



Case Western Reserve University

Department of Environmental Health & Safety

CWRU Biosafety Manual

Special Considerations for Recombinant or Synthetic Nucleic Acids

Background/NIH

Experiments which utilize recombinant or synthetic nucleic acids that alter gene expressing in cell culture, animals or humans are regulated by the National Institutes of Health, Office of Science Policy (NIH OSP). These materials are considered to be biohazards by the NIH, Centers for Disease Control (CDC) and CWRU. Information regarding these regulations can be found in the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids (NIH Guidelines)*. As described in the NIH Guidelines, such research must be reviewed and approved by the CWRU Institutional Biosafety Committee (IBC). This portion of the CWRU Biosafety Manual addresses these experiments and procedures explicitly.

Risk Assessments/ ECPs

All laboratories utilizing recombinant or synthetic nucleic acids (further referred to as 'recombinant materials') must submit an Exposure Control Plan (ECP) to the Department of Environmental Health and Safety. The specific recombinant materials to be used must be listed in the biohazard inventory. The Risk Group can be determined by consulting Appendix B of the NIH Guidelines. Risk Group determination must be based on the wildtype organism even if the proposed research involves attenuated or defective organisms or single genes from pathogenic organisms. The nature of the wildtype biohazard, any mutated or inserted genes and the ways in which the materials will be manipulated must be considered when performing a risk assessment and making a determination of Biosafety Level Containment (BSL).

The ECP should indicate potential exposure information. If the laboratory is working with a pathogenic organism it is strongly encouraged that the laboratory creates post-exposure plans and shares this information with all lab staff and University Health Services. A hard copy should be available and easily accessible in the laboratory in the event that an exposure was to occur while Health Services is closed. This will allow the University Hospitals Emergency Room staff to treat the injured person.

The ECP must be updated annually or when there is a change in personnel, procedures or biohazardous materials.

General Handling of Recombinant Materials

It is expected that all CWRU Laboratories will follow the recommendations set forth in the CDC's Biosafety in Microbiological and Biomedical Laboratories, 5th Edition (BMBL). The additional general handling requirements are as follows:

1. All procedures must be performed carefully to minimize the creation of splashes or aerosols. Experimental procedures should be practiced, using non-hazardous materials until desired technique is mastered.
2. Work surfaces should be decontaminated at least once a day and after any spill of viable or hazardous material. Work surfaces should be decontaminated prior to the start of a procedure to ensure the materials in use and the laboratory workers do not become contaminated.
3. All cultures, stocks, and other regulated wastes must be decontaminated before disposal by an approved decontamination method, such as autoclaving or disinfection with bleach. Materials to be decontaminated outside of the immediate laboratory must be double-bagged in a labeled red biohazard bag, placed in a durable, leak-proof container and closed for transport from the laboratory.
4. Laboratory personnel should receive appropriate immunizations or tests for the agents handled in the laboratory. (Hepatitis B vaccine or TB skin testing).
5. Only needle-locking syringes or disposable syringe-needle units are to be used for injection or aspiration of infectious materials.

Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. They are to be placed in a red rigid sharps container for disposal.

6. Do not handle broken glassware directly by hand. The glassware should be removed by mechanical means such as a brush and dustpan, tongs, or forceps.
7. All cultures, tissues, or specimens of body fluids must be placed in appropriate container(s) that prevent(s) leakage during collection, handling, processing, storage, transport, or shipping. Secondary specimen containment must be utilized when transporting infectious agents within the campus community.
8. Spills and accidents involving recombinant materials should be immediately reported to the PI, the EHS office and the IBC.

Decontamination and Waste Handling

All materials containing recombinant or synthetic nucleic acids must be properly inactivated and decontaminated prior to disposal. The following are the approved methods for proper decontamination and waste handling of recombinant materials:

1. Chemical
 - a. Sodium Hypochlorite is the most common component used for disinfection and usually consists of a 1:10 dilution of household bleach. Surfaces contaminated with blood or OPIM should be cleaned using a freshly prepared 1:10 dilution of household chlorine bleach solution that is prepared at least daily. The contaminated area should be flooded with the bleach solution and then cleaned up using paper towels. Ten minutes of exposure is

required for disinfection. Gloves should be worn during the clean-up procedures. Chlorine bleach can corrode some items and surfaces, so items treated with chlorine should be rinsed thoroughly. Bleach has a shelf life, and the concentration of sodium hypochlorite diminishes over time. Always make a fresh bleach dilution at least once daily and properly discard bleach over one year from date of manufacture. When using bleach to disinfect biohazardous agents or bloodborne pathogens for disposal, remember to keep an accurate log.

