# Laboratory Biosafety Plan

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I. Purpose

The Albert Einstein College of Medicine (Einstein) is committed to providing a safe and healthful work environment. This manual which is a further extension of this commitment is designed to provide general operating and emergency procedures for working safely with biohazardous agents or materials including Recombinant DNA (rDNA) that come under the purview of Einstein’s Institutional Biosafety Committee (IBC).

This Biosafety Plan for Laboratories is based on guidelines provided in the CDC/NIH publication Biosafety in Microbiological and Biomedical Laboratories (5th Edition, 2009) and the NIH Guidelines. The plan describes standard practices, emergency procedures, safety equipment and facilities and biosafety levels which has been determined based on risk assessment of the agents/materials and the laboratories’ proposed procedures.

The operational procedures described in this manual have been designed to provide a high degree of protection to all individuals and the surrounding environment. We would like to emphasize that even the best containment facility and equipment is rendered ineffectual with improper laboratory procedures. Any person working with biohazardous agents or materials including rDNA must be trained and proficient in safe microbial practices and techniques before handling infectious or potentially-hazardous materials. The Principal Investigator is directly responsible for providing worker training and supervision in their laboratory and for ensuring that each employee has received appropriate training and experience before commencing work.

II. Scope

This policy and the procedures outlined herein apply to all Einstein faculty, staff, and students.

III. Plan

III.A. Roles and Responsibilities

III.A.1. Institutional Biosafety Committee – Statement of Purpose

The Institutional Biosafety Committee acts on behalf of the Albert Einstein College of Medicine (Einstein) to:

1. Review and support the activities of the Department of Environmental Health and Safety in providing guidance on the safe use, procurement, storage, and disposal of biohazards including recombinant DNA (rDNA).
2. Review the activities of the Biohazard Facility (BSL3).
3. Review recombinant DNA research to ensure compliance with the NIH Guidelines.
4. Promote a greater awareness and understanding by faculty and staff for the need to:
   a. Conduct all laboratory procedures and activities with attention to personal and environmental health and safety
   b. Comply with governmental health and safety regulations and laws.
5. Review new safety and health regulations and provide guidance on their application to Einstein.
6. Review research activities which raise safety and health issues.
7. Review those engineering facilities designed to protect the worker from biohazards.
8. Ensure that administrative controls on the use of biohazards, e.g., written guidelines, monitoring personal protection practices, are available and followed.

9. Recommend to the Dean (and the Senior Associate Dean of Academic Affairs), measures to decrease the exposure of the Einstein Community to biohazards.

10. Act as an interface between research faculty and the Department of Environmental Health and Safety.

III.A.2. The Department of Environmental Health and Safety

The Department of Environmental Health and Safety (EH&S) along with other groups (e.g., IAS), assists the PI in developing procedures that will result in safe operations as they pertain to biohazards including rDNA and can determine if an employee is unable to work with biohazardous agents or materials including rDNA, take out of service any malfunctioning containment equipment, and insure compliance with CDC/NIH guidelines and other health and safety regulations. EH&S will be responsible for coordinating meetings of the IBC, providing technical guidance and training materials to PIs, workers, and IBC members regarding biosafety, revising procedures as experience dictates, inspecting laboratories, supervising any needed emergency response or drills, investigating and reporting, as needed, to the Center for Disease Control (CDC)/National Institute of Health (NIH).

III.A.3. Principal Investigator

The Principal Investigator (PI) is immediately responsible to ensure that the purpose of this manual, and all other applicable guidelines, are fulfilled. The PI should verify that all staff members are properly trained who are conducting research, have been given the Biosafety Plan and follow the specific protocols and policies. The PI must inform the laboratory staff of any potential hazards associated with their work including, biological, chemical, and radioactive hazards. The PI is responsible for immediate notification to EH&S of any accidents or incidents involving biohazardous materials including rDNA.

III.A.4. Laboratory Worker

All laboratory workers must have sufficient training and experience to work safely with biohazardous agents or materials including rDNA. All personnel working with biological agents or materials including rDNA should read and comply with the procedures of this manual and meet with their PI and EH&S for training before starting work. In addition, they should be clearly instructed by their PI as to the procedures they must follow while performing research. The laboratory worker is responsible for immediate notification to the PI and EH&S of any accidents or incidents involving their work. Any unsafe act or malfunctioning equipment should be brought to the immediate attention of the PI and EH&S and should be repaired or taken out of service. Employees should also report any instances which constitute an exposure, theft or other unusual event involving biohazardous agents or materials including rDNA.

III.A.5. Institute for Animal Studies

The Institute for Animal Studies (IAS) is responsible for humane care and husbandry of all animals used in research at Einstein and for ensuring that this research is conducted in a safe manner and according to regulations. The animal care program and all research in which animals are used are overseen by the Institutional Animal Care and Use Committee (IACUC). The IACUC reviews all research projects using animals, inspects animal rooms, as well as all areas where animals may be taken for procedures, and provides training jointly with the IAS to anyone working with animals (investigators, students, fellows, technicians, and animal handlers).
III.A.6.   Einstein

Einstein, in conjunction with the PIs, EH&S, and the IAS assumes the responsibility for ensuring compliance with all guidelines within this manual and other applicable guidelines including but not limited to:

- CDC/NIH: Biosafety in Microbiological and Biomedical Laboratories, 5th edition, 2009
- OSHA regulations in 29CFR parts 1910.1200 and 1910.1450
- NIH Guidelines for Research Involving Recombinant DNA Molecules
- City, State and Federal waste disposal guidelines

III.A.7.   National Institute of Health

The National Institute of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) mandates the establishment of an Institutional Biosafety Committee (IBC) for the review and oversight of biological research. The NIH outlines the roles and responsibilities for biosafety and establishes the practices, procedures and conditions under which rDNA work must be conducted.

