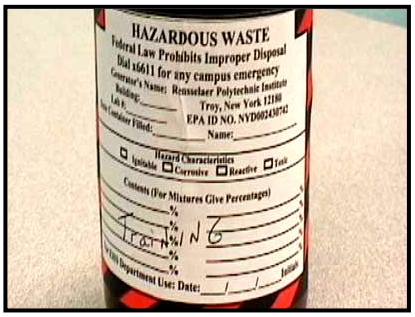
9.3 Combination Wastes

If biohazardous or non-infectious biological waste (either Regulated Medical waste or Biosafety Level 1 waste) also contains hazardous chemicals or radioactive components, the hazardous chemical/radiation constituents take precedence during disposal procedures. Proper procedures for disposal of combination wastes are as follows:

- During waste generation, ensure that a properly labeled container is used. All Hazardous Waste containers at Rensselaer must be labeled with the following Hazardous Waste label:



- When the container is close to full, the biological component must be decontaminated. Proper decontamination procedures will depend upon the composition of the waste – waste containing volatile, flammable, radioactive compounds, or compounds that form hazardous gases upon heating must not be autoclaved. In these cases, bleach or other disinfectant decontamination procedures should be followed. Check the Material Safety Data Sheet for information regarding the hazards of using heat treatment for the materials, and consult the *Office of Environmental Health and Safety* with any questions. If a disinfectant is used, be sure to add it to the hazardous waste label. All constituents on Hazardous Waste labels must be in words, not chemical formula.
- Utilizing Material Safety Data Sheets, ensure that compatibility is observed during the decontamination process.
- Now that the biological hazard has been eliminated, follow regular hazardous waste or radioactive waste procedures for disposal.

Section 10. Shipping/Receiving Procedures

Interstate shipment of etiologic agents is regulated by the U.S. Department of Transportation and the Department of Health. Packaging and shipping of biological materials must be done in a way that prevents leakage and ensures that the package arrives to its destination in good condition. Improper shipping of hazardous/biological/radioactive materials can jeopardize the Institute's regulatory compliance and result in civil charges against individuals. The Office of Environmental Health and Safety must be contacted to approve all hazardous/biological/radioactive materials shipments originating from Rensselaer.

10.1 Receiving

10.3.1 Packages containing etiological materials must be promptly unpacked and the materials stored as directed.

10.3.2 Remove the etiologic agents shipping label before reusing, recycling, or disposing of the outer shipping container.

10.3.2 If a package containing etiologic materials is received and evidence of leakage or any other damage is discovered, isolate the package and contact the Office of Environmental Health and Safety immediately.

Section 11. Training

11.1 Principal Investigator Training Responsibilities

11.1.1 The PI must ensure that all laboratory personnel are trained in:
1) Laboratory specific standard microbiological techniques
2) Laboratory specific protocols
3) The hazards of the agents and materials involved in the research.

11.1.2 The PI must ensure that all laboratory personnel are aware of and attend the training sessions offered by the Office of Environmental Health and Safety, listed below.

11.2 The Office of Environmental Health and Safety Training Responsibilities

The Office of Environmental Health and Safety provides the training courses applicable to laboratory work listed below. Courses mandatory for laboratory personnel are listed in **bold**.

11.2.1 **General Laboratory Safety, including Hazardous Communication**
11.2.2 **Hazardous Waste Management**
11.2.3 **Bloodborne Pathogen training (required for all personnel handling human-derived materials, including cell lines)**
11.2.4 Lockout/Tagout – control of hazardous energy
11.2.5 Radiation Safety
11.2.6 Driver Safety Awareness
11.2.7 Back Safety and Ergonomics
11.2.8 Respiratory Protection
11.2.9 Other courses as needed, upon request.

Employees of Rensselaer can access their individual Environmental Health and Safety course curriculum at www.rpi.traincaster.com

A current Environmental Health and Safety seminar can be viewed at http://www.rpi.edu/dept/hr/ehas/safety/schedule.html

For questions or additional scheduling information, please contact Will Fahey (Training and Program Development Specialist) at extension 2318 or by electronic mail at faheyw@rpi.edu.

Section 12. Terrorism Prevention

Due to the potential for illegitimate use of infectious agents, the **USA Patriot Act** of 2001 established provisions that regulate the possession, usage, and transfer of select agents

While possession of biological agents, toxin, or delivery systems is permitted when reasonably justified by a prophylactic, protective, bona fide research, or other peaceful purpose, the following restrictions apply:

1. Persons identified as "restricted" by the **USA Patriot Act** are not permitted to ship, transport, possess, or receive select agents.
2. Facilities receiving or transferring select agents must be registered with the Centers for Disease Control and Prevention.
3. Select agents must be kept secure – access to the select agent area must be controlled with card keys or other similar devices and laboratory doors, freezers, incubators, etc. must be locked.
4. Only authorized individuals may access select agent areas. Entries into the area must be recorded, including one-time or occasional entries for maintenance, repairs, etc.
5. Records must be kept of transfers, acquisitions, purchases, inventories, stocks, use, and destruction of select agents. An annual inventory of select agents must be conducted and documented.
6. Work must be conducted at the Biosafety Level recommended by the CDC/NIH Publication "Biosafety in Microbiological and Biomedical Laboratories".