- b. Sodium Hydroxide is used for decontamination of mycobacterium tuberculosis and prions. The Petroff method uses a 4% solution for TB, while 40% is used to disinfect prions. Sodium hydroxide is extremely caustic and must be handled correctly, using the proper PPE, and neutralization procedures prior to disposal.
- c. Formaldehyde is an OSHA-regulated chemical that is a suspected carcinogen, so its use as a disinfectant is not recommended.
- d. Iodophors that are registered with the EPA may be effective hard-surface decontaminants when used per manufacturer's instructions, but iodophors formulated as antiseptics are not suitable for use as disinfectants.
- e. Peracetic (peroxyacetic) acid and hydrogen peroxide mixtures minimize the negative effects of corrosiveness sometimes seen with chlorine compounds and high concentrations of peracetic acid alone. A limited number of trade-name products containing <0.1% peracetic acid and <1.0% hydrogen peroxide and registered with the EPA as sterilants/disinfectants are available. The benefit of these products is their rapid action and broad-spectrum of germicidal activity, in addition to the reduced corrosiveness.
- f. Quaternary ammonium compounds are low-level disinfectants and are not recommended for spills of human blood, blood products, or other potentially infectious materials.

Any chemical disinfectant on the [EPA's list of approved tuberculocidal disinfectants \(List B\)](#) may be used and must be included in the ECP.

- 2. Autoclaving utilizes heat, pressure and time to sterilize infectious waste. Autoclaves must operate at a MINIMUM temperature of 121°C (250°F) at a MINIMUM pressure of 15 PSIG for a MINIMUM time of 60 minutes during a treatment cycle (Ohio EPA, Ohio Administrative Code 3745-27-32(D)(1)(a)). These parameters must exist simultaneously (time starts when the proper temperature and pressure are met). Variations of these parameters may be used if it can be validated that the combination of time, temperature, and pressure achieves a performance standard of a four log (base ten) reduction in *Bacillus stearothermophilus* spores. Other variables to consider include autoclaves utilizing gravity versus vacuum displacement. ALWAYS follow manufacture's guidelines for proper operation. Autoclaves must not be loaded beyond the total treatable volume of infectious wastes and must be validated periodically, using a noninfectious "dummy" load, to ensure efficacy. Cycle records must be kept and filed. Only approved materials may be autoclaved. Contact EHS concerning any questions pertaining to autoclave protocols.

Quality assurance and validation testing are required periodically (at least monthly, or immediately following any maintenance) to ensure the sterilization cycle is effective.

Remember to keep an accurate log of all cycles.

3. Incineration is no longer available at Case Western Reserve University. Biohazardous waste including recombinant materials, including transgenic animals and/or animals inoculated with recombinant materials, are transported and incinerated off campus by an approved contractor. Waste must be properly packaged and labeled. In most cases, biohazardous waste must be decontaminated by the laboratory generating it prior to being picked up for incineration. This ensures safe transport within the University community. Contact EHS if there are any questions on proper disposal of biohazardous waste.

Liquids Containing Recombinant Materials

After decontamination of biohazardous liquids containing recombinant materials, the liquid can be poured down the sanitary sewer drain with copious amounts of water. Following decontamination, decant all solids (tissue, etc.) prior to pouring. If biohazardous liquids contain other hazardous materials (chemical or radiological, etc.), other additional waste disposal regulations may exist (EPA, ODH, etc.). Even though decontaminated biohazardous liquids can be poured down the drain, the chemical used for decontamination may need to be neutralized or disposed of in another manner. BE CAREFUL to follow proper procedures and check with EHS before proceeding if there are any questions. Remember, an accurate disinfection log must be kept.

Solids Used with Recombinant Materials

Biohazardous solids that have been used with or contain recombinant materials include all plastic Petri dishes and plastic tissue culture vessels containing media, whether contaminated or not; cultures and stocks of infectious agents; devices used to transfer, inoculate or mix such agents; paper or cloth contaminated with biohazardous agents. All biohazardous solids must be autoclaved in red bags, marked, sterilized and tagged with the investigators' name and date of sterilization.

The autoclaved waste must remain in the laboratory for pickup by the biowaste crew. Call Customer Service at 216-368-2580 before 4:30 PM for evening pickup. An accurate waste log should be kept.

