MIT BIOSAFETY MANUAL

MIT Biosafety Program

Biosafety Program
Environmental Health and Safety Office
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1 Important Phone Numbers

Environmental Health and Safety Office	617 452-3477
MIT Police	617 253-1212
MIT Police Emergency Number (on-campus)	100
Medical - 24 hour Emergency	617 253-1311
Facilities	617 253-4948 617 253-1500

2 Abbreviations

ATCC	American Type Culture Collection
BL	Biosafety Level
BBP	Bloodborne Pathogens
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BPS	Bloodborne Pathogens Standard (OSHA)
BSC	Biosafety Cabinet
BSO	Institutional Biosafety Officer
BSP	Biosafety Program
CAB/ESCRO	Committee on Assessment of Biohazards and Embryonic Stem Cell Research Oversight
CAC	Committee on Animal Care
CBC	Cambridge Biosafety Committee
CDC	Centers for Disease Control
CFM	Cubic feet per minute
CMV	Cytomegalovirus
COUHES	Committee on the Use of Humans as Experimental Subjects
DCM	Division of Comparative Medicine
DLC	Departments, Labs and Centers
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GMBH
DURC	Dual Use Research of Concern
EBV	Epstein Barr Virus
ECP	Exposure Control Plan
EHS	Environmental Health and Safety Office
EMP	Environmental Management Program
FPM	Feet Per Minute
IBC	Institutional Biosafety Committee
IHP	Industrial Hygiene Program
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HEPA	High Efficiency Particulate Air
HIV	Human Immunodeficiency Virus (the numbers 1&2 refers to different strains)
HTLV	Human T Cell Lymphotropic Virus (the numbers 1&2 refer to different strains)
MIT	Massachusetts Institute of Technology
NAS	National Academy of Sciences
NIH	National Institutes of Health
NSF	National Sanitation Foundation
OBA	Office of Biotechnology Activities (NIH)

OSHA	Occupational Safety and Health Administration
PI	Principal Investigator
rDNA	Recombinant DNA
RPP	Radiation Protection Program
rRNA	Recombinant RNA
WIBR	Whitehead Institute for Biomedical Research
USDA	United States Department of Agriculture

3 Introduction

It is the policy of the Massachusetts Institute of Technology (MIT) that the Principal Investigator (PI) is the responsible person for all activities that occur in his or her laboratory. However, safety is a shared responsibility among all of the laboratory staff. Individuals are expected to act in a responsible manner while working in the laboratory. Researchers are expected to follow established and agreed upon procedures, use personal protective equipment (PPE) as appropriate to the risks inherent in the research and as established as policy by the PI, clean up after each experiment, report problems to their supervisor, and notify their supervisor and/or the Director of the Occupational Medicine at 3-8552, of any medical situation that they are experiencing which may have an impact on their safety in the laboratory. Many resources exist at MIT to assist members of the Institute in multiple aspects of their work and home lives. If you have questions or concerns, please do not hesitate to contact the Biosafety Program at x2-3477 or x3-1740 or bsp@mit.edu. We can refer you to, or assist you in, finding the needed MIT resources.

Much of the information contained in this document is taken from the National Institute of Health (NIH) and Centers for Disease Control (CDC) publication *Biosafety In Microbiological and Biomedical Laboratories (BMBL),* 2009. The information contained in the BMBL will not be copied in detail into this document. Instead, this manual is meant to be used as a guide to web-based materials and information and will not merely reiterate already available text. The web-links will be given so that MIT personnel can readily find the detailed information.

Work with vertebrate animals is covered separately by the Division of Comparative Medicine (DCM), at x3-1756. Information on CAB/ESCRO Policies concerning use of biological materials including rDNA/synthetic nucleic acids in animals can be found at the CAB/ESCRO web page (http://web.mit.edu/cab/).

If you need additional information or have questions, contact BSP at 617-452-3477.

4 Responsibilities

4.1 Massachusetts Institute of Technology

MIT is responsible for ensuring that all research is carried out in a safe and prudent manner and in full conformity with the provisions of Federal, State and Local regulations such as the Cambridge Ordinance, the State Plumbing Code, OSHA BBP Regulations, and MA State Infectious Waste and Sanitary Laws. In order to fulfill this responsibility, MIT has:

Established and implemented policies that provide for the safe conduct of research and that ensure compliance with the applicable regulations. MIT, as part of its general responsibilities for implementing these regulations, has established additional procedures deemed necessary to govern the Institution and its components in the discharge of its responsibilities under these regulations.

Established an IBC, the Committee on Assessment of Biohazards and Embryonic Stem Cell Research Oversight that meets the requirements set forth by the NIH, the City of Cambridge, and the recommendations of the NAS.

Required investigators engaged in research subject to the NIH Guidelines, OSHA Bloodborne Pathogen Standard (OSHA BBP Standard), the City of Cambridge Recombinant DNA ordinance and other

regulations to comply with the provisions of those regulations. MIT Biosafety Program will assist investigators in meeting their responsibilities.

Ensured appropriate training for research personnel regarding the NIH Guidelines and OSHA BBP standard, their implementation, and laboratory safety. Responsibility for training laboratory staff is carried by the PI but may be delegated to the Biosafety Program.

Assigned the responsibility for determining the necessity for health surveillance of research personnel to the CAB/ESCRO and the MIT Medical Department Occupational Health group.

Assigned to the CAB/ESCRO the responsibility for reporting within 30 days to the NIH's Office of Biotechnology Activities (OBA), and the City of Cambridge Biosafety Committee any significant research related accidents or illnesses and problems with, or violations of the NIH Guidelines.

4.2 Membership and Procedures of the CAB/ESCRO

MIT has established the CAB/ESCRO, which meets the NIH Guidelines, the City of Cambridge Ordinance, and the National Academy of Science requirements for an IBC and an ESCRO committee. For detailed information about the scope, policies and procedures of the CAB/ESCRO, please go to the CAB/ESCRO web page (http://web.mit.edu/cab/)

The CAB/ESCRO comprised of members selected to collectively provide experience and expertise appropriate to assess the safety of research experiments performed by MIT investigators and any associated potential risk to public health or the environment. At least 2 members are not affiliated with MIT (apart from their membership on the IBC) and represent the interest of the surrounding community with respect to health and protection of investigators and the environment. Membership is structured to meet the NIH requirements as outlined in the NIH Guidelines and certain MIT recommendations. For example, MIT asks that all Presidential committees have a member representing graduate students and postdoctoral fellows.

No member of the IBC is involved (except to provide information requested by the IBC) in the review or approval of a project in which he or she is, has been, or expects to be engaged, or has a direct financial interest.

MIT has made available to the Cambridge Biosafety Committee (CBC) all minutes of IBC meetings and any documents submitted to or received from funding agencies which the former are required to make available to the CBC (e.g., reports of Guideline violations and significant research related accidents, and agency directives to modify projects).

4.3 Functions of the CAB/ESCRO

The CAB/ESCRO is responsible for: reviewing, for compliance with the NIH Guidelines and the City of Cambridge rDNA Ordinance, all rDNA and synthetic biology research conducted at or sponsored by MIT; for approving those research projects that it finds are in conformity with the NIH Guidelines and CBC; and the expected best practices and procedures.

The committee review includes:

An independent assessment of the containment levels required for the proposed research and of potential DURC issues associated with the proposed work.

An assessment of the facilities, procedures, and practices, as well as the training and expertise of personnel.

Notification to the PI of the results of their review.

Continuing period review of biological research being conducted at MIT to ensure the requirements of the NIH Guidelines, Cambridge rDNA Ordinance, and other relevant regulations are being fulfilled.

Assessment of emergency plans covering accidental spills and personnel contamination resulting from such research. Guidance information on these procedures is available in this document and via the CAB/ESCRO web page.

An assessment of the appropriate immunizations and vaccines to be offered to research personnel based on the nature of the work.

4.4 Principal Investigator (PI)

On behalf of MIT, the PI is responsible for complying fully with both the NIH Guidelines and City of Cambridge Ordinance for research that involves rDNA and synthetic biology and all other applicable local, state and federal regulations. Please see Section IV B 5 of the NIH Guidelines for specific PI responsibilities. Each PI is responsible for the preparation and submission of a written Biological Research Registration the covers all proposed biological research experiments. Forms are available on the CAB/ESCRO web site (http://web.mit.edu/cab/), from BSP staff, or from the BSO. A completed registration must be sent to the Biosafety Program for inclusion on the agenda for the next committee meeting. CAB/ESCRO review and approval must be obtained prior to initiation of the research project.

Each PI is responsible for reporting any significant alteration or modification of a registered biological research registration to the Biosafety Program for review and approval prior to implementing the changes or modifications. Depending on the extent of the change(s), a new or revised application maybe required. The Biosafety Program will advise the PI accordingly. All amendments are reviewed by the CAB/ESCRO or may be given administrative approval by the Institutional Biosafety Officer. When documentation of registration submission and approval is required by an outside contractor, institution, or granting agency, the BSO can provide the required information.

What follows are some of the other important responsibilities.

Refrain from initiating or modifying biological research subject to the NIH Guidelines or Cambridge Ordinance until that research, or the proposed modification has been approved.

Be adequately trained in good microbiological techniques.

Adhere to CAB/ESCRO approved emergency plans for dealing with accidental spills and personnel contamination.

Comply with shipping requirements for recombinant or synthetic materials, microbes, animal and human tissues and cells.

Make the initial determination of the required levels of physical and biological containment in accordance with the NIH Guidelines.

Select appropriate microbiological practices and laboratory techniques to be used in the research. Ensure the integrity of containment measures and procedures, and the genotypic and phenotypic characteristics of the biological materials in use in the laboratory.

Make available to the laboratory staff copies of the approved biological research registrations that describe the potential biohazards and the precautions to be taken.

Instruct, train, and supervise the staff in the practices and techniques required to ensure safety and in the procedures for dealing with accidents. Correct work errors and conditions that could result in breaches of the NIH Guidelines and Cambridge Ordinance.

Notify the CAB/ESCRO of any changes in personnel or research location.

5 Personnel Training Requirements

5.1 Research Personnel

All MIT research personnel must have General Biosafety training prior to starting work in their respective labs. Biosafety refresher training is also offered to lab groups along with either the annual OSHA BBP retraining or separately at the request of the laboratory PI. Staff members must be adequately trained prior to beginning any work with etiologic agents, recombinant or synthetic nucleic acid containing organisms, and human source materials (OSHA required training must be documented, contact the Biosafety Program at 3 1740 for details). This training will be provided on an individual basis either by the PI or Biosafety Program staff.

Lab group training for researchers will include:

Good aseptic technique

The biology of the materials and/or microbes used in experiments with emphasis on potential biohazards

Proper techniques and disinfectants for decontamination/disinfection of lab benches, cultures, glass, etc.

Appropriate waste disposal procedures

Emergency procedures, spill clean-up, response to personnel contamination

Development of a culture of safety within the group

5.2 Training Requirements for Non-technical Staff:

All non-technical staff members are familiarized with the potential hazards associated with biological research in general. This is a basic "awareness" training. All such workers are shown the universal biohazard signs and instructed to avoid, if at all possible, areas posted with such signs. In general, all non-technical staff members (except glassware washers and custodial staff) should not enter the laboratory research area unless properly supervised by a technical staff member or a member of the EHS Office.

In addition to the above training, custodians and glassware washers are familiarized with the laboratory area where they must go in order to perform their duties. They are instructed not to touch anything on laboratory benches, equipment, nor handle any containers designated as radioactive, chemical, or biological waste, and to avoid refrigerators, freezers, and other containers designated for storage of biological materials including rDNA/synthetic nucleic acids. All custodians, repair and maintenance personnel, grounds staff, campus police, housekeeping, residence staff, and athletics trainers are given BBP training and offered the Hepatitis B (HBV) vaccination. They are also informed of what to do in case of an incident, accident, or possible exposure.