Because of the above restrictions and the fact that all but one of the select agents (see Appendix A of 42 CFR 72 for the complete list) must be used and stored in Biosafety Level 3 or 4 facilities not available at Rensselaer, **research involving select agents is not permitted at Rensselaer.**

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APPENDIX 1

BIOLOGICAL RESEARCH REGISTRATION APPROVAL FORM

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APPROVED BIOLOGICAL RESEARCH REVISION FORM

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Institutional Biosafety Committee
Troy, NY

IBC Reg. NO.: _____ Biosafety Level: _____

Principal Investigator (PI): _____ Department: _____

phone: _____ e-mail: _____ office: _____

Alternate Contact Person: _____
Department: _____

phone: _____ e-mail: _____ office: _____

Laboratory Location(s): _____

_____

Project Title: _____

Date of submission: _____

Please check all that apply:

__ rDNA / RNA __ Pathogen __ Select Agents / Toxins __ Human cells / tissues
__ Viruses

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APPROVED BIOLOGICAL RESEARCH REVISION FORM

GENERAL INSTRUCTIONS: The intent of this form is to ensure compliance with the CDC/NIH guidelines for biosafety in research laboratories. In completing this form you must convey to the Institutional Biosafety Committee (IBC) that you: understand the potential hazards of the proposed research, have designed the experiments to minimize potential hazards, and have communicated potential hazards to others who may come in contact with the products you propose to use or generate.

In some cases, it is acceptable to combine multiple experiments or organisms onto the same registration form. If the form can be clearly understood when more than one organism/experiment is listed, then a PI may combine. Please contact the IBC if you have questions about this form.

SUBMITTING THIS FORM TO THE IBC: Submit the completed form to the IBC. The IBC will submit comments and questions to the PI for a response. The PI's response will be sent to the IBC for further review.

After all comments have been addressed, the protocol will be voted on at the next available IBC meeting. The IBC meets bi-monthly to review protocols. After the protocol has passed the IBC's vote, the PI must mail a signed hard copy to the IBC, which then will provide an approval letter for the PI.

Please mark which of the following sections you will be completing:

_____ **Part A: Recombinant DNA Experiments.** Select the category that best reflects the type of experiment that you are conducting. Indicate the possible adverse effects of the DNA, quantity of culture used, and a description of the experiment. Also, provide detailed information regarding the DNA inserts, vectors and host cells being used in your rDNA system.

_____ **Part B: Pathogenic Microorganisms.** Agents capable of causing disease in immune-normal, healthy adults must be registered in Part B. These agents include organisms classified as biosafety level 2 (BSL-2) or higher in the latest edition of the CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) publication. **Registration is required for BSL-2 organisms or higher.**

_____ **Part C: Human Blood, Human Cell Lines and Tissues or Other Potentially Infectious Materials (OPIM).** These items, including established human/primate cell lines must be listed in Part C. Human and primate cell lines obtained from commercial sources are also included in this requirement. OPIM is material with the potential for transmission of HIV, HBV, HCV, and other bloodborne diseases, including tissue from animals known to be infected with any of these agents, microbial stocks and cultures, certain body fluids, unfixed human tissue, primary tissue/cell cultures and must also be registered in Part B. These must be handled under BSL-2 conditions as if they were primary cells or tissues. For further information, please visit the CDC website:
http://www.cdc.gov/od/ohs/biosfty/bmbl4/b4ah.htm

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_____ **Part D: Possession, Use and Transfer of Select Agents, Toxins, High Consequence Livestock or Plant Pathogens.** The use of these agents, toxins or pathogens is regulated by the CDC Select Agent Regulation, 42 CFR 73, and the USDA Select Agent Rule 7 CFR 331/9 CFR 121. Facility Registration is required and is administered by the Centers for Disease Control, and/or the USDA. If you anticipate using these materials complete Part C of this form. Additional requirements of the "USA Patriot Act" and the "Public Health Security, Bioterrorism and Response Act of 2002" also must be satisfied.

_____ **Part E: Administration to animals of any of the above selections.** Administration of any of the above agents to animals requires approval of the IACUC and may also require that the animals be housed in specialty cages and handled under BSL-2 or BSL-3 conditions.

__XX__ **Part F: Safety Measures.** This section must be completed for all registrations.

__XX__ **Part G: Affirmation.** This section must be completed for all registrations.

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Part A: <u>RECOMBINANT DNA</u>

Please identify the type of experiment described in this registration form by checking the appropriate category in column (E).

(A) If your experiment involves:	(B) Registration w/NIH required?	(C) Registration w/IBC required?	(D) IBC must receive registration	(E) Experiment described on this form involves
Cloning of DNA encoding toxin molecules lethal to vertebrates at an LD_{50} of less than 100 ng/kg	Yes	Yes	Prior to initiation	☐
Human gene therapy	Yes	Yes	Prior to initiation	☐
Transfer of drug resistance to organisms not known to naturally acquire the trait, if such acquisition could compromise use of the drug to control disease in humans, veterinary medicine, or agriculture	Yes	Yes	Prior to initiation	☐
Risk Group 2, 3, or 4 agents as host-vector systems	No	Yes	Prior to initiation	☐
Cloning of DNA from risk group 2, 3, or 4 microorganisms into nonpathogenic prokaryotic or lower eukaryotic host-vector systems	No	Yes	Prior to initiation	☐
Use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems	No	Yes	Prior to initiation	☐
Production of transgenic animals at the UMDNJ Transgenic Core Facility	No	Yes	Prior to initiation	☐
Use of transgenic animals at BL-2, 3 or 4	No	Yes	Prior to initiation	☐
Use of viable rDNA-modified microorganisms involving whole animals or whole plants	No	Yes	Prior to initiation	☐
Administration of rDNA to animals	No	Yes	Prior to initiation	☐
More than 10 liters of culture	No	Yes	Prior to initiation	☐
Propagation and maintenance in tissue culture of r-DNA containing <2/3 of the genome of any eukaryotic virus in the **demonstrable** absence of helper virus, or of a virus that has been established to be non-replicating	No	Yes	At initiation	☐
Propagation and maintenance in tissue culture of r-DNA containing a virus that has been established to be non-replicating	No	Yes	At initiation	☐
Formation of rDNA containing no more than 2/3 the	No	Yes	At	☐