Regulated Sharps

1. All syringes, needles, scalpel blades, and razor blades are considered an infectious waste according to the Ohio Infectious Waste Law and, whether contaminated or not, MUST always be disposed of in a properly identified rigid container. These containers must also be used for:
 - a. Contaminated and uncontaminated lancets
 - b. Contaminated and uncontaminated microtome blades
 - c. Contaminated and uncontaminated IV tubing with needles attached
 - d. Contaminated glass Pasteur pipettes
 - e. Contaminated broken and unbroken glass and plastic ware

- f. Any other contaminated items that could potentially puncture a bag
- g. Contaminated disposable plastic pipettes and pipette tips

Place the sharps in a leak-proof, puncture-proof container with a lid. A fluorescent orange or orange-red label that has the biohazardous symbol in a contrasting color must be affixed to the container. Be sure to label the container with the word "SHARPS". Most biological laboratories use the preferred "red rigid SHARPS container", which can be purchased with proper labeling affixed. Once the containers reach capacity, call Customer Service at 216-368-2580 before 4:30 PM for evening pickup. NEVER OVERFILL SHARPS CONTAINERS.

- 2. Non-biohazardous plastic pipettes and pipette tips can be placed in a sturdy corrugated box lined with a plastic bag (non-red, orange or yellow). All previous identifying markings must be removed or defaced from the box and the words "Non-biohazardous SHARPS" must be marked. The box must be securely taped. Again, call Customer Service at 216-368-2580 before 4:30 PM for evening pickup.

Mixed Hazardous Waste

Special care must be taken when dealing with biohazardous waste that contains recombinant materials and other hazardous materials. For instance, for radioactive biohazardous waste, one of two procedures must be conducted:

- 1. Radioactivity must be held for 10 half lives before material can be disposed of as a biohazardous waste.
- 2. The material must be decontaminated before it can be disposed of as radioactive waste. NOTE: If decontamination utilizes the use of a hazardous chemical, the result will be a chemical/radioactive mixed waste.

When dealing with a biohazardous material containing a hazardous chemical, such as a carcinogen, the material must be decontaminated prior to hazardous chemical disposal.

ALWAYS call EHS for proper waste disposal concerns, and include all waste disposal protocols in the CHP and ECP.

Spills, Exposures, Reporting and Emergency Response

Accidents and Exposures

Laboratory personnel who are accidentally exposed to a potentially infectious agent or material should report the incident as soon as possible to the PI and EHS. The PI will see that necessary treatment or health monitoring is obtained without delay. University Health Services will provide follow-up and counseling on risk of infection and its consequences. The Risk Management Employee Illness or Injury Report form must be completed for all workplace injuries and illnesses.

It is strongly recommended that laboratories working with recombinant materials and other biohazards have written post-exposure procedures in place. These procedures should be on file with University Health Services and a hard copy should be located in an easily accessible location in the laboratory in case the exposure occurs after-hours and the researcher must go to the University Hospitals emergency room for treatment.

Post-Incident Reporting

Spills, accidents, exposures and loss of containment involving recombinant materials may require reporting of the incident to the NIH OSP. Appendix G-II-B-2-k of the *NIH Guidelines* states:

Spills and accidents which result in overt exposures to organisms containing recombinant or synthetic nucleic acid molecules are immediately reported to the CWRU Institutional Biosafety Committee and NIH/OSP. Reports to NIH/OSP shall be sent to the Office of Science Policy, National Institutes of Health, preferable by email to NIHGuidelines@od.nih.gov, additional contact information is also available here and on the OSP website (www.osp.od.nih.gov). Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained. After contacting Health Services, Security Services, or Environmental Health and Safety for proper handling of the spill and any safety issues, please contact the CWRU IBC office to discuss the spill and/or accident. The CWRU IBC can be reached at 216-368-0838.

As CWRU does not have recombinant research involving large scale quantities (>10L), Risk Group 4 organisms, Biosafety Level 4 Containment or Risk Group 3 strains of Influenza, the University's additional reporting requirements fall under Section IV-B-2-b-(7) of the *NIH Guidelines*. This section states:

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/OSP within 30 days, unless the Institutional Biosafety Committee determines that a report has already been filed by the Principal Investigator. Reports to NIH/OSP shall be sent to the Office of Science Policy, National Institutes of Health, preferable by email to NIHGuidelines@od.nih.gov; additional contact information is also available here and on the OSP website (www.osp.od.nih.gov). All incidents involving recombinant materials will therefore be presented to the IBC who will investigate and determine if significant exposure or violations occurred, at which time a report will be filed with the NIH OSP.