III.A.8.   Center for Disease Control

The Center for Disease Control (CDC) Guidelines are taken from the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) and addresses the appropriate measures and facilities for work with all microbial agents, including bacterial, viral, fungal, and parasitic and rickettsia agents.

III.A.9.   Occupational Safety and Health Administration

The Occupational Safety and Health Administration (OSHA) sets forth the requirements for working safely with human blood and other potentially infectious materials. This is covered under the Bloodborne Pathogen Standard; more information can be found in Albert Einstein College of Medicine’s Exposure Control Plan available on the EH&S website at: www.einstein.yu.edu/ehs.

III.B.   General Laboratory Safety

Laboratories are designed so that they can be easily cleaned; bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat. Both laboratories and animal rooms have a hand washing sink. Autoclaves for decontaminating laboratory wastes are available throughout various floors of Einstein.

Laboratory coats, gowns, or smocks must be worn while in the laboratory and before beginning work with biohazardous agents or materials including rDNA. No shorts, skirts or open-toed shoes are to be worn when working in the laboratory. In the animal room, disposable protective clothing can be donned outside the animal room, removed when exiting, and disposed in the proper waste container.

Upon completion of work, all surfaces and waste, liquid wastes and solids are decontaminated with an appropriate disinfectant such as 10% bleach or other waste can be bagged for decontamination in the autoclave.
III.B.1. Equipment

Equipment to be utilized in the laboratory must meet the appropriate electrical safety standards, which refer to local and national codes. It is critical that equipment be selected which:

1. Does not contribute to the spread of biohazards
2. Does not present a hazard to personnel or facility during operation

It is essential that all equipment be properly maintained. If equipment malfunctions, it should not be used. Responsibility for cost, decontamination, or repair of equipment in individual laboratories falls to the Principal Investigators.

III.B.2. Biological Safety Cabinet

A Class II Biological Safety Cabinet (BSC) or other engineering control devices, and the appropriate personal protective equipment (PPE), are used whenever procedures with a high potential for creating aerosols are conducted. These activities may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, intranasal inoculation of animals, and harvesting infected tissues from animals.

The Class II BSCs are designed for work with moderate- to high-risk agents and are among the most effective, as well as the most commonly used primary containment devices in laboratories. These cabinets, when used in conjunction with good microbiological techniques and PPE, provide an effective containment system for safe manipulation of moderate and some high-risk microorganisms and recombinant materials. The design features of this hood include an air barrier along the work opening to prevent the escape of biological agents into the laboratory, High Efficiency Particulate Air (HEPA)-filtered supply air across the work surface, and HEPA-filtered exhaust air.

Annual certification, decontamination, and maintenance must be performed by a certified (accredited by the National Sanitation Foundation) professional. Arrangement and payment for any of the above work is to be scheduled by the Principal Investigator or the department.

III.B.3. C. Biological Agents

Any biohazardous agents or materials including rDNA used at Einstein must have approval of the IBC. The approval encompasses a total review of the proposed project using biohazardous agents or materials. A hazard warning sign, incorporating the universal biohazard symbol, should be posted on all the access doors into laboratory rooms with risk group 2 or higher. The hazard warning sign should identify the biohazard, list the name and telephone number of the PI or other contact person, and indicate the special requirements for entering the laboratory. Transportation of biohazardous agents or materials including rDNA within the facility must be in a secured leak-proof, unbreakable container with an appropriate label. An absorbent material should be added to the container in case of breakage.

III.C. Medical Considerations

All laboratory workers have had a pre-placement physical by either Einstein’s Occupational Health Center or private physician. Hepatitis-B vaccine is offered through Occupational Health to all at-risk personnel and is provided to the worker at no cost.
III.C.1. Medical Restrictions

1. Pregnant women, immune-suppressed individuals, persons on steroid therapy, or immunosuppressive drugs should consider the risks involved with their work with biohazards before commencing work. The decision to allow these persons to work must be made by an appropriate physician and the responsible PI with notification to Occupational Health and EH&S.

2. Persons with a fresh or healing laceration or skin lesions should not work with biohazardous agents or materials including rDNA unless the injury is completely protected. Personnel with injuries of this type must notify their PI prior to working.

III.C.2. Reporting

Emergency telephone numbers must be posted near entrances or by the telephones in high-containment areas. Telephone numbers must include the PI, lab contact person, EH&S Office and an emergency number. A Safety Officer is available 24/7 by telephone by contacting Security at the front desk of Forchheimer (x4111 or x2019). Additional emergency information is in the Emergency Response Guide which is posted in all laboratories.

1. Any illnesses or symptoms known to be associated with biohazardous agents or materials including rDNA, should be immediately reported to the PI, Occupational Health Services (347-498-2401) and EH&S (X3560).

2. Any incident must be immediately reported to the PI and EH&S. Incidents include:
   a. Physical injury - e.g., cut, burn, broken bone, slip/fall, bite, other.
   b. Biohazardous agents or materials including rDNA exposure – parenteral exposure, exposure via aerosol, ingestion, or mucous membrane or skin contact.
   c. Any spill involving biohazardous agents or materials including rDNA.

