BIOSAFETY MANUAL

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MGH Biosafety Manual modified for McLean, March 2010
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**FOR IMMEDIATE ADVICE ABOUT ANY CHEMICAL EXPOSURE:**

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INTRODUCTION

The contents of this manual include general guidance on biosafety practices and procedures in a microbiological or biomedical research lab. The primary goal of biosafety is to reduce or eliminate exposure and the risk associated with handling potentially hazardous biological agents. It is meant as a guide and resource for staff working in a research lab and has been established to accomplish the following goals:

- Educate personnel on Biosafety practices and procedures
- Protect personnel from exposure to infectious agents and recombinant DNA
- Provide an environment of high quality research while maintaining a safe work place
- Prevent environmental contamination
- Comply with applicable federal, state and local requirements

The success of any Biosafety Program depends on the combined efforts of the lab staff and supervisors as well as support personnel. Planning for and implementing a sound Biosafety Program is a foundation on which quality research can be performed on infectious agents and recombinant DNA while decreasing the potential of accidental exposure to hazardous biological material.

THE REGULATIONS

Laboratory procedures and facilities used in protecting laboratory workers and the general public from potential health hazards associated with biological agents are governed by federal, state and local regulations. Some of these rules such as those from Occupational Health and Safety have the force of law while those from the NIH and CDC are guidelines. Many granting agencies require grantees certify that they adhere to both the suggested federal guidelines and the legally mandated requirements.

FEDERAL REGULATIONS

Occupational Health and Safety Administration (OSHA): Bloodborne Pathogen (BBP) Standard. The OSHA law requires several measures to protect workers and the public from accidental infection by these agents and requires staff with potential exposure to bloodborne pathogens to attend annual training. Bloodborne pathogens of greatest consequence include human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV). All laboratories that work with human blood, tissues, and body fluids must adhere to the OSHA BBP Standard (http://www.osha.gov/SLTC/bloodbornepathogens/index.html).

The Center for Disease Controls and Prevention (CDC) and the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) regulate use of Select Agents and Toxins. The “CDC/USDA Select Agents” regulations focus on the safety and
security of those biological agents and toxins (list of Select agents: http://www.cdc.gov/od/sap/docs/salist.pdf ) that could pose a threat to human, animal and plant health and safety. These regulations require institutions that possess, use or transfer “Select Agents” to be registered and approved by the CDC and/or APHIS.

National Institutes of Health (NIH): “Guidelines for Research Involving Recombinant DNA Molecules” available at http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html This document provides guidelines for constructing and handling recombinant DNA molecules (rDNA) and organisms containing rDNA. It is MGH policy that all laboratories adhere to these guidelines.

The CDC and the NIH publish a set of guidelines for work with infectious organisms. The publication, entitled “Biosafety in Microbiological and Biomedical Laboratories” is available at http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm. It is the policy of McLean Hospital and MGH that all laboratories adhere to the CDC guidelines.

COMMONWEALTH OF MASSACHUSETTS REGULATIONS

Regulations from the State of Massachusetts (105 CMR 480.000—Minimum Requirements for the Management of Medical or Biological Waste State Sanitary Code Chapter VIII), http://www.mass.gov/Eeohhs2/docs/dph/regs/105cmr480.pdf primarily focus on the management and disposal of infectious waste. The site defines infectious waste and gives details on how to dispose of it. The state statutes agree with the NIH and CDC definition of biohazardous waste. The state defines discarded Pasteur pipettes, no matter what their use, as Medical Waste and they must be dealt with appropriately.

BELMONT REGULATIONS

Belmont adopted regulations for the use of Recombinant DNA (rDNA) Molecule Technology and Nonrecombinant Infectious Agents in December 2000. Text of the regulation, which prohibits research requiring BL3/4 containment, is available through Research Administration. All work done in Belmont has to be registered with the Town of Belmont Department of Public Health and must be approved by the Harvard Committee on Microbiological Safety (COMS). In general the Town of Belmont agrees with NIH-CDC guidelines.

MCLEAN HOSPITAL REGULATIONS

McLean Hospital accepts the OSHA, NIH, CDC and USDA guidelines as Hospital policy. The NIH places the responsibility for implementing its rDNA Guidelines in the hands of an Institutional Biosafety Committee (IBC). The Institutional Biosafety Committee for McLean reports to the Belmont Biosafety Committee (BBSC) and the Committee on Microbiological Safety (COMS), which serves Harvard Medical School and affiliated hospitals. McLean’s IBC membership includes the Belmont Director of Health, McLean Representation, and one community representative. BBSC membership includes the Belmont Director of Health, the Chair of the Board of Health, and three community members. COMS membership includes representatives of the general public and members of HMS faculty and staff. It is McLean policy that all research involving the use of recombinant DNA and infectious agents, including Bloodborne Pathogens, Select Agents and Toxins be registered with the Committee on Microbiological Safety.
The primary purpose of COMS is to uphold the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines), to ensure the safe handling and management of recombinant DNA and potentially hazardous biological materials.

The primary responsibilities of the COMS are to:

- Promote the best practices for the safe handling and disposal of potentially hazardous and infectious biological materials.
- Ensure compliance with all relevant federal, state, and local regulations for work with biohazardous materials.

The functions of the COMS are to:

- Recommend appropriate biosafety-related policies and procedures for management of potentially hazardous biological materials.
- Serve as a resource for technical information for biological risk assessment and reduction of exposures to biohazards.
- Keep current on regulations pertaining to the use of potentially biohazardous materials.
- Review and audit laboratories to ensure compliance with biosafety guidelines.
- Review biosafety training provided to employees and record attendance.
- Assist investigators in identifying technical resources and relevant information related to biosafety.

REGISTERING RESEARCH PROTOCOLS WITH COMS

Work with biological material including infectious agents, recombinant DNA and human material is overseen by the Institutional Biosafety Committee and “The Committee on Microbiological Safety” (COMS) and serves Harvard Medical School and Harvard Affiliated Institutions. More information on the role of the Committee can be found at: http://www.hms.harvard.edu/orsp/coms/index.htm

All research involving the use of the following must be registered with the COMS Committee:

- Recombinant DNA materials
- Infectious microorganisms, regardless of their pathogenicity, and biological toxins
- Human or non-human primate blood, cell lines or unfixed tissues

In order to expedite the review of your COMS application, please review these guidelines. By providing appropriate information in the application, the review process by the MGH Biosafety Officer and COMS will be completed in as timely a manner as possible. If you have questions regarding completing the application or the approval process, please contact the MGH Biosafety Officer, Anne Sallee asallee@partners.org
Filling Out a COMS Registration

- On the front page, make sure to give an accurate mailing address. Once your application has been approved, COMS sends approval letters through the MGH Biosafety Office.

- Read the Memorandum of Understanding and Agreement section carefully. The signatory agrees that they will provide biosafety training, maintain a safe work environment, and notify COMS immediately of accidental exposures or if a staff member develops symptoms related to the agents used in the laboratory.

- In the “Describe the Experiment in Detail” section, provide a detailed, but brief description of the experiment. Include information on how all biological agents will be used. Do not cut and paste information from other documents such as animal protocols or grants into this section, as these documents may have information that is not relevant to the biological safety of the project.

- If you include citations, or reference scientific papers in your application, please provide a copy of the full publication, if possible.

- List the specific names and sources (i.e. commercial company or research laboratory) of all biological agents and transgenic animals.