All non-technical staff are prohibited from entering any areas designated for BL2+ activity unless accompanied by a member of the laboratory or the EHS Office.

Glassware washers are fully informed of all waste disposal procedures, particularly autoclaving methods, as well as emergency procedures for handling spills of biological materials including rDNA/synthetic nucleic acids.

6 Medical Surveillance and Occupational Health

All workers are responsible for reporting changes in their health status that may affect their safety within the laboratory. Workers should discuss any special medical situations with both their supervisor and the Director of the MIT Occupational Health program at 3-7625. Such circumstances may include pregnancy, skin cuts, or abrasions which may provide a route for infection, long term treatment with antibiotics, immunodeficiency or immunosuppression. It is important that your supervisor and/or the

Occupational Health physician and Nurse Practitioner are aware of any concern you may have so that the situation can be evaluated.

MIT provides free medical monitoring to all employees who face workplace risks. The program is designed to monitor potential health hazards associated with research and development activity, including rDNA, synthetic nucleic acids, etiologic agents, human materials, toxins, controlled substances, nanoparticles, hazardous chemicals and heavy metals. The program is coordinated by an occupational physician. A serum sample may be drawn from an employee working with certain classes of organisms and stored to provide a reference should any medical problems arise.

If personnel have health concerns that may result in an increased risk when working in the laboratory, they should consult with a physician and other health and safety professionals. This team will do a thorough risk assessment, and make recommendations that will reduce the risks as needed. BSP will work with the PI to ensure that all laboratory personnel are aware of the risks inherent in the ongoing and proposed research, and any mitigation measures that might be needed.

Any changes in the health status of research personnel or illness which lasts four days or longer should be reported to the PI and MIT Medical Dept. If there is any suspicion of rDNA/synthetic nucleic acids involvement, a physician will be consulted. If it is determined that the illness is related to working with rDNA or synthetic DNA containing organisms then the results will be reported to the CAB/ESCRO, CBC, and NIH OBA and the Cambridge Public Health Dept.

If a safe and effective vaccine is available against an organism with which a person must work, MIT will provide it for free and at the person's option. The most common example is Hepatitis B immunization. Personnel using human source materials are urged to be immunized against Hepatitis B. Participation in this program is voluntary and individuals should assess the risk/benefit ratio after consultations with health care providers. The vaccination series is free of charge and administered by Medical Department personnel. Following the final injection, the CAB/ESCRO recommends that individuals have a blood sample taken in order to evaluate the immunization effectiveness. The post-immunization titer is free of charge as well.

If unusual treatments or vaccines are needed in case of an accident, the Medical Department should be apprised of the situation before work begins in order for the appropriate materials to be available for emergency treatment.

If an accident, injury or potential exposure to any biological material including rDNA/synthetic nucleic acids occurs in the laboratory personnel are expected to wash or flush the affected area with water, inform the lab manager or PI, and seek medical attention at MIT Medical. The supervisor or lab manager or PI will complete a supervisor’s report of injury. If this did involve an exposure to biological material including rDNA and synthetic nuclei acids, a PDF of the Medical Dept assessment form will be sent to the Biosafety Program for incident follow up. If the exposure involved recombinant or synthetic nucleic acid materials a report to NIH and CBC will be sent.

7 Principles of Biosafety

Many microorganisms and viruses can be infective under certain conditions. Persons working with microorganisms should be aware of the specific risks of their work and take proper precaution. Infection may occur through inhalation, ingestion, exposure to mucous membranes, cutaneous absorption, or percutaneous exposure. Safety precautions must be taken to ensure that experiments are conducted in such a way that prevents exposure via these routes of infection. Furthermore, mechanisms must be in place to prevent microorganisms and viruses from contaminating the laboratory environment or escaping the laboratory and exposing people or the community.

7.1 Risk Assessment

The risk assessment of laboratory activities involving the use of infectious microorganisms or rDNA/synthetic nucleic acids is an iterative process that should be data driven based on a thorough understanding of the research technologies and procedures and how these might create opportunities for potential exposures. The diversity of projects within the Institute makes the classification of experiments and agents into discrete categories and interesting challenge.

A generic risk classification of microbial agents has been done by the NIH and CDC. The risk group classification is based on the morbidity and mortality associated with each agent in humans. This risk group classification is an important piece of information about an infectious agent but does not take into account the special conditions and procedures used in microbiological research laboratories. A comprehensive risk assessment must include a thorough understanding of the research. MIT has only one RG3 virus (HIV) on campus. All other biological agents are RG2 or lower, although the CAB/ESCRO does sometimes require additional containment measures for work with certain RG2 agents, e.g. MRSA or HCV.

Table 1. Classification of Infectious Organisms by Risk Group

Risk Group Classification	NIH Guidelines for Research 2013	World Health Organization Biosafety Manual 4th edition, 2009
RG1	Agents not associated with disease in healthy adult humans.	No to low individual and community risk. A microorganism unlikely to cause human or animal disease.
RG2	Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available.	Moderate individual risk, low community risk; a pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Lab exposures may cause serious disease but effective treatment and preventive measures are available and the risk of spread of the disease is limited.
RG3	Agents associated with serious or lethal disease for which preventive or therapeutic interventions may be available.	High individual risk, low community risk; a pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
RG4	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic measures are not usually available.	High individual risk and high community risk, a pathogen that usually causes serious or lethal disease but may spread from one infected individual to another. Effective treatment and preventive measures are not usually available.

There are four basic components to a risk assessment, summarized as follows:

Hazard Identification is an assessment of whether a substance or process poses any hazard. It employs epidemiological studies, animal bioassays, and other confirmatory assays.

Exposure Assessment estimates human exposure, actual or projected, to an agent. Providing such an assessment often includes assumptions of a physical and biochemical nature concerning the stability of the agent in the environment and air movement in the facility.

Hazard Assessment is the estimation of the extent of the pathogenic or toxic effect at a given dose level. This component of the process involves its own set of underlying assumptions, including the presumed validity of high to low dose extrapolation, immune competency of the personnel involved and that of casual visitors to the area.

Risk Characterization represents a total evaluation of all the evidence and usually estimates risk (as a probability) and the number of people likely to be affected by an agent. The risk characterization phase is contingent on all the assumptions that are used in the previous component processes. It typically has an estimation of the statistical uncertainty.

In order to perform the assessment, consideration must be given to the source of the organisms (identification, source, culture, genetic characteristics, pathological, ecological, and physiological traits); whether there will be an alteration of the genome to produce an engineered organism and if there will be expression of the new genome. Consideration must be given to releases to the environment, both intentional and accidental, and the subsequent survival, multiplication, and dissemination in the environment. Undesirable effects of the engineered (or natural) organism on humans, other organisms and the ecosystem should also be evaluated. Finally, consideration must be given to the experimental protocol, paying attention to the quantity of organism(s), storage conditions and locations, disposal of materials (both biological including rDNA/synthetic nucleic acids and non-biological), and aerosol potential.

7.2 Procedures Producing Aerosols

Procedures producing aerosols contribute significantly to the biosafety burden. Aerosol-producing activities include, but are not limited to:

Sonication	Grinding
Vigorous shaking and mixing	Pipetting
Injection	Centrifugation
Aspiration/Washing	Working with materials under pressure

When working with potentially hazardous materials, these procedures should be performed with the proper containment (in a BSC or equivalent) and personal protective clothing and equipment as necessary (gloves, lab coat, fluid-resistant gown, face/eye shield).

The challenge of risk assessment lies in those cases where complete information on risk factors is unavailable. A conservative approach is advised when insufficient information forces a subjective judgment. An example of this is Universal Precautions (an assessment that assumes all viable human samples are potentially infectious and dictates work practices to protect against exposure) are always advised when working with viable or “untreated” human or human-derived materials due to the possible presence of latent infectious agents.

8 Containment

The objective of physical containment is to confine biological agents, materials, or organisms containing rDNA and synthetic nucleic acid, and thus reduce the potential for infection of the laboratory worker and persons outside the laboratory. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. Emphasis should be placed on the primary means of containment which are provided by laboratory practices and containment equipment. Special

laboratory design provides a secondary means of protection against accidental release of organisms outside the laboratory into the general environment.

The CDC and the NIH have established four levels of agent containment for microbiological organisms. MIT's Institutional Biosafety Committee (IBC), the Committee on Assessment of Biohazards and Embryonic Stem Cell Research Oversight (CAB/ESCRO), has accepted these recommendations as minimum standards. Occasionally the CAB/ESCRO may require higher containment levels than recommended in the BMBL (http://web.mit.edu/cab/). Only biological research requiring BL1, BL2 or BL2+ containment is done at MIT main campus. See Tables 1, 2 and 3 for a summary of the standard containment measures. These are all based on how MIT has implemented the recommendations in the NIH Guidelines and the CDC/NIH BMBL (http://www.cdc.gov/biosafety/publications/index.htm).

8.1 Biosafety Level 1 – BL1 Containment

BL1 is suitable for experiments involving agents of no known or of minimal potential hazard to laboratory personnel and the environment. The laboratory is not separated from the general traffic patterns of the building. Work is generally conducted on open bench tops. Special containment equipment is not required nor generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory. The control of potential biohazards at the BL1 level is provided by use of standard microbiological practices. Table 2 gives a description of the standard laboratory practices for BL1 containment.

Table 2. Standard Safety Practices for Bioresearch Laboratories at MIT

Do not mouth pipette.	Laboratory Doors must not be propped open.	If you are working out of hours and alone, be sure someone knows you are in the lab.
No eating, drinking, or smoking in laboratory. Do not chew pencils.	Wash hands frequently, before leaving the laboratory, and after removing gloves.	Know locations of eye wash stations and emergency safety showers. Be sure that they are easily accessible.
Lab benches must be cleaned and decontaminated regularly. Do not forget to wipe phones, keyboards, equipment control panels, etc.	Know what to do and who to call in an emergency. Know how to clean up a spill of biological materials including rDNA/synthetic nucleic acids and to dispose of clean up materials.	Avoid manipulations that create aerosols. Handle infectious agents and solutions in Biosafety Cabinet as needed. Keep your breathing zone clear.
Do not apply cosmetics in the laboratory. Do not touch eyes, nose or mouth while working in the laboratory. Keep your hands away from your face.	Do not wear open-toe shoes in laboratory. Shoes give protection in case of chemical or biological spill.	Plants, pets or animals not involved in the research are not permitted in the laboratory.
Gloves must be worn whenever potentially biological materials including rDNA/synthetic nucleic acids are handled, or to protect a cut or broken skin. Gloves must not be	Glass and sharp objects must be disposed of in puncture proof, specially marked containers, when near full these containers should be closed and may be placed in a biowaste container for	Accidents must be reported to supervisor and or PI. The supervisor must file a report of accident or injury. https://insidemit-apps.mit.edu/apps/injury/. If the incident involves biological materials including rDNA/synthetic nucleic acids,

worn outside the lab in public spaces. This is to prevent the spread of any contaminant.	removal and off site treatment and disposal.	please contact the Biosafety Program staff immediately.
Safety glasses should be worn when in the laboratory. Safety glasses must always be used when working with chemicals. Contact lenses should not be worn in the laboratory unless extra eye protection is used at all times.	Lab coats should be worn and buttoned whenever working in the laboratory regardless of the containment level of the lab, Lab coats must not be worn outside the laboratory. Most Department, Laboratories, and Centers (DLC) have established policies on lab coat use that should be followed.	Biologically contaminated materials including rDNA/synthetic nucleic acids must be decontaminated prior to disposal or for solid research wastes boxed for offsite treatment and destruction. Liquid cultures may be inactivated by incubation with 10%. bleach for at least 30 minutes before disposal to sink.

8.1.1 BL1 Laboratory Facilities

The laboratory is designed so that it can be easily cleaned, with no rugs or upholstery of any kind

Bench tops are impervious to water, resistant to acids, alkali, organic solvents, and moderate heat

Laboratory furniture/ benches, shelving, etc. is sturdy with spaces between benches, cabinets and equipment wide enough to allow cleaning.

