IX. Decontamination and Spills

IX.1. Definitions

- **Sterilization**: the act or process, physical or chemical, which destroys or eliminates all forms of life, especially microorganisms.
- **Decontamination**: reduction of all organisms and the destruction of pathogenic organisms in or on a material so that material is no longer considered to be capable of transmitting disease.
- **Disinfection**: the act of destroying or irreversibly inactivating specific viruses, bacteria, or pathogenic fungi, but not necessarily their spores, on inanimate surfaces. Most disinfectants are not effective sterilizers.
  - High Level Disinfectants kill all viruses and vegetative cells, but they may not kill endospores reliably.
  - Intermediate Level Disinfectants destroy all vegetative cells including Mycobacteria, fungi and most, but not all viruses. They cannot kill endospores.
  - Low Level (General Purpose) Disinfectants destroy vegetative bacteria, except Mycobacteria, fungi and non-enveloped viruses.
- **Antiseptic**: a substance that prevents or arrests the growth or action of microorganisms either by inhibiting their activity or by destroying them. The term is used especially for preparations applied to living tissue.

IX.2. Evaluation

The initial risk assessment for any project should include an evaluation of the processes and/or agents to be used to ensure that the biohazardous materials involved in the research are inactivated during spill cleanup, before cleaning equipment for re-use, and for final disposal.

The OSHA Bloodborne Pathogens Standard requires that all equipment
and environmental and working surfaces shall be cleaned and decontaminated after contact with blood or other potentially infectious materials. The standard also requires decontamination of contaminated work surfaces after completion of procedures, immediately or as soon as feasible after any overt contamination of surfaces or any spill of potentially infectious material, and at the end of the work shift if the work surface has become contaminated. All reusable equipment shall be decontaminated immediately or as soon as feasible after visible contamination.

For any infectious material adequate pre-cleaning of surfaces is important for any disinfection or sterilization procedure. Ten minutes of exposure to a disinfectant is not adequate to disinfect objects that have narrow channels or other areas that can harbor microorganisms. **Alcohols**, 70%, for example, are effective for killing HBV but are not recommended for this purpose because of their rapid evaporation and the consequent difficulty of maintaining proper contact times. Alcohols have been removed from many laboratories because they are flammable. Alcohols should be maintained only in small volumes and may be desirable as an adjunct to skin disinfection.

**Chlorine compounds** are widely used disinfectants in the laboratory. An inexpensive, broad-spectrum disinfectant for use on bench tops and similar surfaces can be prepared by diluting common household bleach (5.25 % sodium hypochlorite solution [some cut-rate brands might not contain this much hypochlorite]) to obtain at least 500 ppm of free available chlorine. (Some bleach solutions available now contain about 1/3 more hypochlorite than the solutions mentioned above. Check to see what the concentration of chlorine in the bleach solution you are using.) A 1:10 dilution of commercial bleach (10%) that produces a solution containing 5000 ppm of free chlorine can be used to disinfect spills. The use of higher concentrations of bleach in chemical fume hoods should be reserved for significant contamination.

**High concentrations of bleach solutions should not go into an autoclave.**
Prepare a fresh solution of bleach each day; discard unused portions down the sink drain and then flush with fresh water. Be aware that chlorine compounds may corrode metals, especially aluminum. To help prevent corrosion after using the bleach solution, rewipe the surfaces with 70% ethanol.

Chlorine dioxide, either as a liquid solution or as a gas, can also be used for decontamination purposes. The liquid form (i.e. Clidox) is most commonly used as a 1:5:1 or 1:18:1 dilution (base:water:activator). Chlorine dioxide gas can be used for large equipment or space decontaminations.

**Iodophors** that are registered with the EPA may be effective hard-surface decontaminants when used per the manufacturer’s instructions, but iodophors formulated as antiseptics are not suitable for use as disinfectants (i.e. Wescodyne).

**Phenolics** that are registered with the EPA may be effective hard-surface decontaminants when used per the manufacturer’s instructions (i.e. Vesphene, Hil-Phene).

**Quaternary ammonium compounds** are low-level disinfectants and are not recommended for spills of human blood, blood products, and other potentially infectious materials (i.e. Conflikt, End-BacII).

The use of such chemicals requires that the laboratory have a current Chemical Hygiene Plan (29 CFR 1910.1450, Occupational Exposure to Hazardous Chemicals in Laboratories). Safety Data Sheets (SDSs) for the chemicals in use must be made available to the individuals in the lab, as well as training on special procedures for handling the chemicals.