First Aid

In the event that a substance enters the mouth, eyes, lungs, or penetrates/comes in contact with the skin follow the instructions below and seek immediate medical attention. (**NOTE:** Always be aware of any special precautions and procedures required to mitigate toxic or infectious responses to materials in use, by reading and understanding the ECP BEFORE starting any work with them.

1. Remove all contaminated clothing and place it in the biological safety cabinet.
2. Warn others of the biohazard.
3. Take a shower or rinse the exposed area with disinfectant.

4. Report the spill to the PI or Laboratory Manager. If an individual is injured during work:
 - a. Go to the University Hospital Emergency Room to obtain emergency care.
 - b. On-site emergency assistance can be obtained by dialing Protective Services at 216-368-3333.
 - c. Persons requiring immediate emergency care should seek it. Preparation of paperwork will be secondary to obtaining prompt medical attention.
5. Post accident serum samples for diagnosis of possible laboratory acquired infection will be coordinated by the PI or Laboratory Manager.
6. The PI or Laboratory Manager should accompany injured personnel to receive a medical evaluation and complete an incident form.
7. The healthcare provider will make an initial assessment of risk.
8. University Health Services will provide follow-up and counseling on risk of infection and its consequences.
9. Personnel working in the laboratory, or who have performed duties in the past 6 months in an area containing infectious materials, must attempt to notify their supervisors before seeking medical attention if they:
 - a. Develop a fever greater than 100 °F; or
 - b. Display initial onset symptoms consistent with contraction of the infectious agent used in the laboratory.

Spills inside biological safety cabinet

The occurrence of a spill in a biological safety cabinet poses less of a problem than a spill in an open laboratory as long as the spilled material is contained in the biological safety cabinet. A Biosafety Cabinet is designed to contain spills and associated aerosols which are released during work within the cabinet. Provided that the Biosafety Cabinet is operating properly and has been inspected and certified, aerosols produced by a spill should be contained. A spill of a biohazardous material should be attended to immediately. Decontamination of the work zone can usually be accomplished by direct application of concentrated liquid disinfectants along with a thorough wipe down procedure. Formaldehyde gas decontamination or vaporized hydrogen peroxide (a service provided by an approved contractor) may be required to treat inaccessible sections of the cabinet interior following a spill. Contact EHS after a major spill to determine the need for decontamination with formaldehyde or hydrogen peroxide. For spills within a BSC, the following steps should be conducted:

1. Alert people in immediate area of spill.
2. Chemical decontamination procedures should be initiated immediately while the biological safety cabinet continues to operate.

3. Contain the spill and decontaminate. All workers using the Biosafety Cabinets should have a supply of absorbent materials and decontaminating agent within the cabinet. This avoids the need to withdraw your arms from within the cabinet should a spill occur and allows you to decontaminate yourself prior to leaving the cabinet.
4. Wear appropriate personal protective equipment during decontamination procedure. The spill should be covered with paper towels or other absorbent materials soaked with a proven decontamination agent (e.g., 1:10 dilution of bleach containing sodium hypochloride) for 15 to 20 minutes.

NOTE: A 10% bleach solution will eradicate most infectious agents. One exception is prions including the select agent bovine spongiform encephalopathy (BSE), or Mad Cow Disease. Decontamination of this infectious substance requires a 40% household bleach solution or a 1N NaOH solution, which must remain in contact with this infectious material for at least one hour.

5. Use paper towels to wipe up the spill, working from the edges into the center.
6. Place paper towels in a red or orange biohazard bag containing the proper universal biohazard label.
7. Thoroughly rinse area with water using clean paper towels or other absorbent materials and dry.
8. Decontaminate equipment and utensils. Items that are not readily or easily surface decontaminated should be carefully placed into autoclave bags and removed for further treatment (e.g., decontamination by autoclaving or other approved methods).
9. Contaminated gloves and clothes must be decontaminated or properly disposed of after decontamination of area is complete.
10. Remove protective gear. Individuals involved in the spill and clean-up should remove protective clothing (either disposing as biohazardous waste or decontaminating), wash their hands and face with an appropriate decontamination soap, and report to the University's Health Services or the University Hospital Emergency Room for any required evaluation or follow-up.