III.C.2(a) Reporting Requirements Involving Recombinant DNA

In addition to the items above, significant problems with, or violations of, the Recombinant DNA Guidelines, and accidents involving recombinant DNA, must be reported to the EH&S, who will, if necessary report the incident to the Institutional Biosafety Committee (IBC) and to the Office of Biotechnology Activities (OBA) within 30 days at the following address:

Office of Biotechnology Activities
National Institutes of Health
Rockledge 1, Suite 750
6705 Rockledge Drive
Bethesda, MD 20817
Phone: 301-496-9838  FAX: 301-496-9839

III.C.3. Medical Follow-Up

In the event of an accidental parenteral exposure to biohazardous agents or materials including rDNA, the worker must wash the affected area with soap and water for at least 15 minutes and promptly report to Occupational Health or the local Emergency Room for treatment.
III.D. Personnel Practices

1. Disposable gloves, provided by the PI, will be worn when handling biohazardous agents or materials including rDNA. The gloves may be taped to the cuff of the protective clothing to prevent any skin exposure. Powder free gloves and double gloving is recommended when handling large quantities of infectious agents or toxin. Gloves should be inspected before donning.

2. Hands should be washed frequently during the day using soap and water. It is mandatory to wash hands:
   - After handling biohazardous agents or materials including rDNA
   - After a spill and appropriate clean up
   - When removing protective gloves
   - When exiting the laboratory
   - If you suspect contamination
   - When gloves are compromised
   - Before you eat or drink

3. Respiratory protection may be recommended when there is the possibility of hazardous aerosol generating procedures or as deemed necessary by EH&S. If you must wear a respirator, then you must be part of the Respiratory Protection Program which includes a medical evaluation and a fit test. A copy of Einstein’s Respiratory Protection Plan is available on the EH&S website at www.einstein.yu.edu/ehs.

4. Contact lenses do not provide eye protection. It is strongly recommended that contact lenses not be worn while conducting work in the laboratory. Safety glasses with side shield, goggles, or a plastic face shield should be worn for eye protection whenever necessary.

5. Eating, drinking, smoking, and/or applying cosmetics is not permitted in any area where biohazardous agents or materials including rDNA work is taking place.

6. Mouth pipetting, under any circumstances, is not permitted.

7. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

8. All wastes from laboratories and animal rooms must be appropriately decontaminated before final disposal.

9. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Needles are never to be recapped or manipulated in any way. Needle and syringe use should be kept to a minimum or eliminated, if possible. If they cannot be eliminated, safe needles/syringes should be evaluated.

10. Broken glassware must not be handled directly but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

11. Personnel must notify the PI and EH&S of any event or incident which may compromise the safety of personnel or work.

III.E. Emergencies

III.E.1. Emergency Contact

All Hours: Dial ext. 4111
   - Includes but not limited to biological, chemical, radioisotope spill, rDNA, fire, or a medical emergency.
• When calling, state the nature of the emergency and the location. If possible, remain nearby to
direct the emergency response team.
• You may find it useful to use the acronym “CALL” when faced with a sudden emergency while
working with biohazardous agents or materials including rDNA:

   C – Cover your work
   A - Abandon your work
   L - Lock agent away
   L – Leave the laboratory

During Office Hours (9:00 a.m. to 5:00 p.m.)

Biosafety Officer…………………………….X3560
EH&S……………………………………….X4150
Engineering……………………………………X3000
Security……………………………………..X2019

ALWAYS NOTIFY THE PI AND EH&S OF AN EMERGENCY OR ACCIDENT.

III.E.2. Injuries and Illnesses

1. Serious injury or sudden illness. When special first aid, resuscitation, transport, or rescue service is
required, do not hesitate:
   a. Dial 911 then x4111.
   b. Clearly describe the situation and your location.
   c. Notify the PI and EH&S.
   d. Secure all biohazardous agents or materials including rDNA containing materials, if possible. If
      not possible have PI or another trained co-worker secure biohazardous agents or materials
      including rDNA.

2. Injury involving biohazardous agents or materials including rDNA:
   a. Immediately wash the site thoroughly with soap and water, and flush mucous membranes with
      water/saline for at least 15 minutes.
   b. Notify PI and EH&S (x3560); after hours contact security at x4111 to reach EH&S.
   c. Between the hours of 8:00 a.m. and 4:00 p.m., go immediately to Occupational Health Services
      Clinic, located at 1180 Morris Park Avenue, 1st Floor for evaluation and treatment or go to the
      nearest Emergency Room; all other times, go to the nearest Emergency room for evaluation.
      i. The attending doctor will provide the appropriate treatment.
      ii. A report will be given to Occupational Health Services for medical follow-up.
      iii. Employee will be counseled to closely monitor themselves for the appearance of any
          clinical signs potentially related to the exposure.

III.E.3. Emergency Response

Emergencies include, but are not limited to, a biohazardous or hazardous, rDNA, chemical spill, fire, BSC
malfunction, natural disaster, or a total power failure. The primary objective in an emergency is
preservation of personal safety and health. Protecting the facility and the experiment are secondary to
personal safety. The sequences of events that should be followed in an emergency are:
1. Immediate personal safety overrides maintenance of containment. Evacuation takes priority. Get people out of the emergency area and close doors. Only if personal safety is not in jeopardy, secure all biohazardous agents or materials including rDNA.
2. The PI and EH&S must be informed of current state of the biohazardous agents or materials including rDNA and will provide any corrective measures if necessary.
3. The Investigator or EH&S is responsible for deciding whether to override containment procedures in case of serious injury or sudden illness.