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genome of any eukaryotic virus			initiation	
Use of transgenic animals at BL-1	No	No	n/a	☐
rDNA that is not in an organism or virus	No	No	n/a	☐
DNA segments from a single non-chromosomal or viral DNA source	No	No	n/a	☐
DNA entirely from a prokaryotic host when propagated only in that host	No	No	n/a	☐
DNA entirely from a prokaryotic host when transferred to another host by well established physiological means	No	No	n/a	☐
DNA from a eukaryotic host when propagated only in that host or a closely related strain of the same species	No	No	n/a	☐
DNA segments from different species that exchange DNA by known physiological processes	No	No	n/a	☐

Please complete the following sections to describe your experiment.

1. Does the donor rDNA, RNA, cDNA source or its vector have any recognized or anticipated pathogenic, toxigenic or virulence potential for animals, plants or humans? **YES** ☐ **NO** ☐
   a. If yes, explain:

   a. If no, please provide a reference to support your conclusion:

2. Quantity of Material to be used:
   a. < 1 Liter ☐
   b. 1-10 Liter ☐
   c. > 10 Liters ☐
3. Location in which rDNA research is to be conducted (building and room #):

4. Specify source and nature of the DNA sequence(s) to be inserted (genus, species, gene name):

   a. Will the inserted gene(s) be expressed? **YES** ☐ **NO** ☐
   b. If yes, what are the gene product effects? Specifically identify its toxicity, physiological activity, allergenicity, oncogenic potential or ability to alter cell cycle:

5. Describe the virus, phage and/or plasmid used for constructing your recombinants (prokaryotic, eukaryotic):

6. If possible, provide a diagram or map illustrating the construct. If appropriate, include Entrez Gene nomenclature (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene).

7. Identify host cell(s) or packaging cell line in which recombinant vector will be amplified:

8. Is the vector replication competent? **YES** ☐ **NO** ☐
9. Are any viral component(s)/sequence(s) present? **YES** ☐ **NO** ☐
   a. If yes, specify the nature of the viral component(s):

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10. Does the insert contain >2/3 of a eukaryotic viral genome?	**YES** ☐	**NO** ☐
11. Is helper virus used?	**YES** ☐	**NO** ☐
a. Specify type:		
12. Is it a retrovirus?	**YES** ☐	**NO** ☐
12. What cells, tissues, animals, humans, insects or plants will be exposed to the recombinant? Indicate type of cell line and species:		
13. Will you work with transgenic animals?	**YES** ☐	**NO** ☐
14. Will human subjects be exposed to rDNA?	**YES** ☐	**NO** ☐

15. Brief description of proposed research (please include enough information to describe project's specific aims):

Part B - <u>PATHOGENIC MICROORGANISMS</u>

To be completed by the Principal Investigator for all laboratories handling or storing pathogenic microorganisms (agents capable of causing disease in immune-normal, healthy adults and includes organisms classified as requiring work at BSL-2 or higher in the latest edition of either the CDC/NIH publication, *Biosafety in Microbiological and Biomedical Laboratories* or the NIH's *Guidelines for Research Involving Recombinant DNA Molecules*.

1. Name of Organism (genus, species, strain description):		
a. Is organism attenuated?	**YES** ☐	**NO** ☐
2. Is a toxin produced?	**YES** ☐	**NO** ☐
a. Work with toxin?	**YES** ☐	**NO** ☐
3. Is drug resistance expressed?	**YES** ☐	**NO** ☐
A. If yes, indicate to which drugs:		
4. Where is organism stored? Bldg: _____ Room: _____		
a. Are Biohazard Warning Labels in use?	**YES** ☐	**NO** ☐
5. Building and room where organism is used? Bldg: _____ Room: _____		
6. Is a stock culture prepared?	**YES** ☐	**NO** ☐

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If yes, indicate:

a. Total volume of stock culture _____
b. Volume alliquoted per individual vial _____
c. Concentration /ml individual vial _____
d. Maximum volume used in an experiment _____

7. Is organism inactivated prior to use? **YES** ☐ **NO** ☐
   a. Specify Method: _____
8. Do you concentrate the organism in your protocol? **YES** ☐ **NO** ☐
   a. Specify method: centrifugation ☐
      precipitation ☐
      filtration ☐
      other: _____
9. Does the laboratory work with human blood or blood products, unfixed human tissue, or human or other primate cells? **If yes, fill out Part B below.** **YES** ☐ **NO** ☐
10. Are cultures, stocks, and contaminated items decontaminated prior to disposal? **YES** ☐ **NO** ☐
    a. Method:
       autoclave ☐
       chemical disinfectant ☐
       other
(specify):_____

Brief description of proposed research (please include enough information to describe project's specific aims):

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Part C - <u>HUMAN CELLS AND TISSUES</u>

Please list the cell lines and tissues that you will be using, attach additional sheets if needed. Include established human or primate ATCC cell lines and any other potentially infectious materials.