Each laboratory contains a sink for hand washing, as well as an eyewash station.

8.1.2 Additional Requirements of MIT for BL1 Laboratories

All newly constructed or renovated laboratory spaces have net directional inward airflow from less contaminated public spaces into the laboratory. A minimum 6-8 air changes per hour of single pass air is provided.

BL1 signs are posted on the outside of doors. The universal biohazard symbol is posted on all equipment used to store or grow RG1 or recombinant/synthetic nucleic acid containing microbes.

Biosafety cabinets or chemical fume hoods or other ventilated enclosures should be checked for airflow, or certified at least annually.

Needles and Syringes should be kept secure (see CAB/ESCRO policy on security requirements for needles and syringes)

All laboratories must post a green card indicating name and contact information for personnel to be contacted in case of an incident or emergency. This information should be kept up to date and on file with Facilities Operations Center.

8.2 Biosafety Level 2 – BL2 Containment

BL2 is suitable for experiments involving agents of moderate potential hazard to personnel and the environment. Access to the laboratory is limited when experiments are being conducted. Procedures involving large volumes or high concentrations of agents, or in which aerosols are likely to be created, are conducted in BSC. The control of potential biohazards at the BL2 level is provided by use of standard microbiological practices, use of a BSC to prevent exposure to aerosols, and the use of personnel protective equipment (lab coat and gloves). The following are procedures to be used with BL2 containment requirements

8.2.1 Standard laboratory practices are the same as at BL1 (see above) plus the following:

Access to the BL2 laboratory is restricted while work is in progress.

Reusable materials may be autoclaved at the appropriate temperature and pressure for 30 minutes or chemically decontaminated. Autoclaves should be validated using biological indicator vials on a periodic basis to ensure that autoclave conditions are sufficiently robust and it is operating correctly. All autoclaves must be part of the MIT Autoclave Validation Program. If materials (that are not able to withstand autoclaving) are to be chemically decontaminated, care should be taken to ensure that all surfaces of the material are in contact with the decontaminant solution for the appropriate length of time.

8.2.2 Special Practices

Animal carcasses, tissues, and caging materials should be returned to DCM for appropriate disposal and/or cleaning.

Research animals may not be held overnight in laboratories without special CAC approval for the sake of the animal's health.

BL2 signs are posted on outside doors. When the infectious agent(s) in use requires special provisions for entry, e.g. immunizations, a hazard warning sign should be posted. The hazard warning sign identifies the agent(s) in use in the laboratory and what is required for entry.

Laboratory personnel receive appropriate immunizations for agents/materials used in the laboratory (HBV vaccinations if human cells are used in the laboratory).

Personnel are advised of special hazards and the risks involved in the research using the biological agents including rDNA/synthetic nucleic acids. They are required to read and follow instructions on practices and procedures required for the safe handling of the particular agents to be used in the research.

Laboratory personnel receive training on the potential hazards, the work involved, and receive periodic updates as the work progresses.

Sharps, needles and syringe use is minimized. If they must be used, great care is taken with contaminated sharps and they are disposed of directly after use.

Plastic ware should be substituted for glass whenever possible.

Syringes which re-sheathe the needle, needle-less systems, and other safe devices should be used whenever possible. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is an integral part to the syringe) are used for injection or aspiration of infectious or rDNA/synthetic nucleic acids materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport for decontamination.

Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.

Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.

Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious or rDNA/synthetic nucleic acid materials is finished, and especially after overt spills, splashes, or other contamination by infectious or rDNA/synthetic nucleic acid materials. Contaminated equipment must be decontaminated according to any local, state or federal

regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

Spills and accidents which result in overt exposures to infectious or rDNA/synthetic nucleic acid materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are kept.

Animals not involved in the experiment are not allowed in the laboratory.

8.2.3 Safety Equipment (Primary Barriers)

Properly maintained Biosafety Cabinets (BSC) are used whenever procedures with a potential for creating aerosols or splashes are conducted. These may include centrifugation, shaking, blending, sonication or opening containers in the presence of pressure differentials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.

BSC must be used when high concentration or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed heads or centrifuge safety cups are used and if they are filled, balanced, wiped with disinfectant, and opened after a run only in a BSC.

Goggles, masks, faceshields or other splatter guards are used for anticipated splashes or sprays of infectious or other hazardous materials to the face, when the microorganisms must be manipulated outside the BSC.

Protective laboratory clothing designated for lab use is worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas. All protective clothing is either disposed of in the laboratory or placed in special bags and sent out for laundry by the institution; it should never be taken home by personnel.

Gloves are worn when hands may contact infectious, rDNA or synthetic nucleic acid materials, contaminated surfaces, equipment or working with infected animals. Wearing two pairs of gloves may be appropriate; if a spill or splatter occurs, the hand will be protected after the contaminated glove is removed. Gloves are disposed of when contaminated, removed when work with infectious or rDNA/synthetic nucleic acids materials is completed, and are not worn outside of the laboratory. Disposable gloves are not washed or reused.

8.2.4 Additional MIT requirements for BL2. Items noted for BL1 Level, plus:

Laboratory doors are kept closed while experiments are in progress. Names & phone numbers of laboratory personnel to be contacted in case of emergency must be posted.

Class IIA biosafety cabinets are designed to allow HEPA filtered air to be vented back into the working area. It is the policy of MIT that biosafety cabinets are to be vented, that is thimble connected, to the outside where possible.

8.2.5 Work Practices

Centrifugation should be done in screw cap, centrifuge safety cups with gasket-seal lids. If the inner walls of the centrifuge chamber and the rotor show signs of sample leakage the rotor and lid should be immediately decontaminated.

Laboratory hoods, Class I and Class II Biosafety Cabinets must meet NSF design specifications and MIT performance criteria and be exhausted so as to maintain the appropriate inward airflow velocity across the open work face.

- All BSC must be certified annually. Class II Cabinets also must meet National Sanitation Foundation (NSF) 49 specifications.

- Exhaust air volume (CFM) should be maintained at design condition, regardless of changes in filter resistance or changes in the building HVAC system if the BSC exhausts through a ganged system.
- Laboratories will have the supply and exhaust airflow volumes (CFM) controlled so as to assure a net directional inward airflow into laboratory from adjacent areas.

Growth Chambers

- Shakers for bacteria should be covered. If shaking water baths are used, use copper sulfate (enough to give bright blue color) to prevent growth of microorganisms and mold.
- DO NOT USE SODIUM AZIDE; it will accumulate in the plumbing and will act as an explosive.
- Plastic flasks and bottles should be used whenever possible to avoid breakage. Cotton-plugged flasks for growth of bacterial cultures are not considered open vessels as long as the plugs fit tightly, with no tendency to pop out.

8.3 Biosafety Level 2 plus - BL2+ Containment

Suitable for work with specific RG3 agents which may cause serious disease but do not infect by inhalation. The best known RG3 agent that meets these criteria is HIV. The suitability of BL2+ is decided on a case-by-case basis. The control of potential biohazards is achieved by strict adherence to BL3 practices in a BL2 facility.

8.3.1 Standard Laboratory Practices are the same as BL2, plus the following:

All personnel that work in a BL2+ facility must receive project specific training and undergo a mentoring/hands-on training to ensure technical proficiency. All research personnel that have access to the BL2+ facility must be listed on the PI's Biological Research Registration and receive all required training prior to starting to work in the BL2+ facility.

Work surfaces shall be decontaminated daily and following spills of organisms. A bottle of disinfectant must be kept in every work area for spills on work surface. Any spills must be cleaned up immediately.

Contaminated materials that are to be decontaminated away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory. Materials to be decontaminated at off-site from the laboratory are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.

Liquid wastes of infectious, rDNA or synthetic nucleic acid containing organisms or materials shall be decontaminated before disposal. All liquid wastes generated during BL2+ experiments should be immediately decontaminated by mixing with bleach (10% or higher final concentration) or equivalent disinfectant. After a minimum of 30 minutes in contact with the decontaminant, the solution may be disposed of via the sink. Radioactive wastes should be processed in accordance with already established procedures (see Section 10 of this manual on spill clean up for details).

Solid wastes contaminated with rDNA or synthetic nucleic acid materials or microorganisms must be autoclaved or decontaminated and destroyed off site by an outside contractor. Solid waste containers shall consist of an autoclave bag in a labeled (has a biohazard sign/label), covered, cleanable container that is placed inside the BSC at the start of the work. Before removal from the BSC, the bag should be sealed and the outside sprayed and wiped down with disinfectant several times. The closed bag may be placed in the standard double red bag-lined biowaste box for removal for offsite destruction. If autoclaving wastes prior to disposal to biowaste box, all biohazard bags containing BL2+ contaminated materials should be transported to the autoclave in a leak-proof container, preferably in a cart.

Any sterilization of BL2+ contaminated materials is done in an autoclave located within the BL2+ suite. Autoclaves must be tested periodically for proper temperature and pressure control. If you find that an autoclave which has been certified is not operating properly, notify the facility's engineer or manager

immediately. Once the solid waste has been autoclaved, it may be placed in a standard double bag-lined biowaste box for offsite decontamination and destruction.

If reusable materials are utilized in the work then (1) contaminated materials that are to be reused should be decontaminated by autoclaving for 30 minutes prior to cleaning. Materials should be collected in an autoclavable basin containing a suitable decontaminant. Inner surfaces of items which cannot be submerged should be rinsed with a suitable decontaminant. The basin must be covered before being transported to the autoclave. (2) Non-disposable pipettes should be carefully submerged in a horizontal container filled with a suitable decontaminant. Care should be taken to fill pipettes completely with decontaminant solution. Pipettes should sit in the germicidal liquid for the appropriate contact time before draining and placing in sharps container prior to disposal.

8.3.2 Work Practices

- All work with BL2+ materials should be conducted in a BSC. BL2+ materials that must be removed from the BSC into incubators, for centrifugation, for analysis, should be in secondary containment. For example, use trays with sides to place plates into incubators; centrifugation should only be done using safety cups; depending on the analytical method, the cells may be killed then assayed, or the assay equipment may be placed in a BSC if there is any chance of aerosolization or splash.

8.3.3 Special Practices for BL2+ only

- BL2+ laboratory doors are kept closed at all times. Access is strictly controlled.

- Laboratory Access is controlled by the laboratory supervisor and is restricted to those persons whose presence is required for experimental or support purposes. Persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in BL2+ laboratories. The PI has the final responsibility for assessing each circumstance and determining who may enter or work in the facility. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet specific entry requirements (e.g. immunization), may enter the laboratory.

- BL2+ signs must be posted on the outside of the doors to the BL2+ facility. If the infectious agent(s) in use requires special provisions for entry (e.g. immunization), a biohazard warning sign is posted on the access door(s) to the laboratory work area. The hazard warning sign identifies the infectious agent, lists the name and telephone number of the lab director or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

- A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and to follow instructions on practices and procedures.

- Laboratory personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. In addition, personnel are mentored and receive hands-on training to ensure technical expertise before starting to work with viable BL2+ or RG3 agents. All personnel must demonstrate proficiency in the required practices, procedures, and techniques to the satisfaction of the PI. Personnel receive annual updates, or additional training as necessary for procedural or policy changes.

- A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative. Plastic-ware should be substituted for glassware whenever possible.

- Glassware should be avoided if at all possible. If glassware is used in a BL2+, any broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.

8.3.4 Work Practices

- All manipulations involving BL2+ materials are conducted in BSCs or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.

- Cultures, tissues, cells, organs, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.

- Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious or rDNA/synthetic nucleic acid materials is finished, and especially after overt spills, splashes, or other contamination by infectious or rDNA/synthetic nucleic acid materials. Contaminated equipment must be decontaminated according to any local, state or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

- At the end of every working day, all work surfaces are thoroughly wiped down with a decontaminant.