- In the “Describe the Biohazard Potential of These Experiments” section, list procedures where exposures might occur and the biosafety levels of agents in use, if known. Leaving this section blank or stating “none” is not acceptable. If you are using viral vectors to insert or knockout genes address the following issues in the Biosafety Section:
  
  o Identify any oncogenes, tumor suppressor genes, toxin producing genes, or other genes with the potential to cause harm, if expressed.
  o Indicate if the inserted gene has the potential for altering the cell cycle
  o Indicate if the viral DNA can integrate into the host genome
  o Discuss the probability of generating replication-competent viruses including methods to reduce this probability such as multiple plasmid systems and self-inactivation.
  o Discuss the expected consequences of a human exposure to the vector.

Clinical trials involving human gene therapy, vaccine development, and xenotransplantation must also be registered with COMS. The principal investigator (PI) is responsible for completing the appropriate project registration forms. COMS website is located at http://www.hms.harvard.edu/orsp/coms.
COMS registration forms that can be downloaded:

- Recombinant DNA and Infectious Agents form: 
  [http://www.hms.harvard.edu/orsp/coms/Forms/MGH-Forms/MGH-Registration.doc](http://www.hms.harvard.edu/orsp/coms/Forms/MGH-Forms/MGH-Registration.doc)

- Human Gene Transfer form: 

- Human Xenotransplantation form: 

- Select Agent form: 
  [http://www.hms.harvard.edu/orsp/coms/Forms/MGH-Forms/MGH-Select-Agent.doc](http://www.hms.harvard.edu/orsp/coms/Forms/MGH-Forms/MGH-Select-Agent.doc)

If you have questions about completion of the forms, please contact the MGH Biosafety Officer (BSO), Anne Sallee at [asallee@partners.org](mailto:asallee@partners.org) or by phone at 617-734-4579.

Each registration form will be reviewed by the Biosafety Office to verify its completeness and then submitted to COMS for final approval. The PI will receive an approval letter signed by the COMS Chair that contains specific information about biosafety procedures and containment for the project.

**BIOSAFETY OFFICE RESPONSIBILITIES**

- Perform risk assessments/evaluations on research protocols submitted to COMS by each PI.
- Evaluations should be completed before research work is started and reassessed periodically as new data are obtained.
- Risk assessments should include biosafety considerations posed by the particular organisms and research methods under investigation.
- Provide consultation on operations and methods of procedure for biological research laboratories to ensure compliance with CDC/NIH, OSHA and state criteria.
- Provide initial and annual inspections for labs registered through the COMS.
- Provide advice in the event of a large infectious spill.
- Provide lab safety training for new personnel and subsequent annual training.

**PRINCIPAL INVESTIGATOR’S RESPONSIBILITIES**

The implementation of these practices and procedures is the ultimate responsibility of the Principal Investigator (PI). PI responsibilities include:

- Ensuring all work is initially registered with COMS and updated annually.
- Inform the Biosafety office when changes to the protocol occur.
- The implementation of the applicable biosafety procedures and practices in their laboratories.
- Ensure lab personnel are competent to conduct the work.
- Ensure procedures, equipment and facilities are available for laboratory staff.
• Must have a written laboratory-specific biosafety manual.
• Ensure that all laboratory staff attends initial and annual laboratory safety training which includes the required annual OSHA Bloodborne Pathogens Training (BBP).
• Must be knowledgeable of the potential adverse health effects of the biological materials used in their laboratory, the appropriate biosafety level of the laboratory and apply the accompanying safety practices and procedures for that level.

LABORATORY STAFF RESPONSIBILITIES

Laboratory staff scientists are responsible for:

• Following the McLean/MGH biosafety practices and procedures recommended by the Biosafety office and the PI.
• Compliance with NIH, CDC and OSHA regulations is required.
• Informing the PI, lab manager or the Biosafety office of any potentially hazardous situations or conditions.
• Report any exposures to infectious materials, including animal bites and sharps injuries, to the PI and report to Occupational Health 617-855-2438.

BIOSAFETY LEVELS

The Biosafety in Microbiological and Biomedical Laboratories (BMBL) classifies agents into four Biosafety Levels (BL1, BL2, BL3, and BL4). They are tools used to describe physical containment and work practices for different biosafety risk levels of work. Appropriate facilities and practices for a given set of experiments may fall between two levels or may shift from one level to another as a study proceeds. For instance, some studies with dangerous organisms, such as retroviral vectors containing oncogenic or toxic inserts, often will use BL3 work practices in a BL2 facility. This in-between designation is called BL2 with stipulations (BL2-S) and is a COMS classification and not included in the BMBL.

RISK GROUPS

In 1996 the NIH established a pathogen Risk Group classification scheme. Four Risk Groups (RG) were defined. Agents in RG1 have negligible pathogenicity while those in RG4 have extremely high pathogenicity. There is a rough correspondence between RG and recommended Biosafety Level. However, the NIH gives the local Biosafety Committee great latitude in deciding the appropriate containment levels and laboratory procedures for each study.
### Summary of Biosafety Levels for Infectious Agents (BL-1 to BL-3)

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<tr>
<th><strong>Biosafety Level 1 (BL-1)</strong></th>
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<tr>
<td><strong>Agents:</strong> Not known to cause disease in healthy adults</td>
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<td><strong>Practices:</strong> Standard Microbiological Practices</td>
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<td><strong>Safety Equipment:</strong> None required</td>
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<tr>
<td><strong>Facilities:</strong> Open bench top sink required</td>
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<td><strong>Examples:</strong> E. coli, continuous animal cell lines</td>
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<th><strong>Biosafety Level 2 (BL-2)</strong></th>
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<td><strong>Agents:</strong> Associated with human disease, hazard (exposure) = auto-inoculation, ingestion, mucous membrane exposure</td>
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<td><strong>Practices:</strong> BL-1 practice plus: Limited access; biohazard warning signs; “Sharps” precautions; biosafety manual defining any needed waste decontamination or medical surveillance policies</td>
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<td><strong>Safety Equipment:</strong> Primary barriers = Class I or II Biological Safety Cabinets (BSCs) or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; Personal Protective Equipment (PPE): laboratory coats, gloves, face and eye protection as needed</td>
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<tr>
<td><strong>Facilities:</strong> BL-1 plus: Autoclave available</td>
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<td><strong>Examples:</strong> Human cell lines, Hepatitis B Virus, Salmonella typhi, non-human primate material</td>
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<th><strong>Biosafety Level 3 (BL-3)</strong></th>
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<td><strong>Agents:</strong> Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences</td>
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<td><strong>Practices:</strong> BL-2 practice plus: Controlled access; decontamination of all waste; decontamination of lab clothing before laundering; baseline serum</td>
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<td><strong>Safety Equipment:</strong> Primary barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents; PPE: protective lab clothing, gloves, face and eye protection, and respiratory protection as needed</td>
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<td><strong>Facilities:</strong> BL-2 plus: Physical separation from access corridors; self-closing, double door access; exhausted air not re-circulated, negative airflow into laboratory</td>
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<td><strong>Examples:</strong> Yellow fever, Mycobacterium tuberculosis</td>
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LABORATORY WORK PRACTICES and PHYSICAL CONTAINMENT

Containment can be accomplished through the following means:

- **Primary Containment**: Protection of personnel and the immediate lab environment through the use of good microbiological technique and the use of certain safety equipment such as Biosafety Cabinets.
- **Secondary Containment**: Protection of the environment external to the lab from exposure to infectious materials through a combination of facility design and operational practices.

The checklist below provides general guidance on the requirements for Biosafety Levels 1, 2, 2S. Biosafety Levels 3 and 4 have not been included, as work with these agents is not permitted at McLean.