Note: Use of human cell lines or human source materials may require registration with the Institutional Review Board (IRB). Please contact the IRB to determine if your protocol will require review.

1.	2.	3.
4.	5.	6.
7.	8.	9.

<u>Brief description of proposed research (please include enough information to describe projects's specific aims):</u>

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Part D: POSSESSION, USE OR TRANSFER OF SELECT AGENTS, TOXINS, HIGH CONSEQUENCE LIVESTOCK PATHOGENS, AND PLANT PATHOGENS.

The University is required to register with the CDC or USDA for possession, use or transfer of any of these agents, toxins or pathogens. These agents are regulated by Select Agent Regulation, 42 CFR 73.0 and the Agricultural Bioterrorism Protection Act of 2002. If you anticipate obtaining these materials complete Part C of this form. Additional requirements of the *"USA Patriot Act"* and the *"Public Health Security, Bioterrorism and Response Act of 2002"* must also be satisfied.

Are, or will, any of the following agents, toxins or pathogens be used in your laboratory**:** ☐ **Yes** ☐ **No.**

(If "yes", please indicate which by marking the box next to the item with a check "√" or an "X".)

Viruses (HHS and USDA)	√	**Bacteria (HHS and USDA)**	√
Akabane virus		Bacillus anthracis	
African swine fever virus		*Brucella abortus*	
African horse sickness virus		*Brucella melitensis*	
Avian influenza virus (highly pathogenic)		*Brucella suis*	
Blue tongue virus (Exotic)		*Burkholderia mallei (formerlyPseudomona mallei)*	
Bovine spongiform encephalopathy agent		*Burkholderia pseudomallei*	
Camel pox virus		*Botulinum neurotoxin producing species Clostridium*	
Classical swine fever virus		*Cowdria ruminantium* (Heartwater)	
Crimean-Congo hemorrhagic fever virus		*Coxiella burnetti*	
Eastern Equine Encephalitis virus		*Francisella tularensis*	
Ebola viruses		*Mycoplasma capricolum*/ M.F38/*M. mycoides capri*	
Foot and mouth disease virus		*Mycoplasma mycoides mycoides*	
Goat pox virus		*Rickettsia prowazekii*	
Cercopithecine herpesvirus 1 (Herpes B virus)		*Rickettsia rickettsii*	
Japanese encephalitis virus		*Yersinia pestis*	
Lassa fever virus		**Fungi**	√
Lumpy skin disease virus		*Coccidioides immitis*	
Malignant catarrhal fever virus (Exotic)		*Coccidioides posadasii*	
Marburg virus		*Toxins (HHS and USDA)*	√
Menangle virus		Abrin	
Monkeypox virus		Botulinum neurotoxins	
Newcastle disease virus (VVND)		Conotoxins	
Nipah and Hendra Complex viruses		*Clostridium perfringens* epsilon toxin	
Peste Des Petits Ruminants virus		Diacetoxyscirpenol	
Rift Valley fever virus		Ricin	
Rinderpest virus		Saxitoxin	
Sheep pox virus		Shigatoxin	
South American Hemorrhagic fever viruses		Shiga-like ribosome inactivating proteins	
Junin		Staphylococcal enterotoxins	
Machupo		T-2 toxin	
Sabia		Tetrodotoxin	
Flexal		**USDA Plant Pathogens**	√
Guanarito		*Liberobacter africanus*	
Swine vesicular disease virus		*Liberobacter asiaticus*	
Tick-borne encephalitis complex (flavi) viruses		*Peronosclerospora philippinensis*	
Central European Tick-borne encephalitis		*Phakopsora pachyrhizi*	
Far Eastern tick-borne encephalitis		Plum Pox Potyvirus	
Russian Spring and Summer encephalitis		*Ralstonia solanacearum* race 3, biovar 2	
Kyasanur Forest disease		*Schlerophthora rayssiae* var *zeae*	
Omsk Hemorrhagic Fever		*Synchytrium endobioticum*	
Variola major virus (Smallpox virus)		*Xanthomonas oryzae*	
Variola minor virus (Alastrim)		*Xylella fastidiosa* (citrus variegated chlorosis strain)	
Venezuelan Equine Encephalitis virus			
Vesicular stomatitis virus (Exotic)			

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Genetic Elements, Recombinant Nucleic Acids, and Recombinant Organisms: * If your research involves rDNA, you must complete the rDNA section of this registration form. Contact EOHSS to obtain more information.	√
(1) Select agent viral nucleic acids (synthetic or naturally derived, contiguous or fragmented, in host chromosomes or in expression vectors) that can encode infectious and/or replication competent forms of any of the select agent viruses.	
(2) Nucleic acids (synthetic or naturally derived) that encode for the functional form(s) of any of the toxins listed in if the nucleic acids: (i) are in a vector or host chromosome; (ii) can be expressed in vivo or in vitro; or (iii) are in a vector or host chromosome and can be expressed in vivo or in vitro.	
(3) Viruses, bacteria, fungi, and toxins listed that have been genetically modified.	