- All potentially contaminated waste materials such as gloves and lab coats are placed in autoclave bags in marked waste containers. When near full the bags are closed then sprayed and wiped down several times with disinfectant. The bags are then placed in the double red bag lined biowaste boxes. The biowaste boxes are closed and removed by an outside vendor for treatment and destruction.

- In case of accidents or spills, biological materials including rDNA/synthetic nucleic acids are contained, decontaminated, and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious or potentially infectious material.

- Spills and accidents which result in overt exposures to infectious or rDNA or synthetic nucleic acid materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are kept.

8.3.5 Safety and Personal Protective Equipment

8.3.5.1 Safety Equipment

- All work must be performed in a BSC or other physical containment device. NO work with BL2+ materials is to be conducted on the open bench top. Properly maintained and certified BSC (class II) are used for all manipulation of BL2+ materials, except where equipment designed to provide for containment of the potential aerosols is utilized for specific purposes.

- Safety blenders which have been designed to contain aerosols are available and should be used.

- Use of a fume hood instead of a BSC to control aerosols is not permitted.

8.3.5.2 Personal Protective Equipment

- Laboratory clothing that protects street clothing (e.g., long-sleeve solid-front or wraparound gowns) is worn in the laboratory. Disposable laboratory coats are suggested as these very good and easily disposed of. Front-button laboratory coats are unsuitable. Laboratory coats and gloves may not be

worn outside the laboratory. Cloth laboratory coats must be decontaminated before sending to the laundry. Cloth lab coats must be autoclaved in a terminal biohazard bag for one hour before they are returned to the laundry service. Lab coats should be changed once a week.

- Gloves are worn when handling infected animals and when hands may contact infectious, rDNA or synthetic nucleic acid materials, contaminated surfaces, or equipment. Wearing two pairs of gloves may be appropriate; if a spill or splatter occurs, the hand will be protected after the contaminated glove is removed. Gloves are disposed of when contaminated, removed when work with biological materials including rDNA/synthetic nucleic acids is completed, and are not worn outside of the laboratory. Disposable gloves are not washed or reused.

- Goggles, masks, faceshields or other splatter guards are used for anticipated splashes or sprays of infectious including rDNA/synthetic nucleic acids or other hazardous materials to the face, when the microorganisms must be manipulated outside the BSC.

- Respiratory protection is worn when aerosols cannot be safely contained (i.e. outside of a BSC - aerosol challenge of animals, necropsy of infected animals), and rooms containing infected animals. Researchers will be referred to the Respiratory Protection Program for appropriate functional and fit testing.

8.3.6 Additional MIT Requirements for BL2+ Laboratories

- Items Noted For BL1 & 2 Plus:

- Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory but must conform to all BL2+ practices and procedures.

- No custodial or other maintenance personnel are permitted to enter a lab under BL2+ conditions unless authorization from the PI is obtained and they are escorted all the while they are in the BL2+ lab.

- All spills and accidents with BL2+ material are immediately reported to the PI and to BSO. Medical evaluation, surveillance and treatment are provided as appropriate and written records are maintained. If a potential exposure occurs, a Supervisor's Report of Injury is completed that day. If medical attention is sought, then the Medical Dept. will send over a report of the assessment to BSP for follow up.

8.4 Research Involving Large Scale Volumes

Fermentations of 10 or more liters are considered to be large scale fermentations. MIT is licensed for large scale experiments up to the BL1 LS containment level. The table below summarizes containment considerations for experiments allowed under our license. More detailed information is contained in Appendix K of the NIH Guidelines (http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines).

Table 3. Summary of Large Scale (LS) Containment Requirements

Requirements	**GLSP[1]**	**BL1-LS**
Develop Standard Operating Procedures for safety of personnel	R[2]	R
Written instruction and training of personnel to keep workplace clean and neat and to keep exposure to biological, chemical and physical	R	R

agents below a level that would adversely affect health and safety		
Provide clothes changing and handwashing facilities, provide protective clothing appropriate to the risk	R	
Prohibit eating, drinking, smoking, mouth pipetting, and applying cosmetic in the workplace	R	R
Develop a system and required accident reporting	NR[3]	R
Closed fermentation system or other primary containment	NR	R
Inactivation of organisms prior to removal from system	NR	R
Inactivation of viable material in waste discharge	R[4]	R
Aerosols controlled by engineering or procedural controls to minimize or prevent release of organisms during sampling, additions, transfers, removal of product, culture or effluent	Minimize by procedure	Minimize by engineering
Treatment of exhaust gases from a closed system to minimize or prevent release	NR	Minimize
Closed system that has contained viable organisms not to be opened until sterilized by a validated procedure	NR	R
Emergency plans required for handling large spills	R	R
Access restrictions	NR	Per lab Director

[1] GLSP = Good Large Scale Practice. For an organism to qualify for GLSP, the host must be nonpathogenic, nontoxigenic, contain no adventitious agents and have an extended history of safe large scale use or have built in limitations that permit optimum growth in the large scale setting but limit survival without adverse consequences in the environment.

[2] R = Required

[3] NR = Not required

[4] Required by the State Plumbing Code.

9 Guidelines for Research Involving Viral Vectors, Use of Established Human Cell Lines, and Human Materials

9.1 Guidelines for Use of Viral Vectors

All viral vectors in use in MIT laboratories are replication-defective. As new generations of a particular viral vector system are developed, the CAB/ESCRO has asked that investigators continue to adopt the vector systems with increased safety features. For information about common types of viral vectors on our campus and their characteristics, please contact the Biosafety Program.

Once an investigator has determined the appropriate viral vector system to use and transgenes, promoters, etc, BSP will work with the researcher to develop the appropriate safety and containment measures. Establishing the appropriate containment level for generation and use of the various viral vectors can be difficult. The issues embedded in the generation of a viral vector preparation are twofold: (1) were replication competent viral particles created in the packaging cell line during generation of the rep- viral

vector prep? If so, how will these be detected and what will be used as the positive and negative controls in the test used for detection of replication competent virus? (2) What is the probability that the viral vector (if it integrates into the genome) can be mobilized in vivo and what is the likelihood of insertional mutagenesis in the experimental system?

In the first instance, the CAB/ESCRO requires that the investigator uses multiplasmid packaging systems and test the viral vector preps that are generated in their laboratory to ensure that the system works as expected. Once the generation of viral vector preps becomes routine for the group, the frequency of testing can be altered. The type and sensitivity of the assay and the positive and negative controls must be clearly outlined. It is important that the assay used for detection of replication competent particles be sensitive enough to measure at least one rep+ particle in 10^6 replication defective particles. If investigators purchase their vectors then the vendor must include all testing data including titer and # of replication competent particles per vector particles. The vendor should include a description of the assay and controls used to determine these numbers. This information is in essence the "lot release" criteria and the investigator should address what criteria they must have for their research and for CAB/ESCRO approval.

For the second issue, the CAB/ESCRO asks that investigators use only SIN retroviral or lentiviral vectors. It requires CAB/ESCRO approval to use non-self inactivating retro- or lentiviral vectors and the investigator must show that a vector without this additional safety feature must be used. It is up to the investigator to convince the committee. The researcher must consult with Occupational Health to determine if this different viral vector might require a change in an established treatment protocol in case of a possible exposure.

A containment paradigm was developed within the MIT Biosafety Program about 15 years ago and has been shared with the wider Biosafety community. Various investigators at MIT and the WIBR reviewed this containment assessment at that time. It was subsequently published in 2000, 2005, and is presently in press for the 5th edition of Biological Safety Principles and Practices (ASM). The containment assessments in this table are consistent with the NIH OBA document Biosafety Considerations for Research with Lentiviral Vectors (2006).

Table 4. Containment Levels for Viral Vectors and Various Classes of Transgenes

Gene transfer vector[a]	**Host range**[b]	**Insert or gene function**[c]	**Laboratory containment level**[d]
MMLV based - *gag, pol, env* deleted	Ecotropic	S, E, M, G, CC, T, MP, DR, R, TX, O_v, O_c,	BSL 1*
	Amphotropic, VSV-G pseudotyped	S, E, M, MP, DR, T, G,	BSL 2
		O_v, O_c, R, CC	BSL 2+
		TX	BSL 3
Herpes virus based – non-lytic	Broad host range, nervous system	S, E, M, MP, DR, T, G	BSL 2
		O_v, O_c, R, CC	BSL 2+
		TX	BSL 3

Gene transfer vector[a]	Host range[b]	Insert or gene function[c]	Laboratory containment level[d]
Lentiviral based – HIV, SIV, EIAV, FIV, etc.; *gag*, *pol*, *env*, *nef*, *vpr* deleted	Ecotropic, amphotropic, VSV-G pseudotyped	S, E, M, MP, DR, T, G	BSL 2
		O_c, O_v, R, CC	BSL 2+
		TX	BSL 3
Adenovirus based – serotype 2, 5, 7; E1 and E3 or E4 deleted	Broad host range, infective for many cell types	S, E, M, T, MP, DR, R, G, CC	BSL 2
		O_v, O_c,	BSL 2+
		TX	BSL 3
Rabies virus, SAD B19 strain (veterinary vaccine strain), G protein deleted	Broad mammalian host range, nervous system specific	Marker proteins such as EGFP	BSL 2
		O_v, O_c,	BSL 2+
		TX	BSL 3
Baculovirus based	Broad mammalian host cell range	S, E, M, T, MP, DR, R, G, CC	BSL 1*
		O_v, O_c,	BSL 2
		TX	BSL 2+/BSL3
AAV based - *rep*, *cap* defective	Broad host range, infective for many cell types including neurons	S, E, M, T, MP, DR, G	BSL 1*
		O_v, O_c, R, CC	BSL 2
		TX	BSL 2+/BSL 3
Poxvirus based- Canarypox, Vaccinia[e]	Broad host range	S, E, M, T, DR, MP, CC, R, G,	BSL 2
		O_v, O_c,	BSL 2+
		TX	BSL 3

[a] Refers to the parental or wild type virus and some of the common deletions used in viral vectors.

[b] Refers to ability of vector to infect cells from a range of species. Ecotropic generally means able to infect only cells of species originally isolated from or identified in. Please note that the ecotropic host for HIV, HSV would be human cells. But the ecotropic host for MMLV would be murine cells. Amphotropic and VSV-G pseudotyped virus host range include human cells.

[c] These are general categories of marker or cellular genes and functions. Please note that there are differences in the containment level for the same gene class depending on whether the viral vector integrates into the recipient genome at high rate. The general categories are: EGFP, enhances green fluorescent protein; S, structural proteins: actin, myosin, etc.; E, enzymatic proteins, serum proteases, transferases, oxidases, phosphatases etc.; M, metabolic enzymes: amino acid metabolism, nucleotide synthesis, etc.; G, cell growth, housekeeping; CC, cell cycle, cell division; DR, DNA replication, chromosome segregation, mitosis, meiosis; MP, membrane proteins, ion channels, G-coupled protein receptors, transporters, etc.; T, tracking genes such as GFP, luciferases, photoreactive genes; TX, active subunit genes for toxins such as ricin, botulinum toxin, Shiga & Shiga-like toxins; R, regulatory genes, transcription and cell activators such as cytokines, lymphokines, tumor suppressors; Ov and Oc , oncogenes identified via transforming potential of viral and cellular analogs, or mutations in tumor suppressor genes resulting

in a protein that inhibits/moderates the normal cellular wild type protein. This does not include SV40 T antigen. SV40 T antigen containing cells should not be considered more hazardous that the intact virus. SV40 is considered a Risk Level 1 agent (the lowest level) according to NIH Guidelines (Appendix B). The prevalence of SV40 infection in the US population due to contaminated polio vaccine does not seem to have caused a statistically significant increase in the rate of cancers. However, the data from various studies on SV40 association with cancer is equivocal (Strickler et al., 1998; Butel and Lednicky, 1999, Dang-Tan et al., 2004).

