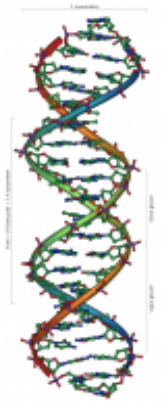
Amherst College



Health and Safety Manual for the Use of

Recombinant DNA

and

Biological Agents

Current as of January 8, 2019

Introduction

This manual contains the basic procedures to be followed for all work with recombinant DNA (rDNA) and/or biological agents in accordance with the requirements of the National Institute of Health (NIH), Centers for Disease Control and Prevention (CDC), the Amherst Board of Health, the Massachusetts Department of Public Health, the Occupational Safety and Health Administration and Amherst College's policies, procedures and best management practices. If you are being asked to read this manual, your research involves the use of rDNA or a potentially infectious biological agent. The CDC, NIH and Board of Health define rDNA as "(1) molecules which are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell; or (2) DNA molecules which result from the replication of a molecule described in (1) above". An infectious biological agent is defined as "any microorganism or infectious substance, or any naturally occurring or bioengineered or synthesized component of any such microorganism or infectious substance capable of causing death, disease or other biological adverse effect in a human, animal, plant or other living organism and/or is deleterious to the environment".

As these definitions suggest, it is important for the health and safety of the campus (and broader) community, and for the preservation of the environment, that dangerous rDNA, rDNA containing organisms and biological agents are handled appropriately. In addition to following the guidelines in this manual, you should always be trained by your P.I. (principal investigator, the professor with whom you are working) in all laboratory specific hazards and good microbiological practices. Additional information can also be obtained from "Biosafety in Microbiological and Biomedical Laboratories" (BMBL) available from the CDC website at https://www.cdc.gov/biosafety/publications/bmbl5/index.htm, or from "Guidelines for Research Involving Recombinant DNA Molecules" available from the NIH website at https://osp.od.nih.gov/biotechnology/biosafety-and-recombinant-dna-activities/

I. Biosafety Levels

All of the laboratories at Amherst College that utilize rDNA or biological agents are assigned a biosafety level (BSL) based on a risk assessment of each laboratory. The risk assessment considers both the hazards of the organisms or agents involved (such as transmission route, infective dose, severity of illness, availability of vaccines), and of the procedures used in the laboratory (such as open centrifuging and sonicating, which can lead to aerosol formation). There are currently four general biosafety levels, numbered 1-4, with 1 presenting the lowest risk. Brief descriptions of these (from the Amherst Board of Health) are given below.

BSL-1: Suitable for work involving well characterized agents not known to consistently cause disease in immunocompetent adult humans, and presents minimal potential hazard to laboratory personnel and the environment.

BSL-2: Builds upon BSL-1. Suitable for work involving agents that pose moderate hazards to personnel and the environment.

BSL-3: Applicable to clinical, diagnostic, teaching, research or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure.

BSL-4: Required for work with dangerous or exotic agents that pose a high individual risk of life-threatening disease, aerosol transmission, or related agent with unknown risk of transmission.

Most of our laboratories working with rDNA or biological agents fall into the BSL-1 category, with the exception of one laboratory with two BSL-2 rooms. Your P.I. will inform you of your laboratory's Biosafety level. Additionally, this information can be found on the safety information card located on the door to your laboratory. Your laboratory must, at a minimum, follow the requirements listed in this manual for your laboratories biosafety level classification. Be aware that there may be additional requirements if you are also working with hazardous chemicals, or in a laboratory where these materials are in use. Please consult the chemical hygiene plan on the Environmental Health and Safety website for additional information. Other hazards (radioactive materials, work with animals, etc.) may also require additional precautions. ***In all cases, the most stringent relevant requirements must be followed.***

II. General Laboratory Practices

All laboratories are required to use standard microbiological practices and techniques to minimize potential hazards. Appropriate training in these techniques, all laboratory specific hazards and proper operation of equipment shall be provided by the P.I. Safety equipment, such as gloves, lab coats, chemical splash goggles, closed containers and biological safety cabinets (BSC), shall be used as appropriate to minimize all potential exposures

BSL-1 General Laboratory Practices

1. Access to the laboratory shall be limited. Guests shall be accompanied by laboratory personnel. Doors to the laboratory shall be closed and locked when it is unoccupied.

2. All laboratory personnel must wash their hands with soap and water after working with rDNA, rDNA organisms or biological agents, and prior to exiting the laboratory.

3. No food or drink is permitted in the laboratory at any time, including gum. Application of cosmetics and handling of contact lenses is also prohibited within the laboratory.

4. Mouth pipetting is strictly prohibited.

5. Broken glassware must not be handled by hand. Dust pans and brushes or tongs may be used. Broken glassware must be placed in the white boxes designated for this purpose. Plasticware should be substituted for glassware when possible. Contaminated glassware shall be disinfected prior to disposal.

6. The use of sharps (needles, razor blades, scalpels) is discouraged. If sharps must be used, the following must be followed. Sharps must be disposed of in appropriate, puncture resistant receptacles designed for this purpose. Filled sharps containers should be referred to the Biology Safety Coordinator (Ms. Maureen Manning, x8328) or the Chemical Hygiene Officer (Mr. Jason Williams, x2736). Needles must not be bent, broken, sheared, recapped, removed from disposable syringes, or otherwise manipulated by hand prior to disposal. Reusable sharps must be stored with all sharp edges covered in block of Styrofoam, or similar material. Contaminated reusable sharps must be decontaminated (chemically or thermally, as appropriate) prior to storage.

7. Gloves must be worn if there is a potential for rDNA, rDNA organisms or biological agents which are hazardous (*i.e.*, infectious) to contact the skin. Gloves shall be removed if contaminated or if the integrity of the protective material has been compromised. Contaminated gloves must be placed in the biohazard bin for autoclaving. Do not reuse gloves. Gloves shall not be worn outside of the laboratory. Hands must be washed thoroughly after gloves are removed.

8. All procedures must be performed such that the generation of aerosols and/or splashes is minimized. Centrifuge tubes containing hazardous (*i.e.*, infectious) materials must be capped prior to centrifuging. Sonication of such solutions must be conducted in a BSC, chemical fume hood, or the solution must be in a loosely covered container during the sonicating procedure. Chemical splash goggles must be worn while performing procedures likely to generate splashes or aerosols. Lab coats must be worn when splashes are likely.

9. All work surfaces must be decontaminated after the completion of work and after any spills. Solutions of 70% ethanol or 10% bleach may be used, as appropriate. All equipment and instruments must be decontaminated prior to service or removal from the laboratory.

10. All cultures, stocks, plates or other items containing non-NIH exempt rDNA, rDNA organisms or biological agents must be disinfected prior to disposal. Bulk quantities of NIH exempt material must also be treated prior to disposal. Liquid phase materials must be brought to a concentration of 10% bleach and allowed to stand for 20 min. (or until the indicator changes color for media) prior to disposal. Material which cannot be chemically disinfected must be sent offsite for treatment. Contact the Biology Safety Coordinator for details on waste that must be treated offsite.

11. Each laboratory P.I. shall provide training to laboratory employees regarding their duties, necessary precautions to prevent exposures and exposure evaluation processes. This shall include risk factors that increase susceptibility to infection. At risk individuals are encouraged to self-identify to health services for counseling and guidance. Training is required at least annually or when procedures change such that additional training is justified.

BSL-2 General Laboratory Practices

1. Access to the laboratory shall be restricted. The doors to the laboratory will remain locked when unoccupied. Only authorized personnel (*i.e.*, those individuals working in the lab) may enter the area.

2. All laboratory personnel must wash their hands with soap and water after working with rDNA, rDNA organisms or biological agents, and prior to exiting the laboratory.

3. No food or drink is permitted in the laboratory at any time, including gum. Application of cosmetics and handling of contact lenses is also prohibited within the laboratory.

4. Mouth pipetting is strictly prohibited.

5. Broken glassware must not be handled by hand. Dust pans and brushes or tongs may be used. Broken glassware must be placed in the white boxes designated for this purpose. Plasticware should be substituted for glassware when possible. Contaminated glassware shall be disinfected prior to disposal.

6. The use of sharps (needles, razor blades, scalpels) is discouraged. If sharps must be used, the follow must be followed. Sharps must be disposed of in appropriate,

puncture resistant receptacles designed for this purpose. Filled sharps containers should be referred to the Biology Safety Coordinator (Ms. Maureen Manning, x8328) or the Chemical Hygiene Officer (Mr. Jason Williams, x2736). Needles must not be bent, broken, sheared, recapped, removed from disposable syringes, or otherwise manipulated by hand prior to disposal. Reusable sharps must be stored with all sharp edges covered in block of Styrofoam, or similar material. Contaminated reusable sharps must be decontaminated (chemically or thermally, as appropriate) prior to storage.

7. In addition to item 7 under *BSL-1 General Laboratory Practices* above, gloves, chemical splash goggles (and face masks or shields, if necessary) and lab coats must be worn. Contaminated protective equipment (goggles, face shields and lab coats) must be properly disinfected (either thermally or chemically) prior to donning again or laundering. Contaminated face masks should be disposed of in the biohazard bin. Used protective equipment must not be stored in non-laboratory areas, such as offices.

8. In addition to item 8 under *BSL-1 General Laboratory Practices* above, all work with live cultures of rDNA organisms or biological agents must be performed inside of a Class II biosafety cabinet (BSC).

9. All work surfaces must be decontaminated after the completion of work and after any spills. Solutions of 70% ethanol or 10% bleach may be used, as appropriate. All equipment and instruments must be decontaminated prior to service or removal from the laboratory.