**BIOSAFETY LEVEL 1:**

**Work Practices and Procedures**

- Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens are in progress.
- Wash hands after handling infectious agents and after taking off gloves.
- Decontaminate work surfaces daily and after spills.
- No eating, drinking, smoking in the lab.
- Use mechanical pipetting devices. No mouth pipetting.
- If you wear contact lenses consider wearing goggles or a face shield while working.
- Policies for safe handling of sharps are instituted.
- Use procedures that minimize aerosol formation.
- Use appropriate personal protective equipment (e.g. laboratory gowns, coats, gloves).
- Vacuum lines must be protected with a hydrophobic or High Efficiency Particulate Absorbing (HEPA) filters.
- Biological spill kit should be available.
- Insect and rodent control program is in effect.

**Physical Containment Check List**

A Basic Laboratory – Open bench work

- Sink for washing hands.
- Designed for easy cleaning; all surfaces are easily disinfected.
- Non-porous, alkali, acid and solvent resistant bench tops.
- Screens on windows if they open.
- Spaces between walls and equipment must be accessible for cleaning
- Each laboratory must have lab specific Biosafety manual.
BIOSAFETY LEVEL 2:

Work Practices - In addition to BL1

- Use biological safety cabinets to contain aerosol-producing procedures. The use of centrifuges with sealed heads or safety cups is preferred.
- Wear protective clothing including a lab coat or protective gown, goggles or face shield (if splashes are possible) and gloves. Leave them behind in the lab when you leave. Change gloves frequently.
- Biohazardous wastes must be decontaminated before leaving the building, usually by autoclaving. McLean policy permits the disposal of untreated BL2 level, non-recombinant, solid waste in containers destined for off site processing.
- Biological spill kit must be located inside the tissue culture room. All staff must be trained to clean/decontaminate spills I centrifuges, incubators. Biosafety cabinets, and in the laboratory (both minor and major spills).
- Keep immunocompromised people out of the lab.
- Staff must receive training in safety procedures appropriate to the organisms being studied. Training sessions should be scheduled annually.
- Offer immunization and/or tests for the agents being used (Hepatitis vaccinations, skin TB tests).
- In some cases it may be appropriate to collect and store baseline and periodic serum samples.
- Accidental exposures must be reported to the laboratory director so that medical evaluation and treatment can be provided.
- Use leak proof containers when transporting infectious materials.

Physical Containment: In addition to the BL1 facility requirements

- A biohazard sign must be posted at the entrance of the laboratory or tissue culture room whenever infectious agents are present. The sign must include the universal biohazard symbol, the appropriate biosafety level, the name of the agent used, and the name and phone number of the PI. The sign should also indicate any special requirements for entering the lab (gowns, goggles ...).
- Biosafety Cabinets (Class II) should be installed and certified annually.
- Eye wash.
- A method for decontaminating wastes must be available. Autoclaves, chemical disinfectants or an incinerator are appropriate.

BIOSAFETY LEVEL 2 with Stipulations (BL2-S):

Biosafety Level 2+ refers to Biosafety Level 2 laboratory facilities, including a BSC and other physical containment devices, utilizing BL3 work practices and procedures.

Work practices: - In addition to BL2
• BL2-S laboratories must be self-contained. If a centrifuge cannot be located in the laboratory, then sealed rotors and centrifuge cups must be opened inside a biosafety cabinet.
• Strict needle and sharps precautions must be observed.
• All work with viable materials must be done in a biosafety cabinet.
• All biohazardous wastes must be autoclaved before disposal.
• Autoclaves must be validated using a biological indicator, Bacillus stearothermophilus, a challenge organism for sterilization validation studies and periodic check of sterilization cycles
• PPE must be worn at all times including disposable solid-front gown and disposable latex or nitrile gloves. All PPE must be autoclaved prior to disposal into a biohazardous waste box.
• Conduct all experiments carefully to minimize aerosol production.

Physical Containment: In addition to BL2 facility requirements
• Laboratory doors are locked at all times, with a secure key code entrance where only authorized employees are allowed to enter. Authorized employees are granted access after the PI documents required training and vaccinations, if needed.
• A log book for personnel entering/exiting the laboratory should be used if key-card access if not available.
• ENTRY/EXIT procedures should be in place:
  o Enter the room and put on disposable solid-front gown, gloves and eye protection.
  o Before leaving a BL2-S room, remove and discard gloves within the room, and wash hands in a hand wash sink in the BL2-S room.
  o Discard disposable gown before leaving the laboratory suite.
  o Notebooks taken into the BL2-S laboratory should be vinyl clad and easily wiped for decontamination.

Infectious materials can be transported to and from the BL2-S laboratory only in sealed primary containers placed inside a secondary container. Infectious materials must only be in the custody of trained laboratory personnel.

BIOSAFETY LEVELS 3 and 4

BL3 and BL4 work is not permitted at McLean.

BIOSAFETY CABINETS

Biosafety cabinets (BSCs) are designed to protect the operator, the laboratory environment and work materials from contamination. The barrier between work and worker is a curtain of sterile air descending from the top of the BSC after passing through a high-efficiency particulate air (HEPA) filter. HEPA filters trap 99.97% of particles of 0.3μm in diameter and
99.99% of particles of greater or smaller size. Air flow is balanced so that some air is taken from the room and, along with sterile cabinet air, sucked into a horizontal grill at the front of the work surface. As with any safety device, Biosafety Cabinets only work correctly when used correctly.

For additional information refer to the CDC publication at [http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm](http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm).

Be sure not to confuse a Biosafety cabinet with the clean air bench (positive pressure laminar flow bench) or a fume hood. These three pieces of equipment have very different functions and when used incorrectly, it can result in exposure to an infectious agent.

There are several types of BSCs. Nearly all the cabinets used at the McLean are Class II Biosafety cabinets. The Class II BSC can be used for work with agents from risk group (RG) 2 and 3. A fraction of the air (70%) is recirculated through a supply HEPA filter back into the cabinet work space. The remaining 30% of the air in the BSC passes through the exhaust filter into the room or to the outside. In Class II BSCs only air from the HEPA-filtered (sterile) supply is allowed to flow over the work surface. Each BSC should be certified when it is installed, each time it is moved or repaired, and annually thereafter.

**Effective use of Biosafety Cabinets:**

- Wash hands and arms with soap thoroughly before and after work in the BSC. Proper PPE should be worn; i.e. long sleeved gowns with tight fitting cuffs and disposable gloves.
- Before beginning work, decontaminate the work surface with a disinfectant (such as 2% Vespheine or 70% ethanol).
- Turn on the Biosafety cabinet and run it for 5-10 minutes, to purge contaminated air, before starting work and let the biosafety cabinet run for 5-10 minutes after work is completed.
  - Most biosafety cabinets are designed to operate 24 hours/day.
- Four ways you can protect the sterile air curtain:
  - Set up the lab so that the cabinet is located away from traffic while work is going on. Position the cabinet away from air vents.
  - Keep the front horizontal grill clear.
  - Minimize arm movement in and out of the cabinet.
  - Make sure the sash is at the indicated height (not too low, not too high).
- Everything needed for the complete procedure should be placed in the BSC before starting work.
- Work supplies are best arranged to segregate clean from dirty materials.
- Do not overcrowd the work area.
- Some BSCs are equipped with ultraviolet lights. All UV lights should be turned off when the laboratory is occupied, to protect eyes and skin from inadvertent exposure. In order to work properly the UV lights should be cleaned weekly to remove dust and dirt that may block germicidal effectiveness. The Biosafety Office suggests the lamp be left off.
  - If you do use the UV light overnight, please be sure to have the sash all the way down to protect custodial and security staff after hours.
• Avoid using open flames in the BSC as this disrupts the unidirectional airflow. The use of Bunsen burners can shorten the lifetime of the HEPA filter and has caused explosions in BSCs.
• Avoid using toxic, flammable, explosive or radioactive
• Containers filled with working concentrations of disinfectants, such as Vesphene, (replenished as necessary to maintain concentration) should be placed inside the hood for the disposal of pipettes while working.
• A large side-arm vacuum flask with 100ml of undiluted Vesphene should be connected in series to another “trap” aspiration flask and then to an in-line HEPA filter.
• Biohazard bags and disinfectant flasks should be placed inside the BSC to avoid breaking the protective air barrier and bringing contaminated items out of the hood into the room.
• If waste aspiration flask must be located outside the hood, make sure it is placed inside a secondary container and labeled with a biohazard sticker.
• Use absorbent pads on the work surface where appropriate to minimize splatter and aerosol generation in the case of a spill. Remove and replace daily.
• Work as far to the back of the BSC as possible.
• Do not work in a BSC when an alarm is signaling.
• When work is finished for the day, close and cover all equipment and materials. Allow the BSC to run for 5 minutes to purge all airborne contaminants.
• Remove all materials cultures and equipment.
• Decontaminate interior surfaces with freshly prepared 70% ethanol or another disinfectant appropriate for the agent being used.
• Periodically decontaminate under work grills and work surfaces if these parts are removable.
• BSCs must be certified annually by a certified technician, McLean uses Air Systems Technology 1-800-477-4175.