III.E.4. Spill of Biohazard Agents or Material Including rDNA

In the event of a spill, a bottle of disinfectant must be available within the room. Wherever and whenever the spill occurs, the PI and EH&S must be notified.

1. Spill in the open lab:
   a. Don’t breathe; evacuate all personnel and close the door, making sure it is locked behind you.
   b. Remove contaminated clothing carefully, folding the contaminated area inward. Place clothing in a bag or directly into the autoclave. Thoroughly wash hands and face and any exposed area of the body.
   c. Notify the PI and EH&S (X3560).
   d. POST SIGNS WARNING OTHERS NOT TO ENTER CONTAMINATED AREA. NO ONE SHOULD ENTER THE ROOM FOR 30 MINUTES TO AN HOUR. (This allows aerosols to be carried away and heavier particles to settle.) Once all personnel have been removed from the area, there is no need to rush into the contaminated area. Time should be taken to formulate a plan to safely decontaminate the area.
   e. Decontamination will involve treatment of gross contamination by local application of disinfectant.
2. Decontamination of a Spill:
   a. Re-entry into the facility must be delayed for a period of 30 minutes to one hour to allow reduction of the potential aerosol generated by the spill.
   b. Dress in protective clothing, including a lab coat, gown or Tyvek suit, booties, eye protection and double gloves, if necessary. Respiratory protection may be worn, and care should be taken during decontamination not to disperse droplets.
   c. Place paper towels or other absorbent material along the outside of the spill and on top of the spill, working from the edges in. Pour the germicidal solution (10% solution of sodium hypochlorite – household bleach) around the spill and allow to flow into the spill. Pour disinfectant onto the absorbent material. For a large liquid spill, use full strength bleach since it will be diluted out. CAUTION: Avoid mixing bleach and ammonia compounds. To prevent aerosols, avoid pouring the germicidal solution directly onto the spill. Try covering the spill with an absorbent pad and apply the decontaminant to the absorbent pad.
   d. Allow disinfectant to sit for 15 - 30 minutes; this will provide enough contact time for adequate disinfection.
   e. Carefully remove the soaked pads, placing them into an autoclave bag. Working toward the center of the spill, use paper towels to wipe up the spill. Discard paper towels as they are used into an autoclave bag.
   f. Pick up any sharp objects with mechanical device, not hands.
g. Using paper towels soaked in disinfectant, wipe beyond the area of visible or suspected splashing, including the floor and vertical surfaces. Discard paper towels in the autoclave bag.

h. Decontamination is complete when the whole area of suspected liquid contamination has been washed with a disinfectant and all excess decontaminant has been mopped up.

i. Place all decontaminated materials into an autoclave bag, this includes gloves, shoe covers, and other protective clothing. Once material has been collected, place bag into a medical waste bin. Medical waste bin will then be closed, sealed and ready for final disposal.

3. When the above procedure is followed, the spill area is considered to be decontaminated.

IIIE.5. Biohazardous Agents or rDNA Spill Inside A Biological Safety Cabinet (BSC)

A spill that is confined to the interior of the BSC should present minimal or no risk to personnel in the area. However, chemical disinfectant procedures should be initiated at once while the cabinet ventilation system continues to operate to prevent escape of contaminant from the cabinet.

1. Keep BSC running.

2. Spray or wipe walls, work surfaces and equipment with a disinfectant. Remove decontaminated equipment from BSC. A 10% solution of sodium hypochlorite-household bleach is recommended. Household bleach can be corrosive to metal therefore; it is recommended that the decontaminated areas be rinsed with 70% ethanol following the bleach application.

3. Flood the work surface of the BSC with sufficient disinfectant solution and allow the disinfectant to sit 15 - 30 minutes before beginning clean up procedures.

4. Make sure to wipe all surfaces including the front intake grill.

5. Repeat above process with distilled water, mild soap and water or 70% ethanol.

6. The disinfectant, gloves, wiping cloth and sponges should be discarded into a medical waste container.

7. This process will not disinfect the filters, blower, air ducts, or other interior parts of the cabinet.

8. Notify PI and EH&S of the spill.

9. EH&S should be consulted to determine if gaseous decontamination of the BSC is necessary.

IIIE.6. Biological Safety Cabinet Malfunction

The following sequence of events will take place if a BSC malfunctions:

1. Terminate work.

2. Cover, secure and lock away all vessels containing biohazardous agents, material or rDNA. Turn off all electrical equipment and services, i.e., gas and vacuum.