**IX.3. Sterilization**

Unless the facility is permitted by the Ohio EPA to treat infectious waste, all terminal treatment is incineration. Consequently, **all pre-treated and untreated biohazards** (i.e., infectious waste) shall be
placed in a burn box. See Appendix C. Consult EHS for assistance.

According to the OEPA, for terminal sterilization to be allowed, the sterilization process (steam autoclaving, dry heat, etc.) must be validated, and the validation documented. Liquid "cold" sterilants may be used to sterilize equipment that will not withstand the heat of steam or the chemical reactivity of ethylene oxide processing.

Additionally, the sterilization process must also be monitored at least weekly (or a quality-control run completed if the autoclave is used less often than weekly) with biological indicators (spore strips, time/temperature charts, etc.), and records of monitoring kept for review.

**Since The Ohio State University has no autoclave licensed for terminal sterilization by the OEPA, individuals using autoclaves must still have their autoclaved waste prepared and sent off-site for incineration per the University contract.** If an autoclave is being used for infectious waste pre-treatment, periodic monitoring of the effectiveness of the sterilization process is still recommended.

### IX.3.1. Steam Sterilization

Steam sterilization (autoclaving) is the primary means of sterilization at the University. The following points must be kept in mind when steam sterilization is to be used:

- Materials affected (*e.g.*, denatured or melted) by heat will be destroyed by this method of sterilization;

- Steam must reach the material for a prescribed period of time (adequate sterilization time) to ensure sterilization. **Containers must be open to allow steam penetration, or water must be placed in the container before placing in the sterilizer.**

- Use extreme caution when opening the autoclave following
the sterilization cycle. Steam can cause serious injury. Additionally, malfunctioning autoclaves can fill with superheated water that will be released when the autoclave is opened.

- Suggested sterilization cycle times:
  - 60 minutes @ 121°C & 15 PSI for decontaminating waste
    - Lengthen time for large or dense loads
  - 30 minutes @ 121°C & 15 PSI for sterilizing clean materials (i.e. glassware)
  - Use slow exhaust for liquids and fast exhaust for glassware

Additional information can be found in the “Safe Use of Autoclaves” training, available on the EHS website.

IX.4. Disinfection

An integral part of the biosafety program is the identification of appropriate disinfectants or decontaminating agents. Such materials are to be kept readily available in the use-dilution required.

The disinfectant and the disinfection process must be validated, and the validation documented. Personnel must be trained in the appropriate use of the approved disinfectant. EHS personnel can assist in the development of an appropriate validation and monitoring process.

Disinfectants must always be used in accordance with the manufacturer’s recommendations. Failure to follow the manufacturer’s recommendations can result in the failure of the disinfectant to perform as expected.

IX.4.1. Disinfection Hazards

Disinfectants are potentially hazardous chemicals and should be handled with care. Check the manufacturer’s Safety Data Sheet (SDS)
before use.

Personnel should be informed of the hazards associated with disinfectant use and provided with appropriate PPE to minimize exposure under use conditions.

Appropriate disposal requirements must be specified for each disinfectant used.

**IX.5. Spills and Spill Cleanup**

Spills of biohazardous materials may constitute a significant health hazard if not handled in an appropriate manner. All personnel working with biohazardous materials must be trained in the specific cleanup and disinfectant procedures to be used for their particular laboratory. Personnel must also be informed of the handling and disposal of contaminated clothing and personal protective devices. All of this information should be included in a Standard Operating Procedure developed by the PI.

A biological spill shall be followed by prompt action to contain and clean up the spill. When a spill occurs, warn everyone in the area and call for assistance as needed. The degree of the risk involved in a spill depends on the volume of the material spilled, the creation of infectious aerosols, the concentration of organisms in the material spilled, the hazard of the organisms involved, the route of infection of the organisms, and the diseases caused by the organisms.

Spills of biological agents can contaminate areas and lead to infection of laboratory workers. Prevention of exposure is the primary goal in spill containment and cleanup, exactly as in chemical spills. In evaluating the risks of spill response, generation of aerosols and droplets is a major consideration.

**IX.5.1. Generic Spill Cleanup Plans**

As part of the laboratory Safety Plan, each laboratory must have a
biological spill kit and a spill cleanup plan detailing specific disinfectants and procedures for agents used in that laboratory. The biological spill kit should contain supplies to clean up any spill of biological origin, including plant, animal or human material and recombinant or synthetic nucleic acids, both infectious and non-infectious. Cleanup of any spill requires the use of appropriate personal protective equipment (i.e., laboratory coat, shoe covers, gloves, and possible respiratory protection). To comply with OEPA regulations, all spills of infectious materials greater than one gallon or one cubic foot must be reported to OEHS. The following procedures should serve as a guide for the development of specific procedures for the laboratory Safety Plan.