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<th>SHARPS GUIDELINES</th>
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The best way to avoid sharps injury is to avoid using sharps. Plastic transfer pipettes may be a good replacement for Pasteur pipettes. Plastic ware can eliminate broken glass problems. Self-sheathing or safety needles are available and can be ordered on PeopleSoft.

Sharps Use
Because the majority of laboratory biohazard injuries are due to hypodermic needles there has been special concern over needle use and disposal. Here are the some safety recommendations:

• Avoid using needles and syringes. Use safety needles when necessary.
• Do not bend or break needles.
• Do not recap needles.
• Do not remove needles from syringes.
• Throw away the entire syringe-needle combination.
• Be careful during clean-up. There may be some sharp items hidden in the garbage.
• If you do stick yourself, encourage the wound to bleed for several minutes, wash the area and then go to Occupational Health.
Sharps Disposal
The idea behind any disposal policy is to protect maintenance workers and the public from being injured by discarded sharps. Procedures at McLean are designed to make it as easy as possible for lab workers to get rid of waste without compromising the safety of people later in the waste stream. To protect yourself and others from injury:

- Place needles, syringes, suture needles, scalpels, and razor blades into standard Sharps containers. These come in many sizes and are thick red containers labeled with a biohazard symbol and can be obtained from Building Services at 617-855-2656.
- Do not overfill the Sharps containers. Close and discard these in the large, cardboard biohazard boxes when they are 3/4 full.
- Place Sharps containers near work areas so they will be used.

Broken medical glassware
Please place clean broken glassware into a standard broken glassware boxes. If the glassware is contaminated it MUST be placed directly into red Sharps containers.

Pasteur pipettes
Pasteur pipettes are a special problem. Massachusetts law requires they be considered biohazardous waste no matter what their previous use. They must be placed directly into Biohazardous Sharps containers. Don’t use broken glassware boxes - they are not incinerated. Pasteur pipettes used in BL2+ studies should be autoclaved before being discarded in the red Biohazard Sharps containers.

BIOLOGICAL WASTE DISPOSAL
Massachusetts law requires that special care be taken when discarding biological waste. It must be in red bags marked with the universal biohazard symbol. Ultimately it will be disinfected and ground up so that it is no longer physically dangerous. Then it’s discarded in a landfill.

Biological waste (“red bag waste”) is collected in Red Bag Waste boxes, which are large cardboard containers provided by Building Services. A red biohazard bag goes in the box, and when the box is ¾ full it should be closed up and picked up by Building Services. Building Services loads it on an outside disposal contractor’s truck. Once processed, the boxes are decontaminated and returned to us for reuse.

Refer to the Waste Disposal Table on the EH&S Website for most recent disposal guidelines. Environmental Health & Safety Department at MGH

Also refer to the Waste Streams Diagram on the McLean Safety website. https://research.mclean.harvard.edu/newsite/safety/Hazwaste.php
CHEMICAL DISINFECTANTS
The following web site is an excellent source of information about disinfectant brands "EPA certified" for general sterilization, tuberculosis (a particularly difficult disinfection problem), HIV and Hepatitis B: http://www.ace.orst.edu/info/nain/lista.htm

General Recommendations
• Decontaminating Liquids.
  o Add hospital approved chlorine bleach to a final 1/10 dilution for 20 minutes or,
  o Add a phenolic to a final dilution of 1/20 for 20 minutes.
• Decontaminating Surfaces
  o Wipe with a 1/10 dilution of chlorine bleach, or
  o Wipe with 70% ethanol, or
  o Wipe with a 1/20 dilution of a phenolic

DISINFECTANTS

Chlorine Bleach
• Mercury-free chlorine bleach (Elite or Austin’s A-1) is the only bleach allowed down the drain at McLean. A 1/10 dilution will inactivate most microorganisms in 20 minutes.
• Some bacteria and most spores are more resistant. Mycobacterium tuberculosis, the organism responsible for tuberculosis, needs a 1/5 dilution for inactivation.
• The concentration needed to decontaminate also depends on the organic load of the material to be treated. More protein - more bleach.
• 10% bleach solutions decompose at room temperature, prepared fresh weekly.

Alcohols
• Ethyl alcohol and isopropyl alcohol diluted to 70 - 85% in water are useful for surface decontamination.
• Alcohols are non-corrosive and are appropriate for decontamination of materials that can be damaged by halogens.
• Alcohols should be used with care. Avoid using them at 100%; a 100% alcohol solution is an excellent desiccant. Desiccation will often preserve, rather than kill, many microorganisms.
• Some organisms, such as Mycobacterium tuberculosis, are not inactivated by 70% ethanol.

Phenolics
• Vesphene II diluted to 2% is used for surface decontamination and chemical disinfection of liquid biohazard wastes.
• 20-30 minutes of contact time is required for effective decontamination.
Summary of Disinfectants and Their Uses:

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Final Concentration**</th>
<th>Effective on:</th>
<th>Ineffective on:</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine Bleaches:</td>
<td>1/10</td>
<td>Bacteria, some spores, viruses, TB^, HIV</td>
<td>Some spores</td>
<td>Prepare weekly. Contact time ~20 minutes to disinfect. Corrosive.</td>
</tr>
<tr>
<td>Iodophors:</td>
<td>1/100</td>
<td>Bacteria, most viruses, TB</td>
<td>Spores</td>
<td>A surface disinfectant. Iodine is insoluble so it’s not good in solutions. Corrosive.</td>
</tr>
<tr>
<td>Alcohols (Ethanol, Isopropanol)</td>
<td>70%</td>
<td>Bacteria, most viruses</td>
<td>Spores, TB</td>
<td>At 100% alcohols are a preservative!! Flammable.</td>
</tr>
</tbody>
</table>

** Concentration of named brands  ^ use 1/5 dilution

MIXED BIOLOGICAL/ CHEMICAL WASTE DISPOSAL

Mixed biohazardous and chemical waste should be first treated to eliminate the biological hazard. Then the waste can be handled as hazardous chemical waste. CAUTION: Some disinfectants, such as chlorine bleach, can react dangerously with chemicals. Check with the McLean Lab Safety Officer if you have any questions.