[d] This is a general assessment of containment levels for laboratory construction and use of these vectors for non-production quantities only. This is based on the NIH Guidelines (2013) and the 2007 CDC/NIH BMBL. Please note this table cannot cover every potential use within a research or laboratory setting; as information is gained risk assessments and containment levels may be changed. Local IBCs should use all available information and their best judgment to determine appropriate containment levels. BSL-1* refers to the containment level based on parent virus risk group. However, most procedures involving the handling and manipulation of the viral vectors are done at BSL-2 to protect cell cultures and viral stocks from contamination.

[e] Certain specific strains of poxviruses, such as MVA, NYVAC ALVAC and TROVAC, are considered low risk agents and can be handled at BSL-2 in certain cases (see section on poxviruses).

Essentially the initial containment level is established by the containment level associated with the use of the parental wild type virus. This takes into account the possibility of formation of replication competent virus in the viral vector preparation. As the potential impact and hazard of the biological function of the transgene increases, the containment level is also shifted upward. This table is used as a starting point for discussion between investigators and the CAB/ESCRO. If new safety features become available in various viral vector systems - and reliable, well controlled, data supports changes to the hazard assessment - the containment level may also be changed. This table is reviewed within the Biosafety Program at least once a year and more frequently by faculty. Also as new vectors are used they are included within the table. Investigators are urged to contribute data to address safety issues so that the assessments remain as relevant as possible.

9.2 Guidelines for Use of Human Materials and OSHA Bloodborne Pathogen Standard

Many investigators use human materials (blood, tissues, or cells) in their research. The most common types of materials are established human cell lines. Work with human materials must be conducted at BL2 containment due to the possibility of the presence of latent viruses in these cells. Risks from working with animal (including human) material in vitro involve the possibilities that the original material may contain viruses or other organisms which may be pathogenic. In addition, the cells themselves may, under certain circumstances, be capable of crossing histocompatibility barrier.

Cells, derived products, and microorganisms which may be present under in vitro conditions, may pose a greater hazard than in the animal (including human) host due to activation or altered properties brought on by in vitro manipulations.

All investigators that work with human materials must be enrolled in the OSHA Bloodborne Pathogen Program and receive initial and annual BBP retraining; be offered the HBV vaccine or if appropriate have their antibody titer assayed, both free of charge; understand what to do in case of an exposure; and know the contents of the Exposure Control Plan (ECP) for their research. The PI of the lab conducting the work with the human materials must prepare an Exposure Control Plan. The Biosafety Program will review the ECP with the PI to ensure that the information is clear and consistent. The completed ECP is used in the lab group annual biosafety refresher training for reference and as a training tool. The ECP form is available at http://ehs.mit.edu/site/content/osha-bloodborne-pathogen. If the PI cannot access the form, the Biosafety liaison for that PI will forward the ECP form. See next section for more detail about the OSHA BBP program.

The CAB/ESCRO asks that PIs list all human established cell lines and the results of any viral testing done for each line in their laboratory as part of the ECP. Where investigators use established human lines

this list becomes part of the research registration. This information and the viral testing data are useful in case of an incident or possible exposure. The Biosafety Program asks that wherever possible investigators use established cell lines that have been tested for the standard range of viral pathogens associated with blood transfusion, e.g., HBV, HCV, HIV1&2, HTLV1&2. In some cases the testing includes EBV, CMV and other viruses as well depending on the commercial source. Recently both the ATCC and DSMZ have started to not only test for viral pathogens but to characterize the cells in their collection as to karyotype and tissue of origin. Use of these well characterized lines can only improve the validity of the science. If in the future journals begin to ask for this information as part of submitted articles investigators will have already addressed this basic scientific issue.

9.3 OSHA Bloodborne Pathogen Standard

OSHA has formulated the BBP Standard for occupational exposure to bloodborne pathogens. The purpose is to reduce or minimize, (to the extent feasible), occupational exposures to bloodborne pathogens. Bloodborne pathogens refer to pathogenic microorganisms that are present in human blood, body fluids and tissues and can cause disease in humans. These pathogens include, but are not limited to: HBV, HIV, HCV. The BBP standard requires that all human blood, tissues and certain body fluids be treated as if potentially infected and requires the consistent use of appropriate PPE. The Standard applies to all occupational exposures to blood and other potentially infectious materials and requires an assessment of all jobs to determine the likelihood of an exposure to human blood.

Many departments conduct research and teaching utilizing blood and other human source material, while certain jobs have duties that include tasks that place the individual at risk of a potential exposure. In both these instances, the OSHA BBP Standard requires that a written Exposure Control Plan (ECP) be generated, that engineering and work practices controls be implemented, that appropriate personal protective equipment be identified and made available free of charge, HBV vaccination and post vaccination titers offered free of charge, as well as initial and annual refresher training are required. The objective is exposure prevention and management and consistent recordkeeping of incidents, exposures and training. Training of personnel must take place prior to beginning work. Currently the BSP conducts both the initial and annual refresher trainings. There are additional requirements for research laboratories and production facilities engaged in the culture, production, concentration and manipulation of HIV and HBV.

OSHA has defined blood as also including human blood components and products made from human blood. Other potentially infectious materials covered under the standard include:

HIV or HBV containing cell or tissue cultures, organ cultures, and culture medium or other solutions; and blood, organs or other tissues from experimental animals infected with HIV or HBV.

Any unfixed tissue or organ (other than intact skin) from a human (living or dead).

The following body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, breast milk, saliva in dental procedures and any body fluid that is visibly contaminated with blood.

BSP will provide training and information programs, a review of laboratory facilities, and practices and procedures for any laboratory or department conducting research or teaching with blood or potentially infectious material (as defined above). The Biosafety Program also provides to training to all groups with potential occupational exposure throughout the MIT campus, e.g. student EMTs, Campus Police, Athletics Dept, custodians, repair and maintenance personnel, and others. Consult your laboratory or departmental ECP for the specific work practices that are in place for your facility.

MIT Biosafety Manual sections 10 and 11:

10 Cleanup of Biological Spills

The information in section 10 applies to all biological materials, rDNA and synthetic nucleic acids, and infectious agents that contain rDNA/synthetic nucleic acids.

10.1 Biological Spill in a Biosafety Cabinet

Decontamination procedures should be initiated at once while the cabinet continues to operate to prevent escape of contaminants from the cabinet.

1. Keep the cabinet running.
2. Remove any contaminated personal protective equipment (PPE).
3. Put on new PPE, i.e. lab coat, gloves and eye protection as needed.
4. Check for broken glass, use forceps to pick up as much as possible and place in red sharps container
5. Cover the whole area of spill and a bit beyond with absorbent material, e.g. paper towels.
6. Surround and saturate the absorbent material with an appropriate disinfectant.
7. Allow to sit for 20 minutes.
8. Pick up soaked paper towels using large forceps or dust pan and squeegee/scraper and place paper towels in biowaste box.
9. Repeat the soaking with disinfectant and cleanup steps as indicated in Steps 4 through 8.
10. Discard absorbent material, gloves into the solid biological waste container, i.e. bio waste box.
11. If using bleach as the disinfectant, wipe down the interior surfaces with a 70% ethanol rinse to remove residue and discard wipe to biowaste box.

10.2 Large Volume Spill within BSC (>200 ml)

1. Keep the cabinet running.
2. Check lab coat for indication of contact with spilled materials, change lab coat if needed, change gloves, put on safety glasses if not wearing them at the time of spill.
3. Cover as much of spill as possible with adsorbent materials.
4. Remove all contents of BSC onto a plastic backed diaper placed outside the BSC but only AFTER spraying each item well and wiping down with disinfectant. Do this several times for each item before removing from the BSC. The BSC needs to be empty in order to do a good job disinfecting and cleaning the spill.
5. Place paper towel over whole surface.
6. Soak the towel with an appropriate disinfectant.
7. Allow to sit for 20 minutes.
8. Scoop up and place soaked paper towels into autoclave bag, repeat this soaking area with disinfectant and cleanup. Then do a final wipe with 70% ethanol to remove bleach (which will eventually pit stainless steel).

9. Check the spill basin below. Lift out tray and removable exhaust grill, and wipe down top and bottom (underside) surfaces with paper towels soaked with a disinfectant. Do a final wipe with 70% ethanol.
10. If there is any evidence of spilled material into catch basin beneath the work surface, use paper towel to absorb it and soak paper towel with disinfectant as above.
11. Let sit for at least 20 minutes (remember odor from disinfectant will circulate into laboratory.

Remove soaked paper towel and place in biowaste box.

Repeat disinfectant cleanup.

Give catch basin, exhaust grill, etc. final wipe down with 70% ethanol before putting back into place.

Discard clean up materials, gloves into biowaste box.

Report spill to Biosafety Office (2-3477)

10.3 Biological Spills in a BL1 or BL2 Containment Laboratory

1. Alert other laboratory personnel of the spill. Leave the area. If anyone is injured they should remove their laboratory coat and glove, wash any affected areas and seek medical attention as soon as possible. Others should begin the spill containment and cleanup process. If there are no injuries the investigator should:
2. Remove contaminated clothing and ensure that you were not splashed with spilled material.
3. Thoroughly wash any potentially contaminated skin with soap and water.
4. Allow 30 minutes for particulates/aerosols to settle before re-entering area or laboratory.
5. Put on new PPE consider whether shoe covers may be needed and decontaminate the spill as follows:
6. Check area to see how far spill might have splashed (is it all on the floor or is some on cabinet doors, etc). Also identify where broken glass is located. Take care to not step into potentially contaminated area.
7. Remove any broken glass or sharps with tongs or forceps, put into sharps container.
8. For larger sharps or glass pieces that do not fit into a small benchtop sharps containers, they need to be placed in a larger rigid container, e.g. bench top mayonnaise jar used for Pasteur pipettes, cardboard box, then sealed and placed into the bio box.
9. Cover the spill with absorbent material such as a paper towel. Spread paper towels over larger area than spill to be sure to catch all the edges and splatters.
10. Saturate paper towel with an appropriate disinfectant (i.e. 10% bleach solution) starting from the outside edge of the paper towels.
11. Allow a contact time of at least 20 minutes.
12. Clean up the spill using dust pan and scraper or tongs, put into autoclave bag (do not use hands as there may be small shards of glass that are hard to see) and dispose of spill materials in biowaste box.
13. Repeat steps 7-11.
14. Rinse floor area with water, and dry. Discard materials into biowaste box.
15. If vertical surfaces were contaminated wipe with 20% bleach solution several times then rinse off bleach residue with several wipes with water. Dry surface. Discard all materials to biowaste box.
16. Remove spill cleanup gloves, etc. and discard into biowaste box.

17. Report the spill to your supervisor. If the spill involved recombinant or synthetic DNA or RNA or a Biosafety Level 2 agent please be sure that is included in the incident report also please let the Biosafety Program know as soon as possible (2-3477). Seek medical attention (MIT Medical E23) in case of a possible exposure or cut.

10.4 Biological Spills in a BL2+ or Higher Concentration/Volumes of RG2

1. Evacuate immediately, holding your breath.
2. Inform supervisor and Laboratory Manager.
3. Remove contaminated clothing (place into a bag) and thoroughly wash any contaminated areas on skin with soap and water. Seek medical attention (MIT Medical E23) in case of an exposure or cut.
4. Spill Decontamination: only trained personnel should don personal protective clothing and decontaminate the spill as follows: if a respirator is to be used during spill cleanup then person needs to have passed the medical assessment, followed by "fit-testing" and be trained as part of the Medical Dept/EHS Respirator Program.
5. Wait 30 minutes for particles to settle before reentry.
6. Put on clean laboratory coat, gloves, safety glasses, shoe covers if available, and respirator if necessary.
7. Remove any broken glass or sharps with tongs and place in sharps container.
8. For large sharp material that does not fit into the small benchtop sharps containers, it needs to go into a rigid container, e.g. benchtop plastic mayonnaise jar used for Pasteur pipettes, or cardboard box, then sealed and placed into the bio box.
9. The rest of the procedure is essentially the same as for spill outside a BSC.
10. Cover the spill with absorbent material such as paper towels. Be sure to cover all edges and splatters.
11. Saturate the towels with an appropriate disinfectant (i.e. 1:10 bleach solution) for a contact time of at least 20 minutes.
12. Clean up the spill and dispose of spill materials in bio box.
13. Repeat the steps 7-12.
14. Give cleaned a final wipe with water or 70% ethanol
15. If spill contaminated vertical surfaces such as cabinet doors, etc, the wipe surface with
16. Report the spill to your supervisor, Laboratory Manager, and BSP. Again, if injured, cut, or a possible exposure occurred, after washing the affected area then seek medical attention.

