

DEPARTMENT OF MOLECULAR AND CELLULAR BIOLOGY

SAFE OPERATING PROCEDURE

RESPONSE TO A BIOHAZARD SPILL

Purpose:

To provide instruction to Research/Teaching staff members on the proper response to a spill of a hazardous biological agent.

Application:

This procedure should be followed in the event of a spill of a potentially biohazardous material. Students, staff and faculty working with these agents should be familiar with the general procedures outlined below.

Procedure: General Information:

- **Keep adequate materials in the lab to contain a spill:** paper towels, readily available disinfectant, Personal Protective Equipment (PPE), a containment waste bucket and accessible instructions for spill clean-up.
- If you are a project or undergraduate student, call a senior person in the lab to help you clean up; if you happen to be working alone, call your advisor (or senior person) to come to the lab.
- **In the event of a major spill;** evacuate the lab and call x2000 from a safe location.
- **Biohazardous material spills on your person:** move to a safe area, remove any contaminated clothing, and thoroughly wash any exposed skin or contact areas.
- **Biohazard agent comes in to contact with the eyes,** flush at the nearest eyewash station for a minimum of 15 minutes.
- Spills on shoes; soak the shoes in a tray with disinfectant. Wipe sole of shoes on disinfected soaked towels.
- **Pre-spill cleanup:** - ensure the user is wearing a lab coat, gloves and eye protection. Ensure the user has a chemical disinfectant, paper towels, and biohazard waste bags. There may also be a need for forceps, a dustpan and broom, and/or a sharps container.
- A typical aqueous 70% Ethanol alcohol solution is not appropriate for surface decontamination because of the evaporative nature of the solution; a contact time of **ten minutes or more** is necessary and not achievable using a 70% (v/v) aqueous solution of ethanol. 70% ethanol can be used to soak small pieces of surgical instruments and for wipe downs following a disinfectant (e.g., 10% bleach) that might leave a corrosive residue.

- When choosing a chemical disinfectant a 1:10 dilution of standard bleach is typically suitable, however:
 - ❖ Be aware that some biological agents are resistant to bleach. If you are unsure of chemical susceptibility, review the Pathogen Safety Data Sheet:
<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php>
 - ❖ Prolonged contact times, higher concentrations and alternate disinfectants may be required. Some bacteria (e.g., *Bacillus anthracis*, *Bacillus cereus*, *Clostridium difficile*) are resistant to chemical disinfection, as are some protozoa (e.g., *Cryptosporidium parvum*, *Giardia lamblia*).
 - ❖ Bleach solutions can be corrosive to metals, including stainless steel. For cleaning of biosafety cabinets, centrifuges, or other metal devices, consider using an alternate chemical disinfectant from Table 1.
 - ❖ Also note the efficacy of bleach is reduced in the presence of significant amounts of organic materials. There may be a need to use additional quantities or select an alternative disinfectant from Table 1.
- Transportation of a biohazardous material through public areas should be performed using a durable, well-sealed primary container, and a leak proof, durable secondary container labeled with a biohazard symbol. In the event a spill occurs in a public space, evacuate the immediate area and call **EHS at x53282** for assistance.

Procedure: Biohazard Spill on a bench top or floor area

- With the potential for aerosols to be produced, evacuate the lab and allow 30 minutes for aerosols to settle.
- Ensure you are wearing the required protective equipment and have all the materials you will need to clean up.
- Cover the spill with paper towels.
- Carefully pour a freshly prepared 1:10 bleach solution or alternative chemical disinfectant (see Table 1) over the area. Working from the outside in and soaking the paper towels to “sop” up the spill. This will prevent the spread of the spill.
- Cordon off the area and leave undisturbed for 20 minutes. This allows adequate contact time for the disinfectant to work.
- Remove any broken glass or sharps using forceps or tweezers and place in a sharps disposal container.
- Pick up the soaked paper towels and other absorbent materials and place in a biohazard waste bag.
- Clean the area again with the bleach solution (or the alternative chemical disinfectant) and paper towel. Wipe up any residues from the spilled material. Place all waste in the biohazard waste bag.
- Ensure all reusable items used in the cleanup (forceps, dustpans, etc.) are bagged and sent for sterilization in the autoclave.
- Notify your supervisor, and report the spill and successful clean-up using EHS Incident Report Form.
<https://www.uoguelph.ca/hr/hr-services-environmental-health-safety/forms>