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Part E: ANIMAL USE

Will biohazardous materials listed above be administered to animals? ***If YES, complete this section; if NO, go to Part E for non-animal work safety concerns.***	**YES**		**NO**	
1. What species will be exposed?				
2. State the Institutional Animal Care and Use Committee (IACUC) active or pending protocol number.				
3. State the max. volume and concentration to be administered per animal.				
4. State the max. volume and concentration to be administered per experiment.				
5. State the ***Animal*** BioSafety Level (ABSL) requested. *Attach detailed procedure if biohazards do not fit conventional Animal Biosafety Level 2* *or3 work practices. Reference CDC/NIH BMBL Animal Biosafety Levels: http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm*				
6. Indicate the proposed routes of administration:				
a. Aerosol	**YES**		**NO**	
b. Indwelling catheter or cannula	**YES**		**NO**	
c. Intranasal	**YES**		**NO**	
d. Parenteral (e.g. IV, IM, IP)	**YES**		**NO**	
e. Other	**YES**		**NO**	
7. Will the animal to be anesthetized or tranquilized during administration?	**YES**		**NO**	
8. Is the agent(s) an **animal** pathogen ?	**YES**		**NO**	
9. Is the agent(s) a **human** pathogen ?	**YES**		**NO**	
10. Is the agent transmitted from animal to animal ?	**YES**		**NO**	
11. Is the agent transmitted from animal to human ?	**YES**		**NO**	
12. Will the agent be inactivated prior to use in animals ?	**YES**		**NO**	
13. Will the animals be housed in microisolator cages?	**YES**		**NO**	
14. Will there be any special procedures or containment needed?	**YES**		**NO**	
a. Describe the special requirements:				
15. Will work with animals be performed in the biosafety cabinet?	**YES**		**NO**	

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Part F: SAFETY MEASURES

Research will be conducted at **Biosafety Level _____** Contact the IBC if you need assistance in determining the appropriate classification. Reference the CDC/NIH BMBL4th Edition available at: http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

1. **Engineering controls:** available to control significant aerosol generating steps for work requiring BL-2 containment or higher (e.g., centrifugation, vortexing, sonication, egg harvesting. Check all that apply:

Indicate the engineering controls used to prevent potential contamination:		
a. Containment Suite (e.g., BL3)	**YES** ☐	**NO** ☐
b. Biocontainment Animal Housing (if applicable)	**YES** ☐	**NO** ☐
c. Class II Biological Safety Cabinet	**YES** ☐	**NO** ☐
i. Type A (A1 or A2) or Type B (B1 or B2)?		
ii. Last date of biological safety cabinet certification (Mo/Yr):		
d. Centrifuge Safety Cups	**YES** ☐	**NO** ☐
e. Other (please specify):		

2. **Sharps**: (e.g., syringes, scalpels, glass) used with BSL-2 and higher organisms must be minimized.

a. Will syringes, scalpels, glass, or other sharps be used?	**YES** ☐	**NO** ☐	
b. Has the research protocol been reviewed to eliminate or minimize the use of sharps where possible?	**YES** ☐	**NO** ☐	
c. Are sharps with integrated safety devices/mechanisms available and used? **If yes, describe device (Type, Model, Brand):**	**YES** ☐	**NO** ☐	**NA** ☐

3. **Personal protective equipment** Indicate work the personal protective equipment required for your work (check all that apply):

a. Tyvek® suit	**YES** ☐	**NO** ☐
b. Lab coat	**YES** ☐	**NO** ☐
c. Apron, rear-fastening gown	**YES** ☐	**NO** ☐
d. Bonnet, hair cover	**YES** ☐	**NO** ☐
e. Powered Air Purifying Respirator (PAPR)	**YES** ☐	**NO** ☐
f. N-95 respirator	**YES** ☐	**NO** ☐
g. N-100 respirator	**YES** ☐	**NO** ☐
h. Surgical mask	**YES** ☐	**NO** ☐
i. Shoe covers	**YES** ☐	**NO** ☐
j. Cover sleeves	**YES** ☐	**NO** ☐
k. Safety glasses	**YES** ☐	**NO** ☐
l. Gloves (check box for all that apply)	**YES** ☐	**NO** ☐
i. nitrile ☐		
ii. non-powdered latex (powdered latex not recommended) ☐		
iii. vinyl ☐		
iv. other:		
m. Other (please list):	**YES** ☐	**NO** ☐

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4. **Decontamination/Disinfection**

a. **Indicate the disinfection method *per application (see columns to the right):***

	Routine cleaning & spills	Solid waste	Liquid waste	Animal Carcasses (if applicable)
1. Autoclave				
2. 1/10 bleach solution				
3. Povidone-iodine product e.g. Betadine ®				
4. 70% ethanol				
5. Phenolic product e.g. Vesphene ®				
6. Chlorine dioxide product e.g. Clidox ®				
7. Quaternary ammonium product e.g. Quatricide ®				
8. Other:				

b. Will radioactive infectious waste be generated? **YES** ☐ **NO** ☐

c. How will contaminated solid waste be disposed?