10.5 Radioactive Biological Spill Outside a Biosafety Cabinet

In the event that a biological spill also includes radioactive material, the cleanup procedure will have to be modified. The biological component of the spill will have to be inactivated prior to disposal of the radioactive waste. Call the Radiation Protection Program (RPP) at 2-3477 for instruction and assistance.

Follow the biological spill clean-up procedures plus the following instructions:

1. Remove protective clothing (lab coat, gloves, etc.) and place in a plastic bag or appropriate radiation waste container.

2. Monitor yourself for radioactive contamination. If contaminated decontaminate yourself and resurvey.
3. Thoroughly wash your hands and face.
4. Monitor the removed protective clothing for radioactive contamination. If positive, isolate this waste and hold for disposal by RPP.
5. Before cleaning the spill area contact RPP at 2-3477 for assistance. If the spill occurs after hours or on weekends, activate the EHS "ON CALL" system by dialing Operations at 3-4948 or MIT Police at 3-1212 (100 from a campus phone).
6. After biological spill is cleaned-up, collect all contaminated materials (paper towels, glass, liquid, gloves, etc.) in a plastic bag. Place the bag in the appropriate RPP waste container.
7. Sharps, forceps, tongs, dust pan and brush should be monitored for radioactive contamination. Decontaminate if possible and resurvey as necessary, otherwise place in RPP waste container.
8. Contact RPP to report the spill.

10.6 Chemical Biological Spill Outside of a Biosafety Cabinet

Inactivate biological component with a disinfectant that will not increase the chemical hazard associated with the waste. Prior to starting your research determine which disinfectant is compatible with the chemical(s) you use. Contact Industrial Hygiene Program (IHP) at 2-3477 for assistance.

After you have determined that the disinfectant is safe to use with the chemical, follow the instructions above for biological spill clean-up.

After the biological material has been inactivated, the cleaned up materials need to be disposed as hazardous waste. Request a chemical waste pick up at http://ehs.mit.edu/site/content/chemical-waste-collection-form.

11 Personnel Exposures and Accidents Involving Biological Materials

The information in section 11 applies to all biological materials, rDNA and synthetic nucleic acids, and infectious agents that contain rDNA/synthetic nucleic acids.

The procedures, activities, personnel behaviors, and equipment that create conditions favorable for occupational laboratory infections are similar to those that lead to the occurrence of industrial type accidents. The extra ingredient is the presence of biological agents including rDNA/synthetic nucleic acids capable of causing human infections. Laboratory events that might create hazards, exposures, or accidents requiring reporting could be classified into two categories:

1. Events occurring during work with biological materials including rDNA/synthetic nucleic acids or in a biological area that could result in physical injury, cuts, burns, abrasions, bites, scrapes, or splashes.
2. Other events occurring during the handling of biological agents including rDNA/synthetic nucleic acids, infected specimens, or animals that could allow release of the agent to the environment or its undesired transfer to, contamination of employees, animals or cultures.

In the first category, the injury site could be contaminated with the biological agent including rDNA/synthetic nucleic acids in use. In the second category, illness or unwanted cross contamination could occur without physical injury, e.g. inadvertent ingestion of contaminated fluids, exposure to aerosols, or penetration of agents through unbroken skin. Therefore, for the purpose of controlling biohazards including rDNA/synthetic nucleic acids, all accidents, known exposures, and potential hazards should be identified and reported.

11.1 Accidental Exposure to Biological Agents including rDNA/Synthetic Nucleic Acids and Agents containing rDNA/Synthetic Nucleic Acids

11.1.1 Reporting Actions

Personnel who, in the course of duty, are accidentally exposed to a biological agent including rDNA/synthetic nucleic acids should immediately wash the potentially contaminated area with soap and water. If the spill is extensive, an emergency decontamination shower should be used. Personnel should immediately report the exposure or incident to his/her immediate supervisor.

In the event that an injury accompanies an exposure or a substance enters the eye, mouth, lungs, or penetrates or comes in contact with the skin, the supervisor should direct disinfecting procedures and ensure that the employee reports without delay to MIT Medical Dept. A physician or medical authority should determine if the risk is significant enough to require medical attention. If the exposure does not require a medical intervention, the employee should still report the exposure to his/her supervisor. Regardless of the medical assessment, the supervisor must complete a Supervisor's Report of Injury or Illness (http://ehs.mit.edu/site/content/occupational-injury-or-illness). If a significant exposure has occurred involving more than three (3) persons, the person most familiar with all details should prepare a list of all exposed persons for the supervisor. The supervisor should call MIT Medical Dept to let them know that a number of people are coming to Urgent Care and give a brief description of the incident and the biological agent or materials including rDNA/synthetic nucleic acids involved. This will aid in more rapid access to a physician.

11.1.2 Individual Responsibility

For the protection of each individual and their coworkers, reporting responsibility begins with any individual involved in an accident, exposure, or suspected hazardous situation. The action taken may vary with the laboratory unit, but in general the individual should inform his/her supervisor as soon as possible in order to begin the reporting process.

11.1.3 Principal Investigator Responsibility

It is the responsibility of the PI to develop or adopt an emergency plan which covers contingencies which may arise in the event of an accidental exposure. If the experiment involves an unusually virulent or uncommon pathogen, then the PI should consult with the Associate Director of MIT Medical Dept. and Occupational Health Division to develop an Occupational

Health Treatment Plan for the agent and the appropriate procedures therapeutics for emergency treatment in case of personnel exposure. A copy of the Treatment Plan should be kept in the laboratory as well as in the Occ. Health Division. The supervisor shall insure that all laboratory personnel are aware of this plan and the possible emergency treatments. This information should also be on file in the Biosafety Program. It is also advisable for individuals to inform their Primary Care physician that they are working with these particular agent(s).

11.1.4 Medical Authority

In the event of an exposure to a biological agent including rDNA/synthetic nucleic acids, the employee shall immediately go to the Medical Department. The attending physician shall determine if the exposure is of sufficient risk to require medical treatment. If medical attention is required, the arrangements for treatment should include an assessment of risk to fellow workers assisting the patient and the precautions required to prevent the exposure of other persons encountered on the route to the medical facility. The Occupational Health physician will send an assessment report to the Biosafety Program so that a follow up investigation can be initiated.

11.2 Requirements for Reporting

As outlined above, all accidents, exposures, potential hazards, hazards and near-misses should be reported. The PI or person designated on behalf of the PI shall report the incident within 24 hours at https://atlas.mit.edu/atlas/Home.action#ehs_2.

Where the incident involves an overt exposure to organisms containing recombinant DNA or synthetic nucleic acids managed at BL2 conditions, the Biosafety Officer will report the incident to NIH OBA, the CAB/ESCRO, and the City of Cambridge Biosafety Committee immediately upon receipt of the report or notification. If the incident requires additional information or follow up a final letter will be sent to NIH OBA and the City of Cambridge Biosafety Committee outlining subsequent findings and conclusions.

All other incidents involving a potential exposure to recombinant DNA or synthetic nucleic acids materials or organisms containing such molecules, will be investigated and reported as required by the *NIH Guidelines* within 30 days of receipt of the notification.

The MIT CAB/ESCRO does not oversee any BL3 or BL4 laboratories. Therefore reporting requirements for these containment levels have been omitted.

11.3 Investigation of Laboratory Accidents

Staff members of the Biosafety Program, in cooperation with the PI and his/her staff, will conduct any necessary investigation of a laboratory incident. The goal of the investigation is two-fold: (a) to obtain information concerning the circumstances and number of employees who may have been exposed, and (2) to evaluate the research protocol, materials and methods, with the objective of preventing similar accidents. In addition, the Biosafety Officer, in consultation with the Occupational Health physician may institute further steps to monitor the health of those who may have been exposed to the agent in question.

As the possible causes of the incident become clear, Biosafety staff will generate a report that is sent to the PI, the injured person, all state and federal agencies that require notification, and the Occupational Health physician.

The reporting of accidents to the PI or laboratory supervisor is the responsibility of the employee who has the accident. The PI or laboratory supervisor should then alert MIT Medical if personnel are going over to Urgent Care for an assessment, and complete the Supervisor's Report of Injury or Illness. Reporting of incidents is for the benefit of the employee, student or staff member. It ensures a record of possible injury is created. If accidents are not reported it will be difficult to control and contain the organisms including rDNA/synthetic nucleic acids involved as well as devise necessary measures to prevent future accidents.

12 Decontamination and Sterilization

12.1 General Procedures

All materials, equipment, or apparatus contaminated with or containing potentially infectious organisms, recombinant DNA or synthetic nucleic acids shall be sterilized on site or discarded into designated waste containers (biowaste boxes). Biowaste boxes are the preferred method. Each individual working with biological materials including rDNA/synthetic nucleic acids is responsible for disposing of their materials into the biowaste box. Autoclaving is an alternative method and if used, each individual is responsible for sterilization of materials before disposal.

To minimize hazards all biological materials including rDNA/synthetic nucleic acids must be placed in an appropriately marked refrigerator, incubator, biowaste box, sterilized, or otherwise confined at the close of each work day.

All autoclaves must be certified for operating efficiency by the periodic use of biological indicator controls and records maintained for three years (required by the MA State Sanitary Code Chapter VIII, 105 CMR 480.000). Contact BSO at 3-1740 for more information and services provided.

Dry hypochlorites, or any other strong oxidizing material, must not be autoclaved with organic materials such as a paper, cloth, or oil: OXIDIZER + ORGANIC MATERIAL + HEAT = POSSIBLE EXPLOSION.

All floors, laboratory benches, and other surfaces in buildings where biological materials including rDNA/synthetic nucleic acids are handled should be disinfected as often as deemed necessary by good laboratory practices and the PI. The surroundings should be disinfected after completion of operations involving plating, pipetting, centrifuging, and similar procedures with biological materials including rDNA/synthetic nucleic acids.

It is the responsibility of the PI to determine that the disinfectant and the time and method of exposure are effective against the biological agent(s) including rDNA/synthetic nucleic acids used in the facility. Documentation of this must be kept on file as per 105 CMR 480.000.

Floor drains, if any, should be flooded with water or disinfectant at least once each week in order to fill traps and thus prevent the backflow of sewer gases.

Floor cleaning procedures which minimize the generation of aerosols should be used. Wet mopping or wet vacuum pick up is recommended. Water used to mop floors should contain a disinfectant or disinfectant detergent. (Dry mopping or dusting should be avoided). Where wet procedures are not practicable, dry vacuum cleaning with a HEPA filter on the exhaust, sweeping compound used with push brooms, or dry dust mop heads treated to suppress aerosolization may be used.

Stock solutions of suitable disinfectants will be maintained in each laboratory for disinfection purposes. NOTE: working dilutions of certain disinfectants have short shelf lives and must be prepared on a regular basis.

12.2 Specific Methods of Sterilization in Use at MIT

12.2.1 Wet Heat

Wet heat is the most dependable procedure for the destruction of all forms of microbial life. Steam sterilization generally denotes heating in an autoclave employing saturated steam under a pressure of approximately 15 psi to achieve a chamber temperature of at least 121^0C (250^0F). The critical factors in insuring the reliability of this sterilization method is: 1) proper temperature and time; and 2) the complete replacement of the air with steam (i.e. no entrapment of air). Some autoclaves utilize a steam activated exhaust valve that remains open during the replacement of air by live steam until the steam triggers the valve to close. Others utilize a pre-cycle vacuum to remove air prior to steam introduction.