MIXED BIOLOGICAL/RADIONUCLIDE WASTE DISPOSAL

Mixed biohazardous and radionuclide waste should be first treated to eliminate the biological hazard. There are three steps in dealing with radioactive waste disposal:

1. Disinfect
2. Check radioactivity
3. Discard as radioactive

CAUTION must be taken, because it is FORBIDDEN to autoclave radioactive material without permission of the MGH Radiation Safety Office (617-726-5128). Also, do NOT disinfect iodinated compounds with chlorine bleach – this method of disinfection of materials labeled with I^{125} can release radioactive gaseous iodine.
Solid Radioactive Biowaste

• Radioactive solid waste should be rinsed (glass or plastic) or sprayed (paper) with chlorine bleach or a phenolic disinfectant. Let the disinfectant work for at least 20 minutes to ensure inactivation.

• After chemical disinfection, examine the items with a radioactivity monitor. If activity exceeds 1.5x the room background, treat the material as radioactive waste and follow proper disposal guidelines.

Liquid Radioactive Biowaste

• Most liquid infectious waste can be inactivated by treating with a 1/10 dilution of chlorine bleach for at least 20 minutes. Add the concentrated bleach to the waste until a final 1/10 dilution is reached. Most iodinated liquid wastes can be safely decontaminated with a 1/10 dilution of common phenolic household cleaners such as Lysol™.

• Monitor the liquid waste for the presence of radioactivity. If levels are within NRC limits, it may be possible to dispose of the isotopes in a designated sink. Be sure to record the disposal. If the activity exceeds the permissible sink disposal limits, pour the liquid waste into an unbreakable container filled with absorbent, and discard as radioactive waste. See the Radiation Safety Manual for further details.

BIOLOGICAL SPILL RESPONSE

Biological Spill Kits

Biological spill response kits should be kept in labs where biological material is used or stored. These kits should be checked frequently to assure the correct material is in place for emergency situations. Contents should include:

• Biological Spill Clean-up Procedure

• Personal Protective Equipment
  o Nitrile disposable gloves (8 mil thickness)
  o Safety goggles and/or Safety shield
  o N95 Dust mask respirator(s)
  o Lab coat/ gown or other covering
  o Disposable shoe covers (booties)

• Small disposable dustpan and broom, or tongs, forceps, or two pieces of cardboard (for picking up broken glass or other contaminated sharps)

• Red Biohazardous waste bags/Autoclavable biohazard bags

• Disinfectant agent suitable for the biological agents being used in the lab
  o Please note: Bleach is not suitable for all agents. Please be sure to check which disinfectant works for the agent you are using

• Spray Container for disinfectant

• Absorbent material, Paper towels or granular absorbent material, etc.

• Warning signs

• Storage container
If a biological spill results in exposure or injury, the exposure or injury should be dealt with immediately. Notify others in the immediate vicinity that there has been a spill and ask for assistance in the spill response while you are attending to the exposure or injury. Post a warning sign if you must leave and no one is available to help will clean-up procedures.

There are several biological spill response scenarios, some requiring a different response based on the hazard of the agent and risk of exposure.

**Biological Spill INSIDE a Biosafety Cabinet**

Regardless of the material or agent spilled inside the BSC, the response will be the same. The primary concern is to minimize exposure via skin contact or percutaneous injury from broken glass or sharps. The risk of exposure via inhalation is minimized by the BSC, which will contain any aerosols generated during the spill.

- Leave the cabinet turned on.
- Put on rubber gloves, lab coat and facial protection. Be sure that all exposed skin, which will be inside the BSC, is covered (i.e. arms and wrists).
- Place absorbent material (paper towels) on top of the spill; pour appropriate disinfectant over the absorbent materials.
- Let disinfectant sit for a minimum of 20 minutes.
- Spray or wipe the cabinet walls, work surfaces, and equipment with disinfectant. If Bunsen Burners are in use, be sure to extinguish prior to using disinfectant.
- If the spill is large, flood the work surface, as well as drain pans and catch basins below the work surface, with disinfectant.
- Drain the catch basin into a container.
- Lift front exhaust grill and tray, and wipe all surfaces.
- Take care that no paper towels or solid debris are blown into the area beneath the grill.
- Discard clean-up materials in biohazard waste bag.
- Wash hands and exposed skin areas with soap and water.
- Let the Biosafety Office know if the spill overflows into the interior of the cabinet. It may be necessary to do a more extensive cabinet decontamination.

**Biological Spill OUTSIDE a Safety Cabinet**

**Minor Spill:** A small volume usually 100mls or less that can easily be absorbed by a few paper towels

- Notify others in the area to stay away from the area to prevent contamination of additional personnel and environment.
- Remove any contaminated clothing and wash exposed skin with soap and water.
- Close door and post a warning sign on door if BL2 or greater.
- For BL2 or greater, if aerosols are created, allow aerosols to disperse and or settle for at least 30 minutes before reentering the laboratory, otherwise continue with clean-up procedure.
- Put on protective clothing (lab coat, N95 respirators/face protection, utility gloves, and booties if necessary).
- Cover the area with absorbent paper towels, and then carefully pour disinfectant around the spill. Avoid enlarging the contaminated area. Use more concentrated disinfectant as it is diluted by the spill. Allow at least a 20-minute contact time.
• Pick up any pieces of broken glass with dustpan, forceps or other mechanical means so that the glass will not come in contact with hands, and place in a sharps container.
• Discard all disposable materials used to clean up the spill into a biohazard bag.
• Wipe surrounding areas (where the spill may have splashed) with disinfectant.
• Wash hands with soap and water.

**Large BSL-1 Spill: Greater than 100mls**

Follow the same procedure for small BSL-1 spills. Even though the spill volume may be large, BSL-1 organisms are not known to cause disease in healthy humans and can be cleaned up safely by lab staff following the designated procedures.

**Large BSL-2 or BL2-S spill: Greater than 100mls**

• Immediately evacuate the lab.
• Any staff contaminated during the spill should remove the contaminated clothing prior to leaving the lab, and wash any exposed skin with soap and water.
• Notify PI and the McLean Emergency (x2222). Biosafety Office at MGH should be called (617-724-4579).
• Based on consultation with the Biosafety Officer, lab staff will be cleared to reenter the room to perform spill clean up. That person will:
  o Post warning sign on the door of the lab. Allow aerosols to settle, if any, approximately 30 minutes.
  o Put on protective clothing (lab coat, gloves and, if indicated, face protection, shoe covers, respiratory protection)
  o Assembles clean-up materials from Bio-spill kit (disinfectant, autoclavable container or bag, forceps and paper towels).
  o Surround the spill with a ring of disinfectant and mix it into the spill. Remember, the disinfectant will become diluted by the spill. Keep its concentration up by adding more concentrated disinfectant.
• After at least 20 minutes contact time, clean-up liquids with absorbent material and dispose of into the red biohazard waste container.
• Re-wipe spill area with disinfectant and dispose of this material into the red biohazard waste container.
• Remove protective clothing and dispose of into the red biohazard waste container.
• Wash hands thoroughly with soap and water.
• Document the spill and forward the information to the Biosafety Officer for review.

**Spills inside a Centrifuge**

• Centrifuges should be equipped with safety cups and rotors to aid in safe clean up if a tube breaks inside the centrifuge.
• Clear area of all personnel if BL2 spill or greater.
• Wait 30 minutes for aerosols to settle before attempting to clean up spill.
• Wear appropriate PPE, lab gown or coat, safety glasses,
• Remove rotors and buckets to nearest BSC for clean up.
• Thoroughly disinfect inside of centrifuge with appropriate disinfectant.
• Clean rotors and buckets with appropriate disinfectant inside the BSC.
• Discard contaminated disposable materials using appropriate biohazardous waste procedures.

Spills outside of Laboratory

• To prevent spills outside the laboratory, all transported biohazardous material must have been labeled with a biohazard sticker and contained inside a primary leak-proof container, then placed in a secondary unbreakable container with a lid. (plastic bin or bucket) and labeled with a biohazard symbol.
• If a spill does occur in a public area, secure the area and keep traffic away from the spill. Seek assistance if necessary.
• Do not attempt to clean the spill without appropriate PPE.
• Use appropriate spill procedures stated in this manual.