5. **Principal Investigator's Assessment of Risk**

a. What is the most serious adverse event you can forsee as a result of this experiment? (For example: recombination, employee exposure, environmental release, activation of latent virus, etc.)

b. How did you determine the appropriate biosafety level for this protocol?

c. Please list the following information about your most recent literature search on the safety of the organisms, reagents and experimental procedures used in this protocol.

i. What is the date of your most recent search?
ii. Which databases did you search?
iii. What keywords did you use?
iv. Please describe any pertinent safety or hazard analysis findings:

d. Is there potential for this material to be contaminated with an organism requiring a higher biosafety level? **YES** ☐ **NO** ☐

i. How would you determine if the material was contaminated with an organism requiring a higher biosafety level?

ii. Is your lab equipped to perform such an evaluation?

e. What was the source of this material (i.e., ATCC, colleague, other)?

i. Can the sender provide background information or quality control data on the material? **YES** ☐ **NO** ☐

ii. Have you already obtained this documentation? **YES** ☐ **NO** ☐

6. **Medical Surveillance** (check all that apply):

_____ 1) Personnel have attended Bloodborne Pathogens (BBP) training within the past year. Personnel have attended bi-annual Laboratory Safety training. Personnel shipping and receiving biohazard material have attended biannual IATA training. Contact EOHSS if more information on training is required.

_____ 2) All personnel who are potentially exposed to blood, body fluids or human cell lines have received Hepatitis B vaccine or proven immunity.

_____ 3) Additional vaccination/surveillance is required for work on this project.

Specify agents and special vaccination/surveillance requirements (attach sheets if necessary):

_____ 4) Individuals at increased risk of susceptibility to agent (e.g., preexisting diseases, medications, compromised immunity, pregnancy or breast feeding) have been referred to RPI Occupational Medicine Services or Employee Health Services for counseling.

_____ 5) There is a known vaccine and/or therapy:

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7. **Project Personnel:** Principal Investigators, use the following table to list all personnel (including any students) in your laboratory who handle or may otherwise be exposed to any of the rDNA, human cell lines, or microorganisms. Please attach additional sheets if necessary.

Name	Title	Date of Last Bloodborne Pathogen Training	Date of Last Lab Safety Training	Signature*

* indicates person who signed this form has been informed of potential hazards and safe work practices

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Part G: AFFIRMATION

I accept responsibility for the safe conduct of work with this material. I accept responsibility for ensuring that all personnel associated with this work have received the appropriate training on the hazards and the level of containment required to perform this research safely. I will report to RPI EH&S any accident or incident that results in a potentially toxic exposure to personnel or any incident releasing recombinant DNA or other potentially hazardous materials into the environment.

Principal/Responsible Investigator: _____

Signature: _____ Date: _____

Grant Agency: _____

Award #: _____

COMMITTEE USE

Approval: ☐ Yes ☐ Yes, approved with modifications *****(see notes below) ☐ No

Committee's Determination of Required Biological Containment-Biosafety Level: _____

Signatures

IBC Chairman / Representative: _____

Date: _____

Biological Safety Officer (optional): _____ Date: _____

Department Chairperson (as appropriate): _____ Date: _____

Occupational Medicine Physician (as appropriate): _____

Date: _____

Veterinarian (as appropriate): _____

Date: _____

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Modifications:

i. IACUC approval required	**YES** ☐	**NO** ☐
a. IACUC tracking #:		
ii. IRB approval required	**YES** ☐	**NO** ☐
a. IRB pending	**YES** ☐	**NO** ☐
b. IRB approved	**YES** ☐	**NO** ☐
c. IRB #:		
iii. Other:		

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APPENDIX 3

PERMITTED AGENTS FOR RESEARCH AT RENSSELAER (BIOSAFETY LEVELS 1 AND 2)

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Appendix 3. Permitted Agents for Research at Rensselaer (Biosafety Levels 1 and 2)

Biosafety Level 1

Biosafety Level 1 agents are usually not placed on a list but include all microorganisms that do not pose a health risk to health adult humans. It must not be assumed that an organism not listed as a Biosafety Level 2, 3, or 4 agent is a Biosafety Level 1 agent – emerging or unknown organisms should be treated as biohazardous until research proves otherwise. Examples of agents in Biosafety Level 1 are: *Bacillus subtilis*, infectious canine hepatitis viruses; influenza reference strains A/PR/8/34, A/WS/33, Escherichia coli K12, Saccharomyces cerevisiae, and other agents listed in Appendix C-II of the NIH Guidelines for Research Involving Recombinant DNA Molecules (1999); and vectors such as Baculovirus.

In addition to the examples listed above, the following low-risk oncogenic viruses have been identified as Biosafety Level 1 agents:

Adenvirus 7-Simian virus 40 (Ad7-SV40
Avian leucosis virus
Bovine leukemia virus
Bovine papilloma virus
Chick-embryo-lethan orphan (CELO) virus (Fowl adenovirus-1)
Dog sarcoma virus
Guinea pig herpes virus
Lucke (Frog) virus
Hamster leukemia virus
Marek's disease virus
Mason-Pfizer monkey virus
Mouse mammary tumor virus
Murine leukemia virus
Murine sarcoma virus
Polyoma virus
Rat leukemia virus
Rous sarcoma virus
Shope fibroma virus
Shope papilloma virus
Simian virus 40 (SV-40)