Physical controls such as pressure gauges and thermometers are widely used but are considered secondary methods of insuring sterilization. The use of appropriate biological indicators at locations throughout the autoclave is considered the best indicator of sterilization. The biological indicator most widely used for wet heat sterilization is Geobacillus sterothermophilus spores.

12.2.2 Dry Heat

This is sometimes used for surgical instruments. Dry heat sterilization is less efficient than wet heat sterilization and requires longer sterilization contact time and/or higher temperature. The specific time and temperature must be determined for each type of material being sterilized. Generous safety factors are usually added to allow for the variables that can influence the efficiency of this method of sterilization. The moisture of the sterilization environment as well as the moisture history of organisms prior to heat exposure appear to affect the efficiency of dry heat sterilization.

Sterilization by dry heat can usually be accomplished at 160 to 170^0C (320 to 338^0F) for periods of 2 to 4 hours. Higher temperatures and shorter times may be used for heat resistant materials. The heat transfer properties and the spatial relation or arrangement of articles in the load are critical in insuring effective sterilization.

12.3 Sterilization Procedures

General criteria for sterilization of typical materials are presented below. Supervisors are encouraged to review the type of materials being handled and to establish standard conditions for sterilization. Treatment conditions to achieve sterility will vary in relation to the volume of material treated, volume of the autoclave, the contamination level, the moisture content, and other factors. Biological materials including rDNA/synthetic nucleic acids should not be placed in autoclaves overnight in anticipation of autoclaving the next day. Special precautions should be taken to prevent accidental removal of material from an autoclave before it has been sterilized or the simultaneous opening of both doors on a double door autoclave.

Full details for how to autoclave waste can be found at the following link:

https://ehs.mit.edu/site/content/autoclaving-your-waste

12.3.1 Steam Autoclave

1. Laundry: 250^0F (121^0C) for a minimum of 30 min.
2. Trash: 250^0F (121^0C) for at least 45 minutes per bag. Size of the autoclave and of the bags greatly affect sterilization time. Large bags in a small autoclave may require 90 minutes or more.
3. Glassware: 250^0F (121^0C) for a minimum of 25 min.
4. Liquids: 250^0F (121^0C) for 25 minutes for each gallon.
5. Animals & bedding: Steam autoclaving not recommended (sterilization time required would be at least 8 hours). Incineration in an approved facility is the recommended treatment of these wastes.

12.3.2 Gas Sterilants

1. Paraformaldehyde: Sixteen hours exposure to a concentration of 1.0 mg/liter at 60% or greater relative humidity and at ambient temperature (70^0F).
2. Vapor hydrogen peroxide is also used for room decontamination without incurring the need for post-decontamination neutralization of residue and cleanup.

12.4 Disinfectants

Choosing the right chemical disinfectant is integral to the protection of laboratory personnel, custodial personnel, biohazardous waste handlers, the public, and the environment. The target organism or biological material including rDNA/synthetic nucleic acids, presence of organic matter, and the situation in which disinfection must be performed will dictate the chemical disinfectant chosen. The disinfectant of choice should be one that quickly and effectively inactivates the biological material including rDNA/synthetic nucleic acids with minimal risk to the user. One must also consider the shelf life, toxicity, corrosiveness, and waste stream of the chemical chosen. A variety of treatments are available, but practicality and effectiveness govern which is most appropriate.

Please visit the following link for more information on laboratory disinfectants:

https://ehs.mit.edu/site/content/laboratory-disinfectants

A comprehensive list of chemical disinfectants, including effectiveness against various biologicals, effective concentrations, toxicity, and corrosiveness has been compiled by the Biosafety Program and can be found at the following link:

https://ehs.mit.edu/site/sites/default/files/files/disinfectant%20table_04_12.pdf

12.4.1 Phenolic Compounds

These are recommended for the killing of vegetative bacteria, including Mycobacterium tuberculosis, fungi and lipid containing viruses. They are ineffective against spores and most nonlipid containing viruses.

1. Low solubility in water unless combined with detergent.
2. Stable in storage.
3. Effective over relatively large pH range.
4. Prolonged contact deteriorates rubber.
5. Many are active against lipophilic viruses.

12.4.2 Quaternary Ammonium Compounds

These are acceptable as general use disinfectants to control vegetative bacteria and nonlipid containing viruses. However, they are not active against bacterial spores.

1. Odorless but act as deodorizers.
2. Effective at temperatures up to 212^0F.
3. Generally ineffective against tubercle bacilli, spores, and hydrophilic viruses.
4. More effective in alkaline than acid solutions.
5. Neutralized by soap and anionic detergents.
6. Effectiveness reduced by organic material.
7. Have built-in detergency properties.

12.4.3 Iodophors

Although these show poor activity against bacterial spores, they are recommended for general use. They are effective against vegetative bacteria, fungi and viruses.

1. Combine iodine with non-ionic detergent.
2. Rapid biocidal action.

3. Effective against Gram positive and Gram negative organisms, and tubercle bacilli.
4. Most effective in acid solutions.
5. Vaporized at 120^0F to 125^0F should not be used in hot water.
6. Iodophors have a built in indicator. If the solution is brown or yellow, it is still active.
7. Iodophors can be readily inactivated and iodophor stains can be readily removed with solutions of sodium thiosulfate.

12.4.4 Alcohols

In concentrations of 70 to 95%, alcoholic solutions are good general use disinfectants, but they exhibit no activity against bacterial spores.

1. Fast acting.
2. Leaves no residue.
3. Compatibly combines with other disinfectants (quaternaries, phenolics, and iodine) to form tinctures, extending alcohol's cidal action.
4. Results from experiments conducted at NIH indicate that a combination of 60% ethanol with 0.01N HCl (pH 4) remarkably improved cidal action against poliovirus and adenovirus.

12.4.5 Aldehydes

Effective against a wide spectrum of bacteria, fungi and viruses. Sporicidal when used properly.

1. **Formaldehyde Solutions.** At a concentration of 8% formaldehyde exhibits good activity against vegetative bacteria, spores, and viruses.
2. **Formaldehyde Alcohol.** Solutions of 8% formaldehyde in 70% alcohol are considered very good for disinfection purposes because of their effectiveness against vegetative bacteria, spores and viruses. For many applications, this is the disinfectant of choice.
3. **Activated Glutaraldehyde.** Two percent solutions exhibit good activity against vegetative bacteria, spores, and viruses. Its use, however, must be limited and controlled because of its toxic properties and the damage to eyes. Limited stability after activation (for alkaline glutaraldehyde).

12.4.6 Chlorine Compounds

These are recommended for certain disinfecting procedures provided the available chlorine needed is considered (i.e., hypochlorites are rapidly inactivated by extraneous organic matter. Available chlorine must be able to exceed chlorine demands). Low concentrations of available chlorine (2 to 500 ppm) are active against vegetative bacteria, fungi, and most viruses. Effectiveness increases with concentration of available chlorine. Rapid sporicidal action can be obtained at about 2500 ppm. The corrosive nature of many of these compounds (especially hypochlorites) and their decay rates limits their use.

1. Broad effectiveness.
2. Solution of 2000 ppm available chlorine commonly used in laboratory as a soak for contaminated equipment.
3. For optimal cidal activity, dilute with warm, acidic water. Resulting solutions are less stable but have a higher biocidal activity.

ALL disinfectants must be used in accordance with manufacturer's recommendations. If you have any concerns or questions please contact BSP at 3-1740.

13 Standard Operating Procedure for Autoclave Validation

Reliable achievement of sterilization by autoclaving requires periodic validation and maintenance. Validation is accomplished by the periodic use of biological indicators and annual parametric testing.

13.1 Autoclave Validation with Biological Indicators

Validation of an autoclave requires that the autoclave loads be standardized. The time required to sterilize a load is dependent not only on temperature, but on the amount of material present within the chamber. Therefore, the bacterial indicators should be included with a standard load. When sterilizing a load of wrapped materials, a wrapped ampoule should also be placed as close to the center of the load as possible. When sterilizing a load of unwrapped material such as bottles or media, an unwrapped/naked ampoule should be placed in the center of the load. When decontaminating materials an unwrapped ampoule should be placed in the center of the biohazard bag and attached to a wire or string for easy retrieval.

A complete protocol for autoclave validation with biological indicators can be found at the following link:

https://ehs.mit.edu/site/content/steps-quarterly-validation

Any evidence of inadequate sterilization should result in an investigation of the autoclave in question. For more information about what to do in the event that an autoclave fails, please visit:

https://ehs.mit.edu/site/content/steps-take-if-autoclave-fails-validation

or Contact BSP at 3-1740 for additional information and assistance.

13.2 Parametric Testing of Autoclaves

Please visit the following link for information on annual parametric testing of autoclaves:

https://ehs.mit.edu/site/content/annual-parametric-calibration

13.3 Autoclave Equipment

The following information has been compiled from manufacturer's recommendations, the literature and in house testing. As many sizes and types of autoclaves exist, no one set of conditions will be valid for every laboratory. Therefore it is important to review individual laboratory procedures in the light of the information presented here and verify the effectiveness of your procedure with biological indicators.

Please visit the following link for more information on autoclave supplies and services at http://ehs.mit.edu/site/content/autoclave-supplies-and-service

13.4 Disposal of Refuse

Please see the following link for more information on biologically contaminated waste including rDNA/synthetic nucleic acids:

https://ehs.mit.edu/site/content/biologically-contaminated-waste

Contaminated animal bedding and animal carcasses from laboratories and animal areas should be collected in impermeable containers which are closed before returning to DCM facilities.

Contaminated solid refuse, such as broken or disposable glassware and plastics, should be placed in bio boxes. See sharps disposal for more information about broken glass disposal.

The primary mechanism for disposal of solid biological waste including rDNA/synthetic nucleic acids on the MIT campus is via a Stericycle biowaste box. These boxes are provided by EHS and may, with approval of the Biosafety Program, be used for the collection of biological waste prior to disinfection. When full, boxes are sealed up and retrieved by EHS staff reducing, the need for laboratory personnel to handle and autoclave waste. There may still be circumstances that require autoclave sterilization of waste or disinfection of waste prior to deposition in the biowaste box. Please reference your Biological Research Registration or contact the Biosafety Program for more information. For complete details on proper autoclave use and maintenance, please see other sections of this manual.

The use of sodium hypochlorite is acceptable for most disinfection of cultures. Common bleach diluted 1:100 is suitable for surface decontamination and a 1:10 dilution is generally acceptable for decontamination of broth or tissue culture solutions. For complete details on chemical disinfectants, please see section 12 of this manual.

13.5 Disposal to Drain

In accordance with the State Plumbing Code, laboratories must sterilize or inactivate all infectious or potentially infectious and all rDNA/synthetic nucleic acids materials before they enters the drainage system. Mechanical garbage disposal units should not be used for disposal of contaminated wastes because these units generate considerable amounts of aerosol.

13.6 Sharps Disposal

Sharps are anything that can easily puncture the skin. Under no circumstances should any sharp object (whole or broken glass, metal, razor blades, hypodermic syringe/needle units, scalpel blades) be placed in regular waste receptacles. In order to prevent injury to MIT or contractor personnel who must handle sharps, the following procedures are to be followed:

14.3.1 Glass

Biologically contaminated: place pieces into a biological sharps container or if too large, seal into a cardboard box and deposit into the bio box.

Clean: place pieces into a cardboard box and label the box “clean broken glass”. Custodial services will remove the box.

14.3.2 Other Sharps

All needle/syringe assemblies are to be disposed of intact. Needles are not recapped, bent or broken. The use of needle chopping devices (guillotines) is not recommended due to the release of aerosols which can contaminate both personnel and surfaces. These devices also require increased needle handling which increases the risk of needlestick injury.

All sharps, regardless of their use, are to be disposed of in a puncture resistant container or a commercially available sharps collector. The safe disposal of sharps depends upon a container that provides an opening or diaphragm that prevents the contents from spilling; is impervious to punctures; and is leak-proof.