BIOLOGICAL/RADIOACTIVE MATERIAL SPILL RESPONSE

CALL EMERGENCY (x2222), the MGH Radiation Safety Office (617-726-5128), and the MGH Biosafety Office (617-724-4579). A biohazardous spill involving radioactivity requires emergency procedures that are different from those used for either material alone.

Before cleaning up, take a moment to think things over. Consider the type of radionuclide, the characteristics of the microorganism, and the volume of the spill. Remember, you can inactivate the biological part of the spill but you are stuck with the radioactivity.

Initial steps to take:
• Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave. Close the door and post warning signs.
• Remove contaminated clothing, turn exposed area inward and put it into a biohazard bag.
• Wash all exposed skin with disinfectant, followed by a 3-minute water rinse.
• Inform your supervisor and the MGH Radiation Safety Office of the spill, and monitor all exposed personnel for radiation.
• Allow aerosols to disperse for at least 30 minutes before reentering the laboratory. Assemble cleanup materials (disinfectant, autoclavable containers, forceps, towels, sponges, etc)
• Confirm with the Radiation Safety Office that it is safe to enter the lab.

Clean-up of Biological Radioactive Spill
• Put on protective clothing. If the spilled material contains airborne pathogens, wear an N95 class of HEPA filtered respirator. NOTE: Medical clearance and fit testing are required before wearing a respirator. Contact the Lab Safety Officer (Elena Chartoff, x2022) or a First Responder (FROL; list of FROLS posted on Chemical Spill cabinets).
• Cover the area with disinfectant-soaked towels, and carefully pour disinfectant around the spill. Avoid enlarging the contaminated area. Allow at least 20 minutes contact time.
• Handle any sharp objects with forceps.
• Wipe the surrounding area, where the spill may have splashed, with disinfectant.
• Soak up the disinfectant and the spill and place the decontaminated materials into an approved radiation waste container and label according to Radiation Safety guidelines.
• Wash your hands and other exposed skin areas with disinfectant. Monitor personnel and spill area for residual radioactive contamination.

DO NOT AUTOCLAVE CONTAMINATED WASTE UNLESS APPROVED BY THE MGH RADIATION SAFETY OFFICE AT 617-726-5128

AUTOCLAVES

Autoclaves work by denaturing biological molecules with superheated steam. Dry heat is not as effective. For example it takes 12 minutes to kill most spores with steam at 121°C while 6 hours are required for dry heat at the same temperature. Autoclaves will sterilize on the basis of:

• Length of time in the cycle
• Temperature
• Contact
• Pressure
• Steam

Autoclave Procedures:

• Bags and containers must be autoclavable.
• Prior to autoclaving the Biohazard bag must be keep closed to prevent airborne contaminants and odors form escaping.
• Leave bag open to allow steam to come in contact with contents during cycle.
• It is recommended to add water to the inside of the bag before autoclaving.
• Do not overload the bag.
• Do not put sharps in the bag.
• Do not mix solids and liquids to be autoclaved in the same bag. Liquid media requires a shorter cycle 15-20 minutes. Solid waste can take up to 1 hour.
• Do not place volatile chemicals in the autoclave, including bleach.
• Place autoclave tape on the outside of the bag. Autoclave tape tells you that a critical temperature was reached. It does not indicate the length of time at the temperature or whether steam was present.
• Place autoclave bags inside a secondary container such as plastic or metal trays that are designed for use in the autoclave.
• Wait for autoclave to cool down before removing items.
• Use heat resistant gloves when unloading autoclave.
• Validate autoclave effectiveness once every month using a Biological Indicator (spore testing).
• Place biological indicator in the center of the load when performing validation tests.
Spore Testing

- Autoclave should be tested and validated regularly.
- Test with a commercial spore culture system (Attest from 3M). These are ampules of Bacillus stearothermophilus in a color indicator solution. The ampules are autoclaved under realistic conditions (usually in the middle of a bag of waste).
- Ampules are incubated for two days at 56°C.
- Record validation results in a log book for inspections.

BLOODBORNE PATHOGENS

OSHA published a Bloodborne Pathogen Standard in December 1991. The Standard has the force of law and must be obeyed by any institution working with blood, blood products, bloody tissue and most body fluids.

- Infection Control has a written Bloodborne Pathogen Exposure Plan located in the Infection Control Manual, which can be obtained through Research Administration and on the McLean intranet (to be up and running 2010). The Objective of the plan is to protect our employees form health hazards associated with Bloodborne pathogens and to provide treatment and counseling should an employee be exposed to Bloodborne pathogens.
- Employees must attend Bloodborne pathogen training annually – now online via HealthStream.
- Standard Precautions –
  - All samples from humans and non-human primates must be treated as potentially infectious.
  - Materials to be handled using Standard precautions
  - All human and non-human primate blood, blood products, other body fluids, non-intact skin, and any unfixed human and non-human primate tissue or organ including both primary and established human cell lines.
- Recommended personal protective equipment to minimize exposure:
  - Gloves - change them whenever they become contaminated.
    Utility gloves - heavier than latex gloves; may be disinfected for reuse if the glove is not cracked, peeling, or torn.
  - Masks, eye protection, face shields - worn whenever splashes spray and/or droplets are a possibility.
  - Lab coats, gowns, aprons - to protect skin surfaces and street clothing.
- Signs and labels
  - The biohazard symbol (see the front cover) must be on containers of biohazardous waste, and on refrigerators, centrifuges and other equipment where blood and other potentially infectious materials are stored.
- Housekeeping and waste disposal
  - Plastic-backed paper may be used to cover benches, but it should be replaced when contaminated, or at the end of the day.
Bench tops should be washed and disinfected at the end of an experiment and after a spill.
Decontaminate reusable items before washing.
Sharps containers should be placed where they can be reached easily when needed (not hidden behind other equipment).
All needles, syringes, razor blades, scalpels, and small pieces of glass such as Pasteur pipettes and slides should be discarded in these containers.
Broken glass that has come into contact with blood, tissue, or other potentially infectious materials is to be discarded in puncture-proof bio-hazard containers.

Potential exposures should be reported to your supervisor. Contact the McLean Occupational Health Services for post-exposure treatment and evaluation (x2438).

HEPATITIS B VIRUS

Hepatitis B infection often causes severe liver disease. Most people recover completely but the infection may incapacitate a person for several months. Hospitalization is required in about 20% of all clinically apparent cases. Rarely, a severe form (85% fatal) of the infection may result.

About 10-15% of those infected with Hep. B virus develop chronic hepatitis, which can progress to a more severe disease such as cirrhosis, or remain clinically asymptomatic. Individuals with chronic hepatitis may be carriers and may transmit the disease to sexual partners, family members, and health care workers. Most Hep. B carriers do not know they harbor the virus. Body fluids, blood, and other tissues from these people are a hidden threat to health care or laboratory workers. The virus that causes this infection is carried primarily in the blood. It does not penetrate intact skin. Direct inoculation of blood under skin or on a mucus membrane is required.

It should be remembered that, even if you are vaccinated and protected against the Hep. B virus, you should still handle blood products with Standard Precautions because you are NOT protected against other possible bloodborne pathogens such as human immunodeficiency virus (HIV), cytomegalovirus (CMV), or hepatitis C viruses.

Recombivax-HB Hepatitis B vaccine
Recombivax-HB is a non-infectious vaccine derived from Hep. B surface antigen (HBsAG) produced in yeast cells. Vaccination side effects are extremely rare. Clinical studies have shown that a complete Recombivax-HB vaccination series induces protective levels of antibody in at least 90% of healthy adults. The recommended course consists of 3 intramuscular injections in the deltoid (arm) muscle at 0, 1, and 6 months. A single booster is recommended every 7 years.