Biosafety Level 2

As described in Section 3 of this document, Biosafety Level 2 agents are of moderate potential hazard to healthy human adults and the environment. Such agents may produce disease of varying degrees of severity from accidental inoculation, injection or other means of cutaneous penetration but can usually be adequately and safely contained by ordinary laboratory techniques. Some agents may cause disease by contact or respiratory routes, but they are self-limiting and do not cause a serious illness, such as the cause of the common cold, the rhinoviruses. The following organisms have been identified as Biosafety Level 2 agents:

Bacteria

Acinetobacter baumannii
Actinobacillus spp
Actinomyces pyogenes
Aeromonas hydrophila
Amycolata autotrophica
Archanobacterium haemolyticum
Arizona hinshawii - all serotypes
Bacteroides spp.
Borrelia recurrentis, B. burgdorferi
Burkholderia (except for those listed in Biosafety Level 3)
Campylobacter coli, C. fetus ssp*. fetus, C. jejuni*
Clostridium chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani
Corynebacterium diphtheriae, C. pseudotuberculosis, C. renale
Dermatophilus congolensis
Edwardsiella tarda
Erysipelothrix rhusiopathiae

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Escherichia coli - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including E. coli O157:H7
Haemophilus ducreyi, H. influenzae
Helicobacter pylori
Klebsiella spp.
Leptospira interrogans - all serotypes
Listeria spp.
Moraxella spp.
Mycobacterium spp. (except those listed in Biosafety Level 3)
Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N. transvalensis
Pasteurella spp.
Plesiomonas shigelloides
Proteus spp.
Rhodococcus equi
Salmonella spp., except *S. typhi*
Shigella spp
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus spp.
Treponema pallidum, T. carateum
Vibrio cholerae, V. parahemolyticus, V. vulnificus
Yersinia enterocolitica

Fungal Agents

Blastomyces dermatitidis
Cladosporium bantianum, C. (Xylohypha) trichoides
Cryptococcus neoformans
Epidermophyton spp.
Exophiala (Wangiella) dermatitidis
Fonsecaea pedrosoi
Microsporum spp.
Ochroconis gallopavum
Paracoccidiodes brasiliensis
Penicillium marneffei
Ramichloridium machenziei
Sporothrix schenckii
Trichophyton spp

Parasitic Agents

Ancylostoma spp.
Ascaris spp.
Babesia spp.
Brugia spp. *filaria*
Coccidia spp.
Cryptosporidium spp.
Cysticercus cellulosae (hydatid cyst, larva *of T. solium*)
Echinococcus spp.
Entamoeba histolytica
Enterobius spp.
Fasciola spp.
Giardia spp.
Heterophyes spp
Hymenolepis spp.
Isospora spp.
Leishmania spp.
Loa loa filaria
Microsporidium spp.
Naegleria fowleri
Necator spp.
Onchoerca spp.*filaria*
Plasmodium spp. including simian species
Sarcocystis spp.
Schistosoma spp.
Strongyloides spp.
Taenia solium
Toxocara spp.
Toxoplasma spp.
Trichinella spiralis
Trypanosoma spp.
Wuchereria bancrofti (filaria)

Viruses

Adenoviruses - human, all types
Adenovirus 2 – Simian virus 40 (Ad2-SV40)
Arboviruses listed in BMBL, Section VII-G, Table 1*
Arenaviruses
Coronaviruses
Coxsackie A and B viruses
Echoviruses - all types
Encephalomyocarditis virus (EMC)
Feline leukemia virus (FeLV)
Feline sarcoma virus (FeLV)
Gibbon leukemia virus (GaLV)
Hepatitis A, D, E
Influenza viruses
Lymphogranuloma venereum agent
Measles virus
Molluscum contagiosum virus
Mumps virus
Papovaviridae including human papilloma viruses

Parainfluenza virus
Paravaccinia virus
Reoviruses, all types
Respiratory syncytial virus
Rhinoviruses, all types
Rubella virus
Simian viruses other then simian immunodeficiency virus, Herpesvirus simiae (Monkey B virus) and Marburg virus

* BMBL = Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, 1999 (CDC/NIH)

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APPENDIX 4

PROHIBITED AGENTS FOR RESEARCH AT RENSSELAER (BIOSAFETY LEVELS 3 AND 4)

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Appendix 4. Prohibited Agents for Research at Rensselaer (Biosafety Levels 3 and 4)

Biosafety Level 3

Bacteria

Bacillus anthracis
Bartonella spp.
Bordetella spp.
Burkholderia mallei, B. pseudomallei
Brucella spp.
Chlamydia psittaci, C. trachomatis, C. pneumoniae
Clostridium botulinum
Francisella turarensis
Legionella spp.
Mycobacterium bovis, M. tuberculosis
Neisseria gonorrhoea, N. meningitides
Salmonella typhi
Yersina pestis

Rickettsia

Coxiella burnetii
Rochalimaea quintana (also known as *Bartonella quintana*)
Rickettsia spp.