3. Notify others in laboratory and leave the room.

4. Post signs warning others of the malfunction.

5. Notify PI and EH&S of the problem.

6. Remain available to provide assistance to the EH&S and support personnel.

IIIE.7. Centrifuge Spill

1. If you notice a spill inside a centrifuge, don’t breathe and close the lid.
2. Allow the centrifuge lid to remain closed for an hour, to allow the aerosols to settle (if using a high-speed centrifuge, evacuate laboratory, locking doors behind you).
3. Post warning signs on door.
4. Notify the PI and EH&S.
5. After an hour, don PPE, move rotor to the BSC, if possible. Decontaminate all rotor surfaces with disinfectant. Allow 15 - 30 minutes contact time with the disinfectant.
6. Wash hands and any exposed skin surfaces with soap and water.
7. Commence decontamination using 10% bleach solution.
8. Wet and wipe the inside of the centrifuge with paper towels soaked with the disinfectant. Allow to sit for up to 15 - 30 minutes.
9. Wipe all surfaces again with distilled water, mild soap or 70% ethanol to remove bleach residue.
10. Place all clean-up materials into a medical waste container which is then closed, sealed, and sent out for treatment.

III.E.8. Fire

Practices to be followed in the event of a fire are as follows:

III.E.8(a) Fire Inside the Laboratory

1. Immediately alert other personnel in the facility to the danger. If possible, without endangering yourself, secure all biohazardous agent or material including rDNA; turn off any burners and laboratory equipment and leave the facility immediately, making sure the door is locked behind you. Exit using stairwells or horizontal building exits after notifying other personnel.
2. Do not use any elevators.
3. Transmit the fire alarm by pulling the nearest pull box which is located near the stairwell. Follow up with a phone call to x4111 PI and EH&S.
4. If the fire appears containable, (e.g., waste basket or on a bench top), use the fire extinguishers located in the laboratory or if in the animal room, fire extinguishers are located in the hallway. These fire extinguishers may be used on any type of fire. Be familiar with the operation of the extinguisher before you must use it. To operate a fire extinguisher:
   a. Always make sure the exit is at your back.
   b. Remove the unit from the wall and carry or drag it to the fire area.
   c. Pull pin.
   d. Aim nozzle at base of the fire.
   e. Squeeze handle.
   f. Sweep nozzle from side to side and gradually progress forward as the flames are extinguished.
5. Once in a position of reasonable safety, notify the PI and EH&S.

Fire Department or other personnel will wear self-contained breathing apparatus when entrance into any laboratory is necessary under emergency conditions. Fire Department and Police are considered unrestricted personnel and may enter a restricted area. Upon resolution of the emergency, the PI and EH&S will determine if decontamination procedures are required.
III.E.8(b) Fire in Another Part of the Building

If an alarm sounds indicating fire in another part of the building:

1. Personnel should immediately secure all biohazardous agent or material including rDNA if possible, turn off any burners and laboratory-type equipment. Exit and lock the laboratory or animal room and go to the fire code chart located by the stairwell to determine the location of the fire. If required, the employee is to leave the area by the most direct route. We encourage evacuation if a fire is reported in your building.
2. If the mandatory evacuation alarm sounds 4-4-4, everyone must evacuate.
3. Do not use any elevators.

III.E.8(c) Emergency Evacuation

Building evacuation may be necessary in certain emergency situations. The 4-4-4 alarm sequence will activate only in extreme emergencies indicating that the building must be evacuated immediately by emergency stairwells or horizontal exits.

III.E.8(d) Electrical Failure

In the event of power failure, all electrical power will be lost for 10 to 15 seconds until the emergency generator is activated. At this time, only those lights and receptacles on the emergency electrical power supply will be reactivated. Power will be lost to all pieces of equipment not connected to the emergency supply. Once a power failure has occurred, individuals should stop work, secure biohazardous agent or material including rDNA, decontaminate surfaces and leave the area, making sure all doors are locked behind you.

III.F. Operational Procedures

III.F.1. General

1. Contaminated supplies, equipment, etc., shall not be removed from the laboratory unless they have been decontaminated.
2. All doors shall be kept closed at all times.
3. Use of needles and syringes should be limited to operations or procedures for which there are no alternatives. All sharps should be disposed in a sharps container. Avoid use of glass.
4. Safe transportation of biohazardous agents or materials including rDNA requires the use of a non-breakable secondary container.
5. Mouth pipetting is not allowed. Appropriate pipettes and pipetting aids are to be provided by the Investigator.
6. PPE worn such as safety glasses, goggles or face shield, must be decontaminated with an appropriate disinfectant such as 10% bleach followed by rinsing with water and or 70% ethanol.
7. Work surfaces shall be decontaminated following spills with 10% bleach solution or a designated disinfectant followed by rinsing with distilled water, mild soapy water or 70% ethanol.
8. In all procedures, care should be taken to minimize the creation of aerosols. Any aerosol-generating procedure must be performed in a BSC.
9. All flasks, test tubes, etc., in which biohazardous agents or materials including rDNA are grown or stored shall be appropriately covered to contain potential spills.

10. To protect the house vacuum system, all vacuum lines must be fitted with an in-line HEPA filter in addition to a secondary liquid disinfectant trap for biological agents or materials including rDNA.
   a. To protect a house vacuum system during aspiration of infectious fluids: the left suction flask (A) is used to collect the contaminated fluids into a suitable decontamination solution; the right flask (B) serves as a fluid overflow collection vessel. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms.

11. Primary suction flasks must contain appropriate liquid disinfectant (i.e., 10% household bleach) before use. Flasks should be of durable quality to prevent any cracking or breaking.