Procedure: Biohazard Spill within a Biological Safety Cabinet (BSC)

- Keep BSC on (or turn BSC on) to control any aerosols that may be created.
- Cover the spill with paper towels or another absorbent material.
- Carefully pour a freshly prepared 1:10 bleach solution (or alternative chemical disinfectant – see table 1) over the area, working from the outside in and soaking the area.
- Close the sash on the BSC and leave the spill undisturbed for 20 minutes. This is to allow adequate contact time for the disinfectant to work.

- Remove any broken glass or sharps using forceps or tweezers and place in a sharps disposal container.
- Pick up the soaked paper towels and other absorbent materials and place in a biohazard waste bag.
- Wipe down the area of the spill and the walls and other surfaces in the area with a paper towel soaked in disinfectant.
- Carefully (ask another person to help) lift the steel tray (i.e., the work surface of the hood) and check for spills. Clean up any spills as described above. If not cleaned, the cabinet will remain in a contaminated state.
- Place all waste in a biohazard waste bag. Ensure all reusable items used in the cleanup (forceps, dustpans, etc.) are bagged and sent for autoclaving.
- Leave cabinet running for an additional 10 minutes.
- Notify your supervisor, and report the spill and successful clean-up using EHS Incident Report Form.
<https://www.uoguelph.ca/hr/hr-services-environmental-health-safety/forms>

Procedure: Biohazard Spill within a Centrifuge:

- When you become aware of a spill in a centrifuge, ensure the lid is closed and do not disturb the centrifuge for 30 minutes to allow aerosols to settle.
- Carefully remove rotors and buckets, seal in a plastic bag, and move them to a nearby biosafety cabinet for further cleaning.
- Remove any sharp debris with forceps or tweezers and place in a sharps container.
- Use paper towel and a suitable chemical disinfectant to clean the inside of the centrifuge. A bleach solution is not recommended as it may corrode sensitive parts of the equipment, so please use Table 1 to select an alternative chemical disinfectant.
- Working in the biosafety cabinet, soak all the removable parts in the disinfectant for 30 min, rinse thoroughly, dry and return them to the centrifuge.
- Place the liquid waste in a container suitable for autoclaving.
- Place all other waste (e.g. wet paper towels) in a biohazard waste bag. Ensure all reusable items used in the cleanup (forceps, dustpans, etc.) are bagged and sent for autoclaving.
- Notify your supervisor, and report the spill and successful clean-up using EHS Incident Report Form.
<https://www.uoguelph.ca/hr/hr-services-environmental-health-safety/forms>

Table 1: Chemical Disinfectants (Centre for Food Security and Public Health, Iowa State University)

The Antimicrobial Spectrum of Disinfectants

Chemical Disinfectants

Note: Removal of organic material must always precede the use of any disinfectant.