A copy of the OEHS “Infectious Waste Spill Containment & Clean Up Procedure” can be obtained by contacting your OEHS Safety Representative. This procedure should be posted in all labs where working with or storing potentially infectious materials, including recombinant or synthetic nucleic acids.

**IX.5.1.1. Spill Cleanup Procedures**

The following procedures are to assist lab personnel with containment and cleanup of spills under specific circumstances.

**Spill Contained Within a Biological Safety Cabinet (BSC)**

- BSC must run during cleanup to contain aerosols & HEPA-filter exhaust air.
- Don appropriate personal protective gear before initiating cleanup.
- Initiate clean up as soon as possible using a 10% bleach solution or other EPA approved tuberculocidal disinfectant.
- If the spill is contained on a bench diaper, remove the contaminated bench diaper & discard as infectious waste.
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- If the spill is on the work area surface, cover spilled material with disinfectant-soaked towels. Allow the appropriate contact time (30 minutes for bleach; other EPA approved tuberculocidal disinfectants follow manufacturer’s recommendations) then remove the contaminated towels & discard as infectious waste.

- Wipe down the interior of the cabinet & any splatter on items within the cabinet with a disinfectant-soaked towel.

- Wipe down non-autoclavable materials with disinfectant. Allow the appropriate contact time (30 minutes for bleach; other EPA approved tuberculocidal disinfectants follow manufacturer’s recommendations) with disinfectant before any items are removed from cabinet.

- Place items designated as contaminated used sharps in an appropriate infectious waste sharps container using tongs/forceps. Place other contaminated disposable materials used in the cleanup process in a biohazard bag. Process as infectious waste.

- Place contaminated re-usable items in autoclave bags, autoclavable pans with lids or wrap them in newspaper. Sterilize, preferably by autoclaving, and clean for re-use.

- If the cabinet has a catch basin beneath the work surface & the spill resulted in liquids flowing into this area, more extensive decontamination is required.

  1) Ensure the drain valve under the cabinet is closed.

  2) Pour disinfectant onto the work surface & through the front and rear grilles into the drain pan. Allow 30 minutes contact time.

  3) Absorb spilled fluid-disinfectant from work surface with paper towels & discard in biohazard bag.
4) Prepare to empty drain pan. Place disinfectant solution in a collection vessel. Attach flexible tubing to the drain valve. The tube should be of sufficient length to allow the open end to be submerged in the collection vessel to minimize aerosol generation.

5) Open the drain valve & empty the drain pan into the collection vessel containing disinfectant. Flush the drain pan with water & remove the flexible tubing. Manage contaminated materials as if they are infectious.

6) Remove protective clothing used during cleanup & dispose of as infectious waste. Wash hands when gloves are removed.

7) Notify Principal Investigator or supervisor. Consult with OEHS (614-292-1284) to determine whether gas decontamination of the cabinet and filters is necessary, especially if a high-risk agent or a major spill of a moderate-risk agent occurred.

8) Run BSC at least 10 minutes after cleanup, before resuming activity in the cabinet.

**Outside the Cabinet, Inside the Laboratory**

- If a spill occurs in a Biosafety Level 2 facility, outside the BSC, notify other individuals in the laboratory to evacuate.

- Exit the laboratory to the hallway, closing the door behind you.

- Remove any contaminated clothing (turn contaminated portion inward) & place it in an autoclave bag.

- Wash all exposed skin.
• Place signs on door(s) to the laboratory warning individuals who may want to enter that a spill occurred & access is denied.

• Allow aerosols to settle for at least 30 minutes before re-entering the laboratory.

**If assistance is needed, contact Spill Response Personnel by calling the facility emergency number 614-292-1284; or 614-292-2121 (from cell phone) or 911 (from campus phone) after hours and remain in the area to provide information regarding the size of the spill and the materials spilled to the responder(s).**

• Assemble supplies (disinfectant, sharps containers, towels, tongs, biohazard bags, etc.) before entering the laboratory.

• Don appropriate personal protective equipment (i.e. disposable gown, protective eyewear, gloves, shoe coverings & respiratory protection [if necessary]).

• Clean up spill with a 10% bleach solution or other EPA approved tuberculocidal disinfectant as follows:
  1) Surround spill area with disinfectant or diking material that is soaked in disinfectant.

  2) Place items designated as contaminated used sharps in an appropriate infectious waste sharps container. Place other disposable materials used in the cleanup process in a biohazard bag. Process as infectious waste.