III.F.2. Biosafety Cabinet

The following procedures should be employed:

1. All interior surfaces of the cabinet should be disinfected by wiping with 70% ethanol, before work. After working with biohazardous agents or materials including rDNA, the BSC must be decontaminated with a disinfectant. If using 10% bleach, follow with a wipe down of 70% ethanol or soap and water.
2. Arrange materials to segregate contaminated and clean items.
3. Minimize movement of contaminated items over clean ones and always work from clean to contaminate.
4. When all materials have been wiped down with 70% ethanol and placed in the cabinet, wait 2-3 minutes before beginning work. This allows sufficient time for the cabinet air to purge airborne contamination from the work area.
5. Never work in the cabinet when it’s malfunctioning or out of certification.
6. The view screen must always be set at the 8-inch opening to ensure sufficient inflow velocity. Adjust chair height so that operator is looking down through the glass.
7. Do not place anything over the front intake or rear exhaust grills. As a general rule, keep equipment at least 4 inches inside the cabinet window and perform manipulation of viable materials as deeply into the cabinet as possible.
8. Use plastic disposable pipettes and lab ware wherever possible.
9. Materials including, but not limited to, bottles, waste containers, pipette canisters, culture flasks and small equipment must be wiped down with disinfectant before removal from cabinet.
10. When removing items from or introducing items into a BSC, move your arms slowly in and out of the cabinet to minimize disruption of the air flow.
11. When disposing pipettes, Petri dishes, decontaminate them before removal from the BSC. If they cannot be chemically disinfected, items should be collected for autoclaving.

12. When using a piece of equipment that creates air turbulence in the BSC, (i.e., micro centrifuge, blender, sonicator), stop other work while equipment is operating.

13. Chemically disinfect or autoclave all waste before discarding into the medical waste container.

III.F.3. Centrifugation – Procedures for Spinning Cells

The following procedure should be followed:

1. Move the safety cups to the BSC.
2. Inspect the centrifuge tubes and safety cups for cracks and deformities. Avoid using glass tubes.
3. In the BSC fill tubes making sure not to overfill the tubes. Leakage invariably occurs when tubes are filled to capacity; therefore, the maximum capacity should be 3/4 full.
4. Wipe the exterior of the tubes with disinfectant prior to loading. We recommend safety cups whenever possible. Seal the safety cups with the safety lids and transport sealed contents to centrifuge.
5. When spinning is complete, check for leakage and remove safety cups to the BSC before removing the safety lids and tubes.

NOTE: The following should all be done within the biosafety cabinet:

• Filling the centrifuge tubes
• Loading the safety cups
• Removing the tubes from the safety cups
• Opening tubes

III.F.4. Water Bath – Procedures

1. Water baths must contain an appropriate disinfectant with water being changed at reasonable intervals.
2. All vessels containing viable organisms must be closed and properly labeled.
3. Avoid using glassware whenever possible.

III.F.5. CO₂ Incubator – Procedures

1. Humidity reservoirs (where necessary) are to be refilled with distilled water.
2. Biohazardous agents or materials including rDNA should be incubated in non-breakable vessels. Transportation of cultures from the incubator to the hood shall be in leak-proof pans, trays, or containers large enough to contain cell fluid in case of leakage.
3. Petri dishes or other inoculated solid media should be transported as above.
4. Minimize culture contamination by disinfecting the incubator with a disinfectant and distilled water on a regular basis. Discard unused cultures on a regular basis.

III.F.6. Non-Disposable Sharps or Reusable Items
1. Decontaminate non-disposable or reusable items (such as equipment, bench tops, etc.) contaminated with biohazardous materials by using a chemical disinfectant, such as 10% bleach.

2. When using non-disposable sharps for necropsy or surgery, tools will be decontaminated between animals, a glass bead instrument may be used. At the completion of work, tools must be decontaminated then washed before being sterilized by the autoclave.

III.F.7. Blenders, Homogenizers, Grinders, and Sonicators Procedures

The equipment listed above creates aerosols when processing materials. When working with any biohazardous agents or materials including rDNA, operate and open the above equipment in a Biological Safety Cabinet. To reduce the amount of aerosol generated, wait 30 seconds or more after the equipment has been turned off before opening and removing the processed material. Disinfect all equipment when procedures are complete.

III.G. Waste Disposal – Procedures

Each authorized user is responsible for the decontaminating of his/her own waste materials. This can be accomplished by autoclaving or using a chemical disinfectant.

III.G.1. General Procedures

Generally, waste will be chemically disinfected in the laboratory where biohazardous agents or materials including rDNA are utilized. The laboratory is responsible for loading and unloading the decontaminated waste from the autoclave. The waste must be discarded in the medical waste bins or, in the case of animal carcasses, taken to the pathological waste container located in one of the Animal Institute’s freezers.

- If the autoclave is in use, or malfunctions, return the waste material to the research laboratory.
- When autoclaving is complete, return promptly and remove your waste from the autoclave.

Pipettes and pipette tips, etc. are to be placed in containers with sufficient disinfectant to allow disinfectant contact with the entire pipette or tip. All waste not chemically disinfected must be autoclaved before disposing in medical waste container.

III.G.1(a) Liquid Waste

1. Liquid waste should be mixed with disinfectant (e.g., final dilution of 10% household bleach) in the biological safety cabinet, whenever possible.
2. After autoclaving or chemical disinfection, liquid wastes may be carefully poured down the sink.
4. Never autoclave household bleach or other chemicals.