HEPATITIS C VIRUS
With the general use of Hep. B vaccine and the subsequent decline in Hep. B incidence, Hepatitis C has become a relatively significant problem among health care workers. There is no vaccine to combat Hep. C. Because the virus mutates quickly it is unlikely that a vaccine will be developed soon.

Hepatitis C is an RNA virus communicated through blood to blood transmission. It is the major cause of post-transfusion Hepatitis in the USA. However, this only accounts for about 5% of the total incidence. As with other viral bloodborne diseases it is most commonly found among injecting drug users and the sexually promiscuous. The disease is commonly found in low socioeconomic status areas.

The most common clinical symptom is jaundice. Chronic infections develop in about 85% of those infected and chronic liver disease eventually appears in 70% of infections. Over all, mortality is about 10%. There is no effective treatment for Hep. C. Antiviral agents and interferon have only limited benefits.

**Diagnosis**

An Enzyme-Linked Immunosorbent Assay (ELISA) for Hep. C is now commonly used to screen blood. This has had a dramatic effect on the incidence of transfusion related transmission. More recently developed PCR tests are also a useful diagnostic tool.

### PREGNANCY

Several infectious diseases are known to affect embryonic fetal development. Women of childbearing age should be aware of the risks associated with studies using these agents.

For the infectious agent to affect embryonic development the disease must be transmitted to the child. In some cases transmission is via the blood through the placenta. If the mother gets sick the child gets sick. Rubella is transmitted in this way. Genital herpes simplex virus, on the other hand, is physically transmitted from the vagina through the cervix to the placenta, and then to the child. In addition the virus can infect a child during vaginal birth or via breast milk.

List of infectious organisms thought to have some adverse effects on human embryo and fetal development:
- Coxsackie virus type B
- Hepatitis B virus
- Herpes Simplex Virus
- Herpes Zoster (Shingles)
- Human Parvovirus B19
- Human Immunodeficiency Virus (HIV)
- Influenza
- Rubella Virus
- Toxoplasma gondii
- Treponema pallidum
- Tuberculosis
- Varicella virus
- Venezuelan Equine Encephalitis virus
These diseases are known to cause birth defects in animals but have not yet been shown to be teratogenic in humans:
Lymphocytic Choriomeningitis
Influenza
Bluetongue virus
Mumps virus
Newcastle Disease virus
Parainfluenza type 2
Feline Panleukopenia virus
Salmonella typhimurium & enteritidis (“Rat virus”)  
Rodent Parvovirus (Minute Virus)
Reovirus type 1
Bovine diarrhea-mucosal disease virus
Hog Cholera virus

**NAKED DNA**

There have been occasional reports that the application of bare DNA to the skin can transform or otherwise affect dermal cells. For example, Burns et al.\(^1\) applied DNA containing an activated oncogene to scarified mouse skin and after 9 weeks found tumors at the site. Skin samples were shown to express the oncogene.

Antibiotics have been found to carry DNA coding for antibiotic resistance sequences from the organism that produced the antibiotic\(^2\). It is possible that this DNA is responsible for some of the widespread antibiotic resistance currently affecting clinical practice today. Therefore, naked DNA can have important environmental consequences.


**SHIPPING AND RECEIVING**

There are specific training requirements for shipping and receiving infectious substances, patient specimens, biological products and recombinant organisms. The U.S. Public Health Service (PHS), U.S. Department of Transportation (DOT), U.S. Department of Agriculture (USDA), and the U.S. Postal Service regulate transport of hazardous materials by rail, air, vessel, and public highway. The International Air Transport Association (IATA) and International Civil Aviation Organization (ICAO) guidelines and regulations apply when shipping by air. Import/Export Permit requirements are regulated by the Bureau of Customs; the Department of Commerce, CDC, and USDA require permits for certain agents.

Prior to any hazardous materials shipment, the shipper must be trained and the training documented. The Biosafety Officer can provide you with this training. Please contact EHS at 617-726-2425 for more information.
EQUIPMENT DISPOSAL

Biosafety Cabinets

- Biosafety cabinets must be decontaminated with paraformaldehyde before being removed from the laboratory. BSC paraformaldehyde decontamination must be done by a certified professional.
- The exterior of the BSC should be decontaminated with a suitable disinfectant.
- All stickers should be removed from the exterior surfaces.
- The PI should retain confirmation (receipt) from service contractor that the BSC has been professionally decontaminated.

Laboratory Equipment: incubators, freezers, refrigerators...

- Wear appropriate PPE: Lab coat, gloves, safety glasses
- Remove all contents; specimens and/or laboratory materials. Dispose of properly.
- Remove all labels or stickers form the outside of the equipment.
- Clean all surfaces both inside and out using an appropriate disinfectant.
- Put all cleaning waste (paper towels, sponge...) in the red biohazard waste containers.
- Affix a certificate stating that you have decontaminated the equipment designated for removal; this should include a responsible person's signature. The form can be found in Appendix A of this manual.
Massachusetts General Hospital

Laboratory Equipment Decontamination Form

Equipment Owner
Date: __________________
Principal Investigator: ____________________________________________
Laboratory Manager: ________________________________________________
Department: ______________________________________________________
Building/Room number: _____________________________________________
Phone number: _____________________________________________________

Potential Contaminants
Biological: _________________________________________________________
Chemical: _________________________________________________________

Decontamination Procedure Performed (include name of product used, remove all hazard labels)
____________________________________________________________________
____________________________________________________________________
____________________________________________________________________

Equipment Service/Disposal
Type of Equipment: _________________________________________________
Serial Number: _____________________________________________________
Department/Company Providing Service: _______________________________
Service Provided: ____________________________________________________
____________________________________________________________________
____________________________________________________________________

EQUIPMENT OWNER: I certify that the above equipment has been cleaned and decontaminated for hazardous chemical and biological agents.

Signature and date: ________________________________________________
Appendix B

Good Microbiological Practices

1. Always keep the laboratory door closed when working with pathogenic material.

2. Only authorized personnel should be in the laboratory.
   • Only individuals advised of the potential hazards should be allowed in the laboratories.
   • Access to animal facilities, BL2-S (and greater) and select agent labs are restricted.
   • Children are not permitted in working laboratory areas.

3. Always use a pipetting aid. Never mouth pipette. Make sure the pipettes are not cracked or chipped at the suction ends. They can damage the seating seals of the pipetting aids and create a hazard.

4. Never eat, drink, apply cosmetics, insert or remove contact lenses, or take medication in the laboratory.

5. Use aseptic technique.
   • All techniques and procedures should be performed to minimize aerosols and splashes.
   • Biosafety cabinets should be used whenever there is the potential for aerosols and splashes to occur.
   • Use BSCs when high concentrations and large volumes of infectious agents are used.
   • Micro-incinerators or disposable transfer loops should be used in BSCs, not Bunsen burners.

6. Thoroughly wash hands after handling cells, mammalian tissues, body fluids, microorganisms, and when leaving the laboratory.

7. Wear proper PPE when working in the laboratory.
   • Lab coats and solid front wrap-around gowns should be worn in the laboratory only, remove when leaving the laboratory.
   • Gloves should be used for all procedures that may involve contact with blood or body fluids, infectious materials or animals. Gloves should be disposed of as biohazardous materials and placed in red biohazard buckets.
   • Safety glasses and face shields should be worn to protect from the possibility of splashes or other harmful substances.
   • Close toed shoes or shoe covers should be worn in the laboratory.