When the sharps container is full, it must be sealed and deposited into the bio box. Containers too large to be placed into the bio box will be removed by EHS. Request for pick-up of bio boxes or sharps containers can be made online at:

https://ehs.mit.edu/site/content/biological-waste-pick

14 Handling of Biological Laboratory Waste

This includes materials, microbes, and animals and animal tissues containing rDNA/synthetic nucleic acids.

14.1 Disposal of Refuse

Please see the following link for more information on biologically contaminated waste including rDNA/synthetic nucleic acids: https://ehs.mit.edu/site/content/biologically-contaminated-waste

- Contaminated animal bedding and animal carcasses from laboratories and animal areas should be collected in impermeable containers which are closed before returning to DCM facilities.
- Contaminated solid refuse, such as broken or disposable glassware and plastics, should be placed in biowaste boxes. See sharps disposal for more information about broken glass disposal.

The primary mechanism for disposal of solid biological waste including rDNA/synthetic nucleic acids on the MIT campus is via a Stericycle biowaste box. These boxes are provided by EHS and may, with approval of the Biosafety Program, be used for the collection of biological waste prior to disinfection. When full, boxes are sealed up and retrieved by EHS staff reducing, the need for laboratory personnel to handle and autoclave waste. There may still be circumstances that require autoclave sterilization of waste or disinfection of waste prior to deposition in the biowaste box. Please reference your Biological Research Registration or contact the Biosafety Program for more information. For complete details on proper autoclave use and maintenance, please see other sections of this manual.

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All sharps, regardless of their use, are to be disposed of in a puncture resistant container or a commercially available sharps collector. The safe disposal of sharps depends upon a container that provides an opening or diaphragm that prevents the contents from spilling; is impervious to punctures; and is leak-proof.

When the sharps container is full, it must be sealed and deposited into the bio box. Containers too large to be placed into the bio box will be removed by EHS. Request for pick-up of bio boxes or sharps containers can be made online at:

https://ehs.mit.edu/site/content/biological-waste-pick

Table 5. Disposal Methods for Biologically including biological materials, rDNA, and synthetic nucleic acids, Contaminated Solid and Liquid Wastes

Waste Type	**Method**
Solid. Plastic plates, paper, gloves, rigid plastic pipettes, tips	Place waste in biowaste box. When full, contact EHS for removal and replacement of box. Alternatively, autoclave waste collected in autoclave bag.
Liquid Cultures, supernatants, media	Chemically decontaminate or autoclave, validate method.
Sharps Needles, syringes, razor blades, scalpel blades, glass slides, Pasteur pipettes, toothpicks	Collect in properly labeled, puncture-resistant commercial sharps container. When full, seal and deposit into the biowaste box.
SPECIAL LAB WASTES	**Method**
Animals, animal bedding	Incinerate. Contact DCM at 3-1757.
Human blood, body fluids, tissues	Incinerate tissues and organs. Solid waste which has come in contact with human blood, body fluids, or tissue must be placed into a biowaste box or autoclaved or incinerated. Contaminated liquid waste may be autoclaved or chemically decontaminated and poured down the drain. Contact BSP at 3-1740.
Mixed Biological including rDNA/synthetic nucleic acids /Hazardous Chemical	Inactivate biological component including rDNA/synthetic nucleic acids with a treatment which will not increase the chemical hazard associated with the waste. Package for shipment as chemical waste. Autoclaving is not recommended, under heat and pressure some chemicals may explode or become volatile. The EHS Office at 2-3477 has Safety Data Sheets (SDS) and chemical waste packaging information. The Industrial Hygiene Program at 2-3477 has advice on chemical compatibilities.
Mixed Biological including rDNA/synthetic nucleic acids /Radioactive	Inactivate biological component including rDNA/synthetic nucleic acids with a treatment which will not volatilize radioactive component of waste. Disinfectant used must be compatible with radiation waste storage and packaging rules (pH, etc.). Package for shipment as radioactive waste. Autoclaving radioactive waste is on a case by case basis. Autoclaving is not recommended where radioactive off gases may be released. Call Radiation Protection Program at 2-3477 for advice.
Recombinant Plants	Incinerate or autoclave all plant materials, soil, and pots. If autoclaving, establish temperature and time necessary to kill most resistant recombinant biologicals involved (e.g., seeds). Contact BSP at 3-1740 for specifics.

15 Shipping and Receiving Regulated Biological Materials including recombinant DNA and Synthetic Nucleic Acids

15.1 Introduction

Transport of biological materials including rDNA/synthetic nucleic acids may be regulated during both shipment and receipt of the material. Therefore both the shipper and recipient should consider the need for a permit any time one plans to ship or receive biological material. The simplest way to find out if your materials require a permit to is seek the assistance of the Biosafety Program. Regulated biological materials may include: soil, plant material, animals, insects, microorganisms (bacteria, fungi, virus, etc.), recombinant organisms, plasmid vectors, DNA/RNA associated with pathogenicity, human blood or tissue or body fluid specimens, cell lines, and media components derived from animal sources. An exhaustive list of all regulated biologicals is not possible here, however, in general, the following four conditions should prompt a permitting review:

all imports from other countries and islands

known or suspected human, animal, or plant pathogens, or unknowns

agents listed in the ATCC or DSMZ catalogues as requiring permits

Other biological materials are subject to regulation. This is a condensed version of the materials most likely seen when receiving shipments. If material received is not on the list, contact EHS for more information.

15.2 Shipping Biological Materials including rDNA/Synthetic Nucleic Acids

Shipments of regulated biological materials including rDNA/synthetic nucleic acids must comply with the International Air Transport Association (IATA) and the Department of Transportation (DOT) shipping regulations. In order to ship regulated biological materials, you must be trained and certified every two years. If biological materials must be shipped, contact BSP to assist you with the shipment. More information about shipping biological material including rDNA/synthetic nucleic acids may be found at ehs.mit.edu/site/content/shipping-biological-materials. To sign up for shipping training, visit ehs.mit.edu/site/training.

15.3 Import and Export Information

15.3.1 Import Permits

Various U.S. agencies regulate the biological materials including rDNA/synthetic nucleic acids. To determine if the material requires a permit and the type of required permit, please go to "Cheat Sheet for Permitting of Biological Materials" at http://ehs.mit.edu/site/content/shipping-biological-materials, and Appendix G "Biological Material Classification Table in SOP: EHS-0062 Shipping Biological Materials ehs.mit.edu/site/content/shipping-biological-materials-0.

When planning to receive specimens from foreign countries, you must determine if a permit is required and if it is required, you must obtain the permit prior to receiving the shipment. The approved permit is sent to the supplying laboratory to be affixed/accompany the package through US Customs. These shipping documents allow entry into the U.S. in a timely fashion. Packages containing biological materials without appropriate clearance paperwork will be held up in Customs.

The following agencies permit the recipient laboratory for the receipt and use of these articles within the United States. For further information on import/export permits see SOG-0095 Guidelines for Import and Export of Biological Materials including rDNA/synthetic nucleic acids at http://ehs.mit.edu/site/content/import-and-export-biological-materials-guidelines

United States Department of Agriculture (USDA)

The USDA regulates the importation and exportation of plant, soil and animal materials associated with pathogenicity and/or foreign source. Some state Departments of Agriculture also require assurances (e.g. California where agriculture is a major industry).

The APHIS Division of Veterinary Services handles all permits for animal pathogens or animal materials. List of animal diseases can be found at: http://www.aphis.usda.gov/wps/portal/footer/topicsofinterest/applyingforpermit?1dmy&urile=wcm%3apath%3a%2FAPHIS_Content_Library%2FSA_Our_Focus%2FSA_Animal_Health%2FSA_Animal_Disease_Information

Animal Products that Do Not Require an Import Permit but will be reviewed at the port of entry: http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/importexport?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_animal_health%2Fsa_import_into_us%2Fsa_apply_for_permits%2Fct_animal_imports_nopermit

Centers for Disease Control and Prevention (CDC)

The importation of etiologic agents is governed by the following federal regulation: USPHS 42 CFR Part 71 Foreign Quarantine. Part 71.54 Etiologic agents, hosts, and vectors. Examples of items that require a CDC Import Permit include: cultures of infectious agents, un-sterilized specimens of human and animal tissues that contain or are suspected to contain human pathogens, and animals capable of being a host or vector of human disease. For further details, please go to cdc.gov/od/eaipp/.

Fish & Wildlife Service and National Marine Fisheries Service

Fish & Wildlife Service permits are required for marine mammals, certain fish and certain live animals including bats. Call 1-800-WILD for further information or visit fws.gov/permits/.

FDA Import Permits

With the exception of most meat and poultry, all food, drugs, biologics, cosmetics, medical devices and electronic products that emit radiation, require a permit or registration before importation into the US. For further details, please visit fda.gov/forindustry/importprogram/default.htm.

National Institutes of Health (NIH)

The NIH requires that federally funded recipient laboratories comply with the NIH Guidelines. NIH also requires that prior to providing rDNA/synthetic nucleic acids materials to collaborators, researchers obtain a letter stating recipient Institutional compliance with the appropriate NIH Guidelines containment level.

Cambridge Biosafety Committee (CBC)

The CBC requires registration of ALL rDNA experiments and compliance with the NIH Guidelines for all institutions in Cambridge.

15.3.2 Export Permits

Exports of biological materials, including rDNA and synthetic nucleic acids may be subject to one or more U.S. controls. For more details, please go to SOG-0095 Guidelines for Import and Export of Biological Materials at http://ehs.mit.edu/site/content/import-and-export-biological-materials-guidelines

David Quimby, dquimby@mit.edu, Export Control Officer can help you determine whether a license is required or there are any restrictions for your item, destination, end user and end use.

Foreign Countries – requirements for receipt of a shipment:

Other countries may require that recipient laboratories obtain import certificates to accompany packages into their country through customs regardless of the value of the package. Recipient Institutions should

be aware of these requirements but it is recommended to communicate with your recipient about the need for such a permit before you send a shipment.

15.4 Timing

PLAN AHEAD when thinking of receiving or sending a regulated strain or material. Permits take time to obtain and may require additional laboratory inspection by outside agencies. Also, keep in mind that transport of some plant, animal and soil organisms is forbidden to and from certain island quarantine regions of the U.S. such as the Virgin Islands, Puerto Rico, Hawaii, and some foreign countries. Contact the Biosafety Program for assistance.

15.5 Recordkeeping

Your lab must keep a log of all materials sent to other labs and a strain collection of materials received. Log entries should include date, recipient, address, copy of recipient's permit, mode of shipping, physical form, volume (amount), material's identity and initials of sender/packager. Strain collection receipt log should note the date, strain/cell line/map or genotype as appropriate, where obtained, media/growth conditions, drug resistance markers, quarantine results (e.g. mycoplasma-free, pure culture, etc.), biosafety level, permit if needed, and recipient-lab storage location.

Before shipping regulated biologicals you need to document that the recipient lab is properly equipped to handle the material by receiving copies of their required permits. When sending rDNA or synthetic nucleic acids where permits are not issued to individual laboratories, a letter from the IBC stating containment approval consistent with the appropriate containment requirements of the NIH Guidelines is needed for all distribution of the materials. If you are transferring materials to another laboratory at MIT, contact BSP to ensure the recipient lab has appropriate registration with the CAB/ESCRO and safe handling procedures are in place.

When filing for permits to receive regulated biological materials including rDNA and synthetic nucleic acids, please copy BSP on all correspondence to permitting agencies. These agencies often contact us to confirm or clarify some issue regarding your permit request. Keep us informed so we can reply promptly/appropriately.

15.6 Packaging and Labeling

If you are trained to ship, please review the packaging requirements in the SOP EHS-0092 Shipping Biological Materials including rDNA and synthetic nucleic acids. Otherwise, please contact BSP to assist with the shipment.

16 Bibliography

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