III.G.1(b) Solid Waste

1. Media bottles, culture flasks, culture tubes, and any other vessels that may have come in contact with biological agents or materials including rDNA must be decontaminated while still inside the Biological Safety Cabinet with sufficient disinfectant before being placed into the medical waste container.
2. Disinfectant should be drawn up into pipettes/tips as they are discarded. Contaminated pipettes may be soaked in sufficient disinfectant for at least 30 minutes before disposal. Assure all surfaces have contact with disinfectant.

3. Sharps may be chemically disinfected before disposal or they may be collected for disinfection by autoclaving.

III.G.1(c) Ordinary Waste

Ordinary trash (paper, wrappers, and cardboard) must be placed in the black bag waste bins for removal by Housekeeping. All ordinary trash that may be contaminated shall be disinfected and removed via medical waste.

III.H. Facility Operations

III.H.1. Access Procedures for Engineering and Other Support Staff

1. Maintenance including routine servicing, repair, and support services will comply with all entry and exit procedures and, if necessary, be escorted into an area by trained individuals.

2. Hands will be washed thoroughly before exiting the laboratory.

3. All potentially contaminated tools will be decontaminated before exiting the laboratory.

4. Custodial personnel shall carry out duties in laboratory rooms.

5. Floors will be washed under the supervision of the laboratory.

6. Thorough floor cleaning, i.e., scrubbing and stripping is to be done as needed and scheduled with the laboratory.

Testing and certification of the Class II Biological Safety Cabinets within the facility is performed annually by an outside contractor. Outside contractor will follow exit and entry laboratory procedures.


III.H.2(a) Material Inbound

No processing is required for inbound materials. Such materials may be introduced via the pathway used by inbound personnel. Special care should be taken when transporting infectious materials or other hazardous material into the facility. Over packing the material with the addition of an absorbent is recommended.

III.H.2(b) Material Outbound

All materials exiting the laboratory must be surface decontaminated and in a closed, plastic autoclave bag, closed buckets, or containers. All material leaving the laboratories must be safe to transport.

III.H.2(c) Laboratory Regulations

Proper recording and control of equipment and safety operations and all safety and operating procedures applied within each laboratory fall under the direct responsibility of the Principal Investigator and must be in agreement with the general defined policies of this manual.
III.H.2(d) Laboratory Hygiene

Housekeeping is to be done daily by those individual personnel assigned to the laboratories. Specific scheduling is to be established by a laboratory manager or Principal Investigator.

Room Cleanliness

Each investigator and laboratory worker is responsible for his assigned area. Each laboratory must be kept neat, clean and uncluttered. Waste bins and medical waste bins will be removed by custodial staff. Bins should not be overflowing. It is the responsibility of the laboratory to ensure bins are safe for removal.

Biological Safety Cabinet Cleanliness

Particular attention to cleanliness and tidiness is necessary within the BSC to permit convenient and uncontaminated laboratory manipulations. Equipment and materials should be limited to essential and current needs. Interior surfaces must be wiped down with disinfectant before and after using the cabinet. Attention to interior and exterior of the window is necessary to maximize visibility of manipulations.

III.I. Practices and Procedures Being Conducted in Animal Room

For complete procedures see IACUC procedures for working with animals and hazards.

The animal room will be posted with the biohazard symbol and will include all hazardous agents in the room along with PPE requirements. PPE required for entry will be found at the entrance of the room and will include: gown, head covering, dust mask, gloves and booties. The animals containing biological agents or materials including rDNA will be identified by a cage card. The cage card will state:

- The name of the PI
- Agent used on the animal
- The number of animals in the cage.

III.I.1. Daily

Workers in the Institute for Animal Studies (IAS) are required by law to do daily inspections of each room where animals are held. Therefore, daily, registered animal caretakers will enter to visually check each cage to see if the animals are sick, wet, dead or need food and water.

If a registered caretaker finds that an animal needs food and water, they will move the animal cage to the biosafety cabinet and replenish the food and water for the animal. If an animal is found sick, the animal caretaker will place a numbered yellow report card on the cage and submit a second copy to the IAS office who will, in turn, notify the Investigator.

If any animals are found dead, the animal caretaker will move the cage to the biosafety cabinet, remove the dead animal, and place it in a clear bag. A three-piece green card will be used to label the cage, the bag, and the third card will be given to the IAS office for record keeping and notification to the Investigator. The animal will be placed in the IAS freezer for the investigator or for final disposal via pathological waste disposal.
Once all work is complete, the caretaker will decontaminate the Biosafety Cabinet before exiting the room.

III.1.2. Weekly

On a weekly basis, animal cages are required to be changed. All dirty bottles, feed, and bedding will be sprayed with 10% bleach solution or autoclaved with animal cages. Cages will be stacked and red bagged before moving to the autoclave room. Once the cages are autoclaved they may be brought to the cage-washing area.

Animal movement will all be done with the aid of the Biosafety Cabinet. Animals moved to new cages will be handled with forceps which have been dipped in bleach.

III.1.3. Monthly

Monthly, the rack holding the animal cages will be moved to the cage washer for a complete cleaning. Animal cages will be moved onto a new rack and the dirty rack will be sprayed down with 10% bleach solution. The rack will then be draped and moved to the cage-washing area.

The room is inspected on a semi-annual basis for any maintenance issues. If the room needs repairs that require the movement of animals out of the room, the investigator is notified.