8. Sharps should be handled with great care. Use safety needles and syringes whenever possible. All sharps, needles, glass, razors, etc. should be placed in appropriate sharp-proof autoclavable containers.

9. Biosafety Level 2 and BL2-S laboratories must have a sign posted on the entry door stating
   • The Biohazard Level
   • agents in use
   • responsible person’s contact information
   • Universal Biohazard Symbol

10. Clean laboratory thoroughly with an approved agent-specific disinfectant. Keep
laboratory neat and free of clutter and materials not pertinent to the work. Intensive cleaning must be done on regular intervals.

11. Always use safe practices during laboratory operations.
   • The PI must provide appropriate training for lab personnel.
   • All lab personnel should attend annual laboratory safety training.
   • The individual laboratory Biosafety Manual should reflect lab specific practices and procedures.

12. Annually certify all Biosafety Cabinets, autoclaves, Clean benches etc.

13. Use Biological Indicators when operating an autoclave. Log books must be kept.

14. Shipping of Biological materials and agents must conform to the latest U.S. Federal Regulations. Anyone shipping or receiving Biological materials should attend a Shipping Training Class.

15. Report all biological spills to the PI.

16. All laboratories are subject to annual inspections by the Biosafety Office.
## Appendix C

### Laboratory Inspection Checklist

Building/Room: __________________________ Date: __________________________

Biosafety Level: _________________________ Conducted By: ____________________

PI:____________________________________ Contact Person: ________________

**Biohazardous Material used in lab:**

<table>
<thead>
<tr>
<th>ITEM</th>
<th>YES</th>
<th>NO</th>
<th>NA</th>
<th>COMMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. Persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. A biohazard sign must be posted on the entrance to the laboratory when biohazardous agents are in use.</td>
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<td>4. The laboratory director ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.</td>
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<tr>
<td>5. Laboratory Benches/work surfaces are cleaned daily. <strong>Method:</strong></td>
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<tr>
<td>6. Persons wash and/or disinfect their hands after they handle viable materials, after removing gloves, before leaving the laboratory and any other appropriate time.</td>
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<tr>
<td>ITEM</td>
<td>YES</td>
<td>NO</td>
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<td>COMMENT</td>
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<td>7. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the work areas. Persons who wear contact lenses in laboratories should also wear goggles or a face shield.</td>
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<td>8. Mouth pipetting is prohibited; mechanical pipetting devices are used.</td>
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<tr>
<td>9. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.</td>
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<td>10. Biosafety Cabinets (BSC) are used to contain aerosols. BSCs are certified at least annually.</td>
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<tr>
<td>11. BSC surfaces are decontaminated at least once a day and after any spill of viable material. Disinfectant used:</td>
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<tr>
<td>12. All solid biohazardous waste is disposed of in red biohazardous bags. It is recommended that labs decontaminate their biohazardous waste before discarding.</td>
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<td>13. Liquid biowastes are decontaminated by autoclaving or chemical disinfection prior to sink disposal. Method:</td>
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<tr>
<td>14. Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.</td>
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<tr>
<td>15. Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory.</td>
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<tr>
<td>16. Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face</td>
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<td>17. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment.</td>
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<tr>
<td>18. Gloves are worn once and then discarded in Biohazard waste boxes.</td>
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<tr>
<td>19. The one glove rule is in affect and lab staff is knowledgeable in this rule.</td>
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<td>ITEM</td>
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<td>20.</td>
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<td>Equipment in which biohazardous material may be present is labeled with the biohazard symbol</td>
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<td>21.</td>
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<td>If biohazardous material is centrifuged outside of a BSC, safety containment cups or sealed rotors with O-rings must be used.</td>
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<td>22.</td>
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<td>Vacuum line filter protection is in place</td>
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</tbody>
</table>
| 23.  |     |    |    | **Glassware is decontaminated before washing.**  
Method: |
| 24.  |     |    |    | Biological spill kit available and properly stocked |
| 25.  |     |    |    | Personnel are knowledgeable about biological spill response |
| 26.  |     |    |    | Accidents are reported to the lab director immediately. Written records are maintained. |
| 27.  |     |    |    | Chemical Hygiene Plan available |
| 28.  |     |    |    | CHP complete and reviewed annually |
| 29.  |     |    |    | Staff knowledgeable about CHP |
| 30.  |     |    |    | Hazardous Chemical Inventory available and updated yearly |
| 31.  |     |    |    | MSDS’s maintained and accessible |
| 32.  |     |    |    | Staff knowledgeable about MSDS’s |
| 33.  |     |    |    | Training Documented for:  
New Employee Orientation  
Lab specific training by PI or designee  
Annual Safety Training |
<p>| 34.  |     |    |    | Emergency Shower available, unobstructed, visually identified and inspected |
| 35.  |     |    |    | Eyewash stations are available, run once a week and documented. |
| 36.  |     |    |    | PPE available and used appropriately |
| 37.  |     |    |    | Closed toed shoes worn at all times |
| 38.  |     |    |    | Fire doors are not propped open. |
| 39.  |     |    |    | Egress route is at least 36” wide. |
| 40.  |     |    |    | Evacuation/Fire Plan is posted in highly visible area and is up to date. |
| 41.  |     |    |    | Personnel are knowledgeable about evacuation plan. |</p>
<table>
<thead>
<tr>
<th>ITEM</th>
<th>YES</th>
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<th>COMMENT</th>
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<tbody>
<tr>
<td>42. Fire safety officer is designated.</td>
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<td>43. Fire extinguishers are available and inspected monthly.</td>
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<td>44. NFPA Diamond posted and current</td>
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<td>45. Fume Hoods are functional, certified annually, free of excess storage and used appropriately</td>
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<td>46. Acids and Bases are segregated and labeled</td>
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<tr>
<td>47. Organic and Inorganic Acids are segregated</td>
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<tr>
<td>48. Flammable liquids and oxidizing agents are segregated</td>
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<td>49. Chemicals are stored away from heat sources or direct sunlight</td>
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<td>50. Benchtop storage of chemicals is limited to small amounts</td>
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<td>51. Containers are stored upright</td>
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<td>52. Flammable amounts are within storage limit for area</td>
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<tr>
<td>53. Flammable liquids are stored in rated cabinets/refrigerators</td>
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<tr>
<td>54. Flammable liquids are stored away from ignition sources</td>
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<tr>
<td>55. Chemical Spill kit available and appropriate</td>
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<td>56. Personnel trained and knowledgeable on spill procedures</td>
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<td>57. Compressed gas cylinders properly secured with one cylinder per securing device</td>
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<td>58. Compressed gas cylinders are labeled, marked full or empty</td>
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<td>59. Liquid nitrogen properly stored</td>
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<td>60. Personnel knowledgeable of Mercury Reduction Program</td>
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<td>61. Mercury Spill Kit if Mercury is located in the lab</td>
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<td>62. Satellite Accumulation Area located in appropriate area and visibly identified</td>
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<td>63. Hazardous waste container compatible with waste being stored and in good condition</td>
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<td>ITEM</td>
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<td><strong>64.</strong> Incompatible chemical wastes are segregated</td>
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<td><strong>65.</strong> Chemical waste containers labeled appropriately</td>
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<td><strong>66.</strong> Hazardous waste labels visible and legible</td>
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<td><strong>67.</strong> Hazardous waste containers closes whenever not in use</td>
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<td><strong>68.</strong> Secondary containment appropriate</td>
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<tr>
<td><strong>69.</strong> Personnel are knowledgeable of hazardous chemical waste disposal procedures</td>
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</table>

If autoclaves are used to decontaminate:

   Cycle length:____________________@__________________(temperature).

   Location:______________________________

   Is the autoclave method of decontamination validated by spore testing on a regular basis?