Spills outside biological safety cabinet

Biological spills outside biological safety cabinets will generate aerosols that can be dispersed in the air throughout the laboratory. Appropriate protective equipment is particularly important in decontaminating spills involving microorganisms.

This equipment includes lab coat with long sleeves, back-fastening gown or coveralls, disposable gloves, disposable shoe covers, and safety goggles and mask or full-face shield. Use of this equipment will prevent contact with contaminated surfaces and protect eyes and mucous membranes from exposure to splattered materials. For minor spills (less than 10 ml and generating little aerosol) on equipment, laboratory benches, walls, or floors:

1. Close laboratory doors and post warning signs to prevent others from entering the laboratory.
2. Thoroughly wash hands and other apparently contaminated areas with soap and water.

3. Put on appropriate personal protective equipment during decontamination procedure.
4. The spill should be covered with paper towels or other absorbent materials soaked with a proven decontamination agent (e.g., 1:10 dilution of bleach containing sodium hypochloride) for 15 to 20 minutes.

NOTE: A 10% bleach solution will eradicate most infectious agents. One exception is prions including the select agent bovine spongiform encephalopathy (BSE), or Mad Cow Disease. Decontamination of this infectious substance requires a 40% household bleach solution or a 1N NaOH solution, which must remain in contact with this infectious material for at least one hour.

5. Use paper towels to wipe up the spill, working from the edges into the center.
6. Place paper towels in a red or orange biohazard bag containing the proper universal biohazard label.
7. Thoroughly rinse area with water using clean paper towels or other absorbent materials and dry, and place paper towels in a red or orange biohazard bag containing the proper universal biohazard label.
8. If using an autoclave, BE CAREFUL to avoid strong oxidizers from coming in contact with organic materials (See Point 10 of "Autoclaves" under "Equipment" in Chapter 5).
9. Wash hands and other apparently contaminated areas again with soap and water.
10. Remove all PPE immediately upon leaving the work area and as soon as possible if overtly contaminated. Contaminated PPE must either be DISPOSED of as biohazardous waste or properly decontaminated.

For major Spills (more than 10 ml or with considerable aerosol):

1. Close laboratory doors and post warning signs to prevent others from entering the laboratory.
2. Leave the biological safety cabinet operating and cultures inside cabinet.
3. Wash hands and other apparently contaminated areas with soap and water.
4. Report the accident to the PI and to the University Biosafety Officer in EHS (216-368-2907).
5. If personal clothing is contaminated, remove all outer clothing and place it in the autoclave or container for autoclaving. Put on clean garments.
6. Leave the laboratory for 20 minutes to allow dissipation of aerosols created by the spill.
7. Upon returning to the laboratory to start decontamination, check to ensure that laboratory doors are closed and appropriate signs are displayed. Put on gloves and other protective equipment as previously mentioned above.
8. Place paper towels soaked with decontamination solution over the spill area. Do not pour decontamination solution directly onto the spill in order to avoid additional release of aerosols.

9. Let decontamination solution / microorganism mixture stand for 30 minutes or longer to allow adequate contact time.
10. Wipe up the spill with the soaked paper towels and properly dispose of towels.
11. Remove all PPE immediately upon leaving the work area and as soon as possible if overtly contaminated. Contaminated PPE must either be DISPOSED of as biohazardous waste or properly decontaminated.

If using an autoclave, BE CAREFUL that strong oxidizers do not come in contact with organic materials (See Point 10 of "Autoclaves" under "Equipment" in Chapter 5).

12. Thoroughly wash hands, face, and other apparently contaminated areas. Special care in decontamination may be necessary. The PI and/or the University Biosafety Officer may require the collection of sample cultures to determine that the area has been effectively decontaminated.

NOTE: A 10% bleach solution will eradicate most infectious agents. One exception is prions including the select agent bovine spongiform encephalopathy (BSE), or Mad Cow Disease. Decontamination of this infectious substance requires a 40% household bleach solution or a 1N NaOH solution, which must remain in contact with this infectious material for at least one hour.

Fire Fighting Procedures

Personal safety is each worker's primary concern in the event of fire.

1. Upon learning of the threat of fire within the building, laboratory personnel will, to the extent possible:
 - a. Turn off all gas burners, biological safety cabinets, electric motors, and other electrical equipment.
 - b. Place containers of infectious materials into autoclaves, incubators, refrigerators, freezers or other storage areas.
 - c. Leave the laboratory as quickly as possible using designated fire evacuation routes.
2. Personnel should be trained by the acting University Fire Marshall in the operation of fire extinguishers.