III.1.4. Spill in Animal Room

If a cage should fall, it will be considered a spill. In the event of a spill:

1. Don’t breathe; evacuate all personnel and close the door, making sure it is locked behind you.
2. Remove contaminated clothing carefully, folding the contaminated area inward. Place clothing in a bag or directly into the autoclave. Thoroughly wash hands and face and any exposed area of the body.
3. Notify the PI, animal care supervisor and EH&S (X3560).
4. Post signs warning others not to enter contaminated area. No one should enter the room for at least 30 minutes to one hour. This allows aerosols to be carried away and heavier particles to settle. Once all personnel have been removed from the area, there is no need to rush into the contaminated area. Time should be taken to formulate a plan to safely decontaminate the area.
5. Decontamination will involve treatment of gross contamination by local application of disinfectant.

III.1.5. Decontamination of a Spill

1. Re-entry into the facility must be delayed for a period of at least 30 minutes to one hour to allow reduction of the potential aerosol generated by the spill.
2. Dress in protective clothing, Tyvek® suit and double gloves, if necessary. Respiratory protection is recommended, and care should be taken during decontamination not to disperse any droplets.
3. Place paper towels or other absorbent material along the outside of the spill and on top of the spill, working from the edges in. Pour the germicidal solution (10% solution of sodium hypochlorite – household bleach) around the spill and allow to flow into the spill. To prevent aerosols, avoid
pouring the germicidal solution directly onto the spill. Try covering the spill with an absorbent pad and apply the decontaminant to the absorbent pad.

4. Allow disinfectant to sit for 15 – 30 minutes; this will provide enough contact time for adequate disinfection.

5. Carefully remove the soaked pads, placing them into an autoclave bag. Working toward the center of the spill, use paper towels to wipe up the spill. Discard paper towels as they are used into an autoclave bag.

6. Pick up sharp objects with mechanical device, not hands.

7. Using paper towels soaked in disinfectant, wipe beyond the area of visible or suspected splashing, including the floor and vertical surfaces. Discard paper towels in the autoclave bag.

8. Decontamination is complete when the whole area of suspected liquid contamination has been washed with a disinfectant and all excess decontamination has been mopped.

9. Place all decontaminated materials into an autoclave bag; this includes: gloves, shoe covers, and other protective clothing. Once material has been collected, place bag into a medical waste bin. Medical waste bin will then be closed, sealed and ready for treatment.

When the above procedure is complete, the spill area is considered to be decontaminated.

III.I.6. Spill Inside a Biological Safety Cabinet (BSC)

1. Keep BSC running.

2. Spray or wipe wall, work surfaces and equipment with a disinfectant. Remove decontaminated equipment from BSC. A 10% solution of sodium hypochlorite-household bleach is recommended. Household bleach can be corrosive to metal, therefore; it is recommended that the decontaminated areas be rinsed with 70% ethanol.

3. Flood the work surface of the BSC with sufficient disinfectant solution and allow the disinfectant to work for up to 15 – 30 minutes before it is cleaned up.

4. Make sure to wipe all surfaces including the front intake grill.

5. Repeat the above process with distilled water, mild soap and water or 70% ethanol.

6. The disinfectant, gloves, wiping cloth and sponges should be discarded into a medical waste container.

7. This process will not disinfect the filters, blower, air ducts, or other interior parts of the cabinet.

8. Notify the PI, animal care supervisor and EH&S of the spill.

9. EH&S should be consulted to determine if gaseous decontamination of the BSC is necessary.

III.I.7. Injuries Involving Animal Containing Biohazardous Agents or Materials Including rDNA

1. Immediately wash the site thoroughly with soap and water, and flush mucous membranes with water/saline for at least 15 minutes.

2. Notify PI and EH&S (x3560), after hours contact security at x4111 to reach EH&S.

3. Between the hours of 8 AM and 4 PM, go immediately to Occupational Health Services clinic, located at 1180 Morris Park Avenue, 1st floor for evaluation and treatment or go to the nearest Emergency Room. All other times, go to the nearest Emergency Room for evaluation.

4. The attending doctor will provide the appropriate treatment.

5. A report will be given to Occupational Health Services for medical follow-up.
6. Employee will be counseled to closely monitor themselves for the appearance of any clinical signs potentially related to the exposure.

III.I.8. Biological Safety Cabinet Malfunction

The following sequence of events will take place if a BSC malfunctions:

1. Terminate work.
2. Cover, secure and lock away all cages.
3. Notify others in the room.
4. Post signs warning others of the malfunction.
5. Notify the PI, animal care supervisor and EH&S of the problem.
6. Remain available to provide assistance to support personnel.

III.I.9. Decontamination of Non-Disposable Sharps (Animal Surgery or Necropsy)

Animal surgery and necropsy will be conducted in approved rooms Chanin 675. Tools for surgery will be autoclaved prior to use. In between animals, instruments may be placed in a bead sterilizer or sterilized using other means.

III.I.10. Exiting Animal Room

Upon exiting the animal room, dispose of booties, gown, gloves, facemask and head covering in the medical waste bin. Once PPE has been disposed, workers must use a hand sanitizer to disinfect hands before washing hands in the laboratory.

IV. Definitions

None.

V. Effective Date

Effective as of: 3 April 2018

VI. Plan Management and Responsibilities

Einstein’s Department of Environmental Health and Safety is the Responsible Office under this Plan. Einstein’s Associate Dean for Finance and Administration is the Responsible Executive. Einstein’s Senior Director of Environmental Health and Safety is the Responsible Officer for the management of this Plan.