  3) Place paper towels over the entire spill area to absorb the spill. Clean the area and dispose of the material as infectious waste.

  4) Apply disinfectant and allow the appropriate contact time (30 minutes for bleach; other EPA approved...
tuberculocidal disinfectants follow manufacturer’s recommendations) with the disinfectant to ensure adequate germicidal action.

5) Wipe down non-autoclavable materials with disinfectant.

6) Place contaminated re-usable items in autoclave bags, autoclavable pans with lids or wrap them in newspaper. Sterilize, preferably by autoclaving, and clean for re-use.

7) Remove protective clothing used during cleanup then place in a biohazard bag for autoclaving.

- Wash hands when gloves are removed.
- Notify Principal Investigator or supervisor & OEHS (614-292-1284)

**Inside a Centrifuge**

*The potential for multiple infections from a single centrifuge accident is high. Aerosols are created when fluid escapes from the rotor or cup while the centrifuge is operating at high speed. All opening of centrifuges must be performed slowly.*

**Unsealed buckets:**

- If a centrifuge tube breaks while the centrifuge is running, turn off motor. Allow the machine to be at rest for 30 minutes before opening. If breakage is discovered after the machine has stopped, re-close the lid immediately & allow the unit to be at rest for 30 minutes.
- Unplug centrifuge before initiating clean up.
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- Don strong, thick, rubber gloves & other PPE before proceeding with clean up.

- Flood centrifuge bowl with a 10% bleach solution or other EPA approved tuberculocidal disinfectant. Place paper towels soaked in a disinfectant over the entire spill area. Allow the appropriate contact time (30 minutes for bleach; other EPA approved tuberculocidal disinfectants follow manufacturer’s recommendations) with the disinfectant. Use mechanical means (such as forceps) to remove broken tubes & glass fragments. Place them in a sharps container for disposal as infectious waste.

- Remove buckets, trunnions & rotor then place in disinfectant for 24 hours or autoclave.

- Unbroken, capped tubes may be placed in disinfectant & recovered after appropriate contact time.

- Use mechanical means to remove remaining disinfectant soaked materials from centrifuge bowl & discard as infectious waste.

- Place paper towels soaked in a disinfectant in the centrifuge bowl & allow it to soak overnight, wipe down again with disinfectant, wash with water & dry. Discard disinfectant soaked materials as infectious waste.

- Remove protective clothing used during cleanup & place in a biohazard bag for autoclaving. Wash hands whenever gloves are removed.

Sealed buckets (safety cups):

- If breakage is suspected, remove the sealed bucket to a biological safety cabinet before opening.
If breakage occurred, replace the cap on the safety cup loosely and autoclave.

Notify Principal Investigator or supervisor & OEHS (614-292-1284).

**Outside the Laboratory; during Transport (on the OSU Campus)**

The major emphasis should be on preventing spills during transport. All transport of infectious materials must be in a rigid, securely sealed, watertight primary container, which is contained within a second rigid, leak proof sealed container. Sufficient absorbent should be added to the second container to absorb contents in case of leakage from the primary container. The outer container must be labeled with the universal biohazard symbol.

If a spill occurs during transport, don gloves and initiate cleanup immediately with a 10% bleach solution or other EPA approved tuberculocidal disinfectant as follows:

- Surround spill area with disinfectant or diking material that is soaked in disinfectant.

- Place **contaminated used sharps** in an appropriate infectious waste sharps container.

- Place paper towels over the entire spill area to absorb the spill. Clean the area and dispose of the material as infectious waste.

- Apply disinfectant and allow the appropriate contact time (30 minutes for bleach; other EPA approved tuberculocidal disinfectants follow manufacturer’s recommendations) with the disinfectant to ensure adequate germicidal action.
• Place all materials used in the cleanup process (including contaminated gloves) in a biohazard bag and process as infectious waste.

• Wash hands as soon as possible.

IX.5.1.2. Biological Spill on a Person

If a biological material is spilled onto a person, emergency response is based on the hazard of the biological agent spilled (including the ability of the organism to penetrate intact skin), 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 should leave the contaminated area immediately. If possible, he or she should go to another laboratory so that hallways and other public areas do not become contaminated.

Contaminated clothing is removed and segregated as biohazard laundry for disinfecting. Contaminated skin shall be thoroughly flushed with water and washed with a disinfectant soap. Showering may be appropriate, depending on the extent of the spill.

For Risk Group 2 and Risk Group 3 pathogens, the employee must report to University Employee Health immediately for evaluation.