	Acids (hydrochloric acid, acetic acid, citric acid)	Alcohols (ethyl alcohol, isopropyl alcohol)	Aldehydes (formaldehyde, paraformaldehyde, glutaraldehyde)	Alkalis (sodium or ammonium hydroxide, sodium carbonate)	Biguanides (chlorhexidine*, Nolvasan*, Chlorhex*, Virosan*, Hibistat*)	Halogens hypochlorite iodine	Oxidizing Agents (hydrogen peroxide, peroxyacetic acid, Trifectant*, Virkon-S*, Oxy-Sept 333*)	Phenolic Compounds (Lysol*, Osl*, Amphyl*, TekTrol*, Pheno-Tek II*)	Quaternary Ammonium Compounds (Roccal*, Zephiran*, DiQuat*, Parvosol*, D-256*)
most susceptible									
mycoplasmas	+	++	++	++	++	++	++	++	+
gram-positive bacteria	+	++	++	+	++	+	+	++	++
gram-negative bacteria	+	++	++	+	++	+	+	++	+
pseudomonads	+	++	++	+	+	+	+	++	-
rickettsiae	+	+	+	+	+	+	+	+	+
enveloped viruses	+	+	++	+	+	+	+	+	+
chlamydiae	+	+	+	+	+	+	+	+	-
non-enveloped viruses	-	-	+	+	-	+	+	-	-
fungal spores	+	+	+	+	+	+	+	+	+
picornaviruses (i.e. FMD)	+	N	+	+	N	N	+	N	N
parvoviruses	N	N	+	N	N	+	+	N	-
acid-fast bacteria	-	+	+	+	-	+	+	+	-
bacterial spores	+	-	+	+	-	+	+	+	-
coccidia	-	-	-	+	-	-	-	+	-
prions	-	-	-	-	-	-	-	-	-
most resistant									

LEGEND

++ highly effective

+ effective

limited activity

- no activity

N information not available

a-varies with composition

b-peracetic acid is sporicidal

c-ammonium hydroxide

d-some have activity against coccidia

DISCLAIMER: The use of trade names does not in any way signify endorsement of a particular product. For additional product names, please consult the most recent Compendium of Veterinary Products. ADAPTED FROM: Linton AH, Hugo WB, Russel AD. Disinfection in Veterinary and Farm Practice. 1987. Blackwell Scientific Publications; Oxford, England; Quinn PJ, Markey BK. Disinfection and Disease Prevention in Veterinary Medicine, In: Block SS, ed., Disinfection, Sterilization and Preservation. 5th edition. 2001. Lippincott, Williams and Wilkins: Philadelphia.

Table 2: Characteristics of Disinfectants: (Centre for Food Security and Public Health, Iowa State University)

Characteristics of Selected Disinfectants

FOR MORE INFORMATION, SEE THE 'DISINFECTION 101' DOCUMENT AT www.cfsph.iastate.edu

Disinfectant Category	Alcohols	Aldehydes	Biguanides	Halogens: Hypochlorites	Halogens: Iodine Compounds	Oxidizing Agents	Phenols	Quaternary Ammonium Compounds (QAC)
Sample Trade Names	Ethyl alcohol Isopropyl alcohol	Formaldehyde Glutaraldehyde	Chlorhexidine Nolvasan [®] Virosan [®]	Bleach	Betadyne [®] Providone [®]	Hydrogen peroxide Peracetic acid Virkon S [®] Oxy-Sept 333 [®]	One-Stroke Environ [®] Pheno-Tek II [®] Tek-Trol [®]	Roccal [®] DiQuat [®] D-256 [®]
Mechanism of Action	•Precipitates proteins •Denatures lipids	•Denatures proteins •Alkylates nucleic acids	•Alters membrane permeability	•Denatures proteins	•Denatures proteins	•Denature proteins and lipids	• Denatures proteins • Alters cell wall permeability	• Denatures proteins • Binds phospholipids of cell membrane
Advantages	•Fast acting •Leaves no residue	•Broad spectrum	•Broad spectrum	•Broad spectrum •Short contact time •Inexpensive	•Stable in storage •Relatively safe	•Broad spectrum	• Good efficacy with organic material • Non-corrosive • Stable in storage	• Stable in storage • Non-irritating to skin • Effective at high temperatures and high pH (9-10)
Disadvantages	•Rapid evaporation •Flammable	•Carcinogenic •Mucous membranes and tissue irritation •Only use in well ventilated areas	•Only functions in limited pH range (5-7) •Toxic to fish (environmental concern)	•Inactivated by sunlight •Requires frequent application •Corrodes metals •Mucous membrane and tissue irritation	•Inactivated by QACs •Requires frequent application •Corrosive •Stains clothes and treated surfaces	•Damaging to some metals	• