



College of the Holy Cross

**Recombinant DNA
Safety Manual**

Triumvirate Environmental,, Inc.

Modified by Jamie Herrick, Biosafety Officer

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Reviewed annually

Recombinant DNA Safety Program

A knowledge and understanding of the information presented in this document is essential for those investigators using or contemplating the use of recombinant DNA technology in their research.

Introduction

Research involving recombinant deoxyribonucleic acid (DNA) shall comply with the National Institute of Health's "Guidelines for Research Involving Recombinant DNA Molecules" (NIH Guidelines) as published in the *Federal Register* (www.nih.gov/od/orda/toc.htm). The recombinant DNA guidelines are applicable to all recombinant DNA research within the United States or its territories, which is conducted at or sponsored by an institution that receives any support for recombinant DNA research from NIH. Any individual receiving support for research involving recombinant DNA must be associated with or sponsored by an institution that can and does assume the responsibilities assigned in the guidelines.

The safe conduct of experiments involving recombinant DNA depends on the individual conducting such activities. The guidelines cannot anticipate every possible situation. The NIH guidelines are intended to assist the institution, institutional biosafety committee (designated as the Recombinant DNA Safety Committee [IBC] at Holy Cross), Chemical and Biological Safety Officer, and principal investigator in determining safeguards that should be implemented. It is the responsibility of Holy Cross and those associated with it to adhere to the intent of the NIH guidelines as well as to its specifics.

The purpose of the *NIH Guidelines* is to specify practices for constructing and handling

- recombinant DNA molecules
- organisms and viruses containing recombinant DNA molecules

In the context of the *NIH Guidelines*, recombinant DNA molecules are defined as

- molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell
- molecules that result from the replication of those described above

Synthetic DNA segments which are likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) are considered as equivalent to their natural DNA counterpart. If the synthetic DNA segment is not expressed *in vivo* as a biologically active polynucleotide or polypeptide product it is exempt from the NIH guidelines. Genomic DNA of plants and bacteria that have acquired a transposable element, even if the latter was donated from a recombinant vector no longer present, are not subject to the NIH guidelines unless the transposon itself contains recombinant DNA.

Responsibilities

General responsibilities relating to health and safety at Holy Cross are described in the Chemical Hygiene Plan, which is distributed to deans, department heads, and principal investigators. Holy Cross is responsible for ensuring that the research is conducted in full conformity with the provisions of the NIH guidelines. This section describes and assigns those responsibilities.

College President

The President of Holy Cross, upon recommendation of the Vice President for Research, shall appoint the Institutional Biosafety Committee.

Institutional Biosafety Committee (IBC)

The [Institutional Biosafety Committee](#) has established and implemented policies that provide for the safe conduct of recombinant DNA research and that ensure compliance with the NIH guidelines. The IBC is comprised of at least five members who collectively have experience and expertise in recombinant DNA technology. They possess the capability to assess the safety of recombinant DNA research and to identify any potential risk to public health or the environment. Two members are not affiliated with the College (apart from their membership on the IBC) and represent the interest of the surrounding community with respect to health and protection of the environment. The Biological Safety Officer also serves as member. The IBC is responsible for reviewing recombinant DNA research conducted at or sponsored by the College for compliance with the NIH guidelines as specified in Section III of the guidelines. The committee approves those research projects that are found to conform with the NIH guidelines. This review shall include:

- an independent assessment of the containment levels required by the NIH guidelines for the proposed research
- assessment of the facilities, procedures, practices, and training and expertise of personnel involved in recombinant DNA research
- notifying the principal investigator of the results of the committee's review
- reviewing recombinant DNA research conducted at Holy Cross to ensure compliance with the NIH guidelines
- adopting emergency plans covering accidental spills and personnel contamination resulting from recombinant DNA research
- reporting any significant problems with or violations of the NIH guidelines and any significant research-related accidents or illnesses to the appropriate institutional official and NIH/Office of Biotechnology Activities (OBA) within 30 days
- establishing subcommittees or ad hoc committees as necessary to carry out its overall responsibilities

Biological Safety Officer

The Biological Safety Officer's duties include, but are not limited to:

- periodic inspections to ensure that laboratory standards are rigorously followed (Note: laboratories are periodically inspected as part of the laboratory safety program)
- reporting to the IBC and the College any significant problems, violations of the NIH guidelines, and any significant research-related accidents or illnesses
- developing emergency plans for handling accidental spills and personnel contamination and investigating laboratory accidents involving recombinant DNA research
- providing advice on laboratory security

- providing technical advice to principal investigators and the IBC on laboratory safety procedures.

Principal Investigator

The principal investigator is responsible for full compliance with the NIH guidelines in the conduct of recombinant DNA research. As part of this responsibility, the principal investigator shall:

- make an 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 for the research
- submit the initial research protocol and any subsequent changes (e.g., changes in the source of DNA or host-vector system), if covered under Sections III-A, III-B, III-C, III-D, or III-E of the guidelines, to the IBC for review and approval or disapproval
- remain in communication with the IBC throughout the duration of the project
- make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken
- instruct and train laboratory staff in the practices and techniques required to ensure safety and the procedures for dealing with accidents
- supervise the safety performance of the laboratory staff to ensure that the required safety practices and techniques are employed
- investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety Officer, the IBC, NIH/OBA, and other appropriate authorities
- correct work errors and conditions that may result in the release of recombinant DNA materials
- ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics)
- initiate or modify no recombinant DNA research which requires IBC approval prior to initiation (see Sections III-A, III-B, III-C, and III-D of the guidelines) until that research or the proposed modification thereof has been approved by the IBC and has met all other requirements of the NIH guidelines
- determine whether experiments are covered by Section III-E of the guidelines and ensure that the appropriate procedures are followed
- report any significant problems, violations of the NIH guidelines, or any significant research-related accidents and illnesses to the Biological Safety Officer, IBC, NIH/OBA, and other appropriate authorities (if applicable) within 30 days
- report any new information bearing on the NIH guidelines to the IBC and to NIH/OBA
- be adequately trained in good microbiological techniques adhere to IBC-approved emergency plans for handling accidental spills and personnel contamination
- comply with shipping requirements for recombinant DNA molecules

Submissions by the Principal Investigator to the NIH/OBA are covered in section IV-B-7-b of the NIH guidelines. For example, these submissions can cover certifying of new host-vector systems or determining level of containment. See the guidelines for exact requirements.

Approval Procedures

The policies set forth below comply with the NIH Guidelines. They apply to all recombinant DNA research conducted at Holy Cross, without regard to source of funding.

Registration Document

Principal investigators intending to use recombinant DNA molecules shall notify the [Institutional Biosafety Committee](#) (IBC) by contacting the biosafety officer for information. The principal investigator shall prepare the registration document according to the nature of the research. A copy of the [current NIH guidelines](#) should be available for reference. All recombinant DNA research falls into one of six classes, described in the NIH guidelines as follows:

- section III-A Experiments that require Institutional Biosafety Committee approval, Recombinant DNA Advisory Committee (RAC) review, and NIH Director approval before initiation
- section III-B Experiments that require NIH/ORDA and Institutional Biosafety Committee approval before initiation
- section III-C Experiments that require Institutional Biosafety Committee approval, Institutional Review Board approval, and NIH/ORDA registration before initiation
- section III-D Experiments that require Institutional Biosafety Committee approval before initiation
- section III-E Experiments that require Institutional Biosafety Committee notice simultaneous with initiation
- section III-F Exempt experiments

If an experiment falls into section III-A, III-B, or III-C and one of the other sections as well, the rules pertaining to section III-A, III-B, or III-C shall be followed. If an experiment falls into section III-F alone, or into section III-F and into section III-D or III-E as well, the experiment is considered exempt from the NIH guidelines.

Status Report

Principal investigators are required to file an annual Status Report for all recombinant DNA research. In addition, principal investigators must file a new or amended registration document when there are changes in their research protocols that require reclassification.

Training

Principal investigators performing recombinant DNA experiments covered by Sections III-A through III-E under the NIH guidelines shall file Statement of Training and Experience forms with the IBC for all active researchers.

Changes in Approved Research

Principal investigators wishing to modify approved research regulated under the NIH guidelines are responsible for notifying the IBC. Modifications to research conducted under Sections III-A, III-B, III-C, and III-D must be approved prior to implementing new procedures. The IBC must be notified of modifications under Section III-E at the time they are implemented. Notification of modifications to exempt research conducted under Section III-F is not required; however, the annual Status Report will reflect the changes.

Laboratory Inspections

Laboratory inspections are required for recombinant DNA experiments that are regulated by the NIH guidelines. The biosafety officer will inspect newly registered laboratories working at biosafety level 2 (BSL2) as part of its normal laboratory inspection functions. Inspection and certification of newly registered laboratories by the IBC are required for recombinant DNA experiments performed at biosafety level 3 (BSL3). The IBC inspection team is comprised of two members of the IBC and a representative of ORS. BSL3 laboratories are inspected annually by the IBC.

Spill Procedures for Recombinant DNA Laboratories

Advance preparation for spill management is essential. Use these procedures to help you preplan your response to spills in the recombinant DNA laboratory. These procedures address minor spills involving small quantities of biological materials. In the case of a large spill or a spill involving a highly infectious agent, contact the biosafety officer for assistance with decontamination and cleanup. More detailed spill management information is available. Call 508-793-2222 for assistance. In all cases the principal investigator is responsible for maintaining an adequate supply of a chemical disinfectant effective against the microorganisms being investigated.

Spill in a Biological Safety Cabinet

A spill that is confined within a biological safety cabinet generally presents little or no hazard to personnel in the area. However, chemical disinfection procedures are to be initiated at once while the cabinet continues to operate. The disinfectant shall be one that is active against the organisms of potential hazard. Flammable liquids, such as ethanol or isopropanol, shall not be used, even if effective, because of the fire hazard of generating dangerous vapor concentrations within the cabinet that could be ignited by an electrical spark or other source.

Spray or wipe the walls, work surfaces, and equipment with the chosen disinfectant. Allow the disinfectant to remain on the surface for the appropriate contact time.

Minimize the generation of aerosols and use sufficient disinfectant to ensure that drain pans and catch basins below the work surface contain disinfectant. The front exhaust shall also be wiped and the disinfectant drained into a container.

- Maintain cabinet ventilation.
- Warn others in the laboratory.
- Notify the principal investigator.
- Wear protective gloves, a lab coat or gown, and eye protection during the procedure.
- Spray or wipe walls, work surfaces, and equipment with appropriate disinfectant. A disinfectant with detergent has the advantage of detergent activity that will help clean the surfaces by removing both dirt and microorganisms.
- Use sufficient disinfectant to ensure that drain pans and catch basins below the work surface contain the disinfectant. Lift the front exhaust grill and tray and wipe all surfaces. Wipe the catch basin and drain the disinfectant into a container.
- Observe the recommended contact time for the disinfectant.
- Dispose of in biowaste container.

This procedure will not disinfect the filters, fans, air ducts, and other interior parts of the cabinet. If the entire interior of the cabinet needs to be disinfected, contact biosafety officer for direction.

Spill in the Open Laboratory

For a spill in the open laboratory outside a biological safety cabinet, the spill response depends on the size of the spill and hazard of the material. A minimally hazardous material spilled without generating appreciable aerosols can be cleaned with a paper towel soaked in a chemical disinfectant.

A spill of a larger volume of hazardous material with aerosol generation requires evacuating the room, waiting for aerosol reduction, donning personal protective gear (including appropriate respiratory protection), selecting a disinfectant effective against the organisms involved, and cleaning as described above. Following cleanup, response personnel shall wash or shower with a disinfectant soap.

For a small spill of biological material in the open laboratory, take the following action:

- Warn others in the laboratory.
- Notify the principal investigator.
- Wear gloves and protective clothing.
- Decontaminate with an appropriate disinfectant.
- Dispose of as described above.
- If clothing is known to be contaminated, carefully remove it, folding the contaminated area inward. Place the clothing into an autoclavable bag.
- Wash arms, face, and hands.

Spill in a Centrifuge

A biological spill in a centrifuge has the potential for producing large volumes of aerosols. On becoming aware that a spill may have occurred within a centrifuge or other piece of equipment, turn off the equipment, warn others in the area, notify the principal investigator, allow aerosols to settle, and decontaminate following the principles described above.

- Turn off the centrifuge and allow time for the aerosols to settle.
- Warn others in the laboratory.
- Notify the principal investigator.
- Wear gloves and protective clothing.
- Decontaminate with an appropriate disinfectant. Place contaminated equipment in a leak proof bag and move it to a biological safety cabinet, if possible, for decontamination.

Spill Involving Radioactive Materials

- Warn others in the laboratory.
- Call 508/793-2222

Biological Spill on a Person

If a biological material is spilled on a person, emergency response is based on the hazard of the biological agent spilled, the amount of material spilled, and whether significant aerosols were generated. If aerosol formation is believed to have been associated with the spill, a contaminated person shall leave the contaminated area immediately. If possible, (s) he should go to another laboratory area so that hallways and other public areas do not become contaminated. Contaminated clothing is removed and placed in red or orange biohazard bags for disinfecting. Contaminated skin shall be flushed with water and thoroughly washed with a disinfectant soap. Showering may be appropriate, depending on the extent of the spill.

NIH Guidelines for Research Involving Recombinant DNA Molecules

http://oba.od.nih.gov/oba/rac/guidelines_02/NIH_Guidelines_Apr_02.htm

B. *Technical description of experiments: Use a separate page to provide a more detailed review of proposed research. Information provided must be sufficient to eliminate the need for reference to other documents or scientific papers. Note approximate starting date of each series of experiments, and be sure to include the following information:*

1. The source of DNA
2. Nature of inserted DNA segments (toxic product, DNA percentage of viral genome, etc.)
3. Hosts and vectors used (bacterial strains, cell lines, plasmids, phage, viruses, etc.)
4. Microorganisms other than recombinant, etiologic agents, human source materials, blood, tissue, body fluids, animal source materials
5. Scale of work, bench scale, >9.9 liters, production scale
6. Specialized equipment needed or used routinely (e.g., centrifuges or Bio-safety Cabinets for controlling aerosols) for processing large quantities of material (more than 1 liter).
7. Chemical aspects of projects which may require prior approval due to the use of large quantities of chemicals or the acutely hazardous or toxic nature of the chemicals involved (e.g., cyanogen bromide, toxin work, mutagenesis, compressed poisonous or flammable gasses, etc.)
8. Radiation aspects of experiments which may require specialized training, monitoring or modification to the Nuclear Regulatory Commission license (e.g., iodinations, use of >1mCi in any one experiment.)

C. Room numbers where project will be conducted:

D. List all associated investigators and support personnel conducting the work. If any are new to the project, please note their experience in handling these or related materials. A copy of each individuals C.V. must be on file with the Safety Office/Institutional Bio-safety Committee.

E. Will any of these materials be shipped between facilities? Contractor or co-investigator at another address:

Name: _____

Address:

Phone:

List if more than one:

Program Director and Principal Investigator

As Program Director/Principal Investigator I certify that the attached application is accurate and complete.

I agree to update this Project Registration Document whenever personnel or material change, or annually.

I understand my responsibilities relating to the items checked above and agree to comply with all permit requirements relevant to this project.

I also understand that I am responsible for the safe, responsible conduct of my program and the design and implications of projects under my direction.

Signature Program Director

Date

Signature Principal Investigator

Date

Section Below Reserved for Safety Office/Institutional Bio-safety Committee Use:

Recombinant DNA Guidelines NIH level evaluation considerations:

IBC review required _____ Date of review: _____

NIH review required _____ Date of review: _____

Recommended health practices and medical surveillance: _____

Date of Review: _____

Animal Care and Use Committee Review

Containment level evaluation considerations: _____

Date of Review: _____

Human Studies Committee Review

Special considerations: _____

Permit Numbers: _____

USDA/APHIS Guidelines for receipt, use, and disposition of regulated biologicals.

Shipping: USDA, US Postal regulations, DOT, Commerce Department Export

License, foreign import certificates.

OSHA Chemical Lab Standard Update Needed (additional hazardous use, new process, volume increase, etc.)

_____ OSHA Bloodborne Pathogen Standard (clinical samples, etc.)

_____ OSHA Hazard Communication Standard (production areas only).

_____ NRC license amendment

Received and reviewed as noted above

Signature

Date

Safety Officer/Institutional Bio-safety Committee Chairman