Fungal Agents

Coccidioides immitis
Histoplasma capsulatum, H. capsulatum var. *duboisii*

Viruses

Arboviruses listed in BMBL, Section VII-G, Tables 3 and 4*
Encephalomyelitis viruses
Hantavirus
Hepatitis B, C viruses
Herpesviruses including Cytomegalovirus, Epstein Barr, Herpes simplex types 1 and 2 and Herpes zoster, except Herpesvirus simiae (Monkey B virus)
Human Immunodeficiency Virus (HIV), all serotypes
Human T-cell lymphotrophic virus
Infectious bronchitis - like virus
Lymphocytic chroiomeningitis virus
Poxviruses - types including Cowpox, Monkeypox or Vaccinia, Tanapox and Yabapox, excepting Alastrim, Smallpox, and Whitepox
Polioviruses
Rabies virus
Simian Immunodeficiency virus
Vesicular Stomatitis virus

Prions

Transmissible Spongioform Encephalopathies (TME) and all other prions

Biosafety Level 4

Viruses

Arboviruses, Arenaviruses, and Filoviruses listed in BMBL, Section VII-G, Table 5*
All known and suspected hemorrhagic fever agents
Hendra and Hendra-like viruses
Herpesvirus simiae (Monkey B virus)
All tick-borne encephalitis virus complex

* BMBL = Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, 1999 (CDC/NIH)

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APPENDIX 5

ETIOLOGIC AGENTS PERMITTED AT RENSSELAER AND REQUIRING SPECIALIZED SHIPPING PROCEDURES

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Appendix 5: Etiologic Agents permitted at Rensselaer and requiring specialized shipping procedures listed in Section 10.1.2 of this Rensselaer's *Biosafety Program*

Bacterial Agents

Acinetobacter calcoaceticus
Actinobacillus - all species
Actinomycetaceae - all members
Aeromonas hydrophilia
Arizona hinshawii - all serotypes
Bacteroides spp
Borrelia recurrentis, B. vincenti
Campylobacter fetus, C. jejuni
Clostridium chauvoei, Cl. haemolyticum, Cl. histolyticum,Cl. novyi, Cl. septicum, Cl. tetani
Corynebacterium diphtheriae, C. equi,C. haemolyticum, C. pseudotuberculosis,C. pyogenes, C. renale
Edwarsiella tarda
Erysipelothrix insidiosa
Escherichia coli, all enteropathogenic serotypes
Haemophilus ducreyi, H. influenzae
Klebsiella - all species and all serotypes
Legionella - all species and all Legionella-like organisms
Leptospira interrogans - all serovars
Listeria - all species
Moraxella - all species
Mycobacterium spp. other than *M. tuberculosis* and *M. bovis*
Mycoplasma - all species
Nocardia asteroids
Pasteurella - all species
Plesiomonas shigelloides
Proteus - all species
Salmonella - all species and all serotypes
Shigella - all species and all serotypes
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus pneumoniae
Streptococcus pyogenes
Treponema careteum, T. pallidum, and T. pertenue
Vibrio cholerae, V. parahaemolyticus
Yersina enterocolitica

Fungal Agents

Blastomyces dermatitidis
Cryptococcus neoformans
Paracoccidioides brasiliensis

Viral Agents

A*denoviruses* - human - all types.
Arboviruses - all types
Coxsackie A and B viruses - all types
Echoviruses - all types
Encephalomyocarditis virus
Hepatitis associated materials (hepatitis A, hepatitis B, hepatitis nonA-nonB)
Influenza viruses - all types
Measles virus
Mumps virus
Parainfluenza viruses - all types
Respiratory syncytial virus
Rhinoviruses - all types
Rubella virus
Simian virus 40

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APPENDIX 6

LABORATORY SPECIFIC STANDARD OPERATING PROCEDURES EXAMPLE

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LABORATORY SPECIFIC STANDARD OPERATING PROCEDURES MANUAL OUTLINE

1. Authorized Laboratory Users
2. Emergency Contact Numbers
3. Materials and chemicals stored at the Facility (Laboratory)
4. Laboratory Specific Training Requirements
5. Authorized Facility Entrance Requirements
6. Laboratory Standard Operating Procedures (procedure and equipment specific)
7. Medical Surveillance (if applicable)
8. Personal Protective Equipment (procedure and equipment specific)
9. Facility Inspection schedule-internal to the laboratory
10. Containment Facility Diagram (Laboratory)
11. Containment Barriers (Biological Safety Cabinets/Protection of Laboratory Vacuum Systems)
12. Movement of Infectious Materials (if applicable)
13. Laboratory Specific Waste Disposal Procedures
14. Autoclave Quality Assurance Program
15. Resources for Pathogen Exposures

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APPENDIX 7

REQUEST FOR HAZARDOUS WASTE PICK-UP FORM

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Request For Hazardous Waste Pick –Up

Instructions: Fill out **ALL** information and fax or e-mail to ext. 2512 (fax) or knutsk@rpi.edu (e-mail)

Date: _____

Building:_____ Room No._____

Exact Location (i.e. fume hood, storage cabinet, etc.):_____
_____

Container Specifications:

Number of Containers:_____ Type: ____Liquid _____Solid

Amount (pounds/gallons):_____ Radioactive Waste: ____YES _____NO

Type of Containers: _____Glass _____Plastic ____Metal _____Other

Containers Identified with Rensselaer Waste Label? _____YES

Contact Person (in laboratory or area):

Name:_____ Phone:_____ E-Mail:_____

Individual Responsible (for laboratory or area):

Name:_____ Phone:_____ E-Mail:_____

For Environmental Health & Safety Use Only

Date Received:_____ Date of Pick-up:_____

Pick-up By: _____

If you have any questions on the procedures above,
Please call x 2092 or x 6114.