10. All cultures, stocks, plates or other items containing non-NIH exempt rDNA, rDNA organisms or biological agents must be disinfected prior to disposal. Bulk quantities of NIH exempt material must also be treated prior to disposal. Liquid phase materials must be brought to a concentration of 10% bleach and allowed to stand for 20 min. (or until the indicator changes color for media) prior to disposal. Material which cannot be chemically disinfected must be sent offsite for disposal. Contact the Biology Safety Coordinator for details on waste that must be treated offsite.

11. In addition to item 11 under *BSL-1 General Laboratory Practices* above, the P.I. shall ensure that no laboratory personnel conduct work involving rDNA, rDNA organisms, or biological agents of a BSL-2 categorization until proficiency in standard and special microbiological practices has been demonstrated.

12. A sign incorporating the universal biohazard symbol and name of the organism in use must be posted on the laboratory door, along with emergency contact information.

III. Infectious Material Spill and Exposure Procedures

The following detail the procedures that must be followed in the event of a spill of material containing infectious organisms or biological agents at BSL-2 containment. All laboratory personnel must be very familiar with these procedures so that successful execution of the protocols can be carried out during emergency situations. Any spill or accident involving rDNA that leads to personal injury (i.e. needle stick), illness or a breach of containment must be reported to the Office of Biotechnology Activities (OBA) at NIH.

Spill Procedure

1. Alert everyone in the lab to the spill and evacuate the immediate area.

2. Wear the appropriate personal protective equipment to clean up the spill. At a minimum this must include gloves, chemical splash goggles, a face mask or shield, and a lab coat. If the spill clean-up requires additional respiratory protection, do not attempt to clean up the spill. Instead, evacuate the lab, closing the door behind you, and report the incident to the campus police at x2111. Identify the organism, the need for respiratory protection and any other hazardous conditions present (eg. hazardous chemicals involved in the spill).

3. Remove broken glass, if any, with tongs or some other mechanical device. Do not use your hands. If a dust pan and brush must be used, attempt to minimize the spread of the contaminated liquid. The collection apparatus and glass should then be decontaminated by soaking in a 10% bleach (or 70% ethanol, if appropriate) disinfectant solution for 20 min., followed by rinsing with water. The glass can then be disposed of in the glass waste box.

4. Place an absorbing towel or spill pillow, as appropriate to the spill size, on the liquid. Carefully disinfect the area with the disinfectant solution. Ensure that all of the liquid is contained by the absorbing material, and let stand for 20 minutes.

5. After the elapsed time, transfer the absorbing material to a biohazard bag.

6. Apply more disinfectant solution to the spill area and contain with absorbing material. After all of the liquid is absorbed, transfer the absorbing material to the biohazard bag.

7. After disinfecting, rinse the spill area with water and paper towels, or mop.
8. Contaminated gloves and face masks should be placed in the biohazard bag. Other contaminated personal protective equipment can be disinfected as appropriate. Lab coats should be autoclaved prior to laundering. The Biology Safety Coordinator should be contacted for disposal of the biohazard bag.
9. Wash hands thoroughly with soap and water before exiting laboratory.
10. If the campus police were not contacted to clean up the spill, report the incident as soon as possible to the Environmental Health and Safety Director (Mr. Rick Mears, x8189).

Exposure Procedure

1. Small spills to the hands or lower arms should be washed thoroughly with soap and water.
2. If a person is splashed in the eyes, assist the person to the eyewash station, and flush the eyes for 15 minutes. Wear gloves, lab coat and chemical splash goggles while assisting the victim.
3. Contaminated clothing should be removed for spills to the body, and placed in a biohazard bag or bin. Wear gloves, lab coat and chemical splash goggles while assisting the victim. Wash the affected area thoroughly with soap and water.
4. Contact the campus police at x2111 to report the incident and request medical evaluation. Report the identity of the organism and type of exposure (i.e, splash to the skin or eye, inhalation, etc.)
5. Reports of all exposures must be made to the P.I. and Institutional Biosafety Committee (IBC).

Spills of non-infectious materials handled at BSL-1 containment levels do not require special clean-up procedures. Exposures to non-infectious materials handled at BSL-1 containment levels should be treated by washing with soap and water for skin contact, or flushing the eyes with water for 15 minutes for eye contact. If irritation develops, seek medical evaluation.

IV. Final Remarks

This manual contains the basic laboratory procedures for working with rDNA, rDNA containing organisms and biological agents. Please note that you must also receive hands-on

training from your P.I. regarding laboratory specific hazards and procedures and standard microbiological practices and techniques. Refresher training is required annually, or more frequently as needed (*e.g.,* when procedures change significantly). Please also keep in mind that this training only covers work with rDNA and/or biological agents. Additional training is required for work with hazardous chemicals, bloodborne pathogens, radioactive materials and for other hazards. If your work involves other hazards, contact the Biology Safety Coordinator or Chemical Hygiene Officer for additional training requirements. We wish you a productive, and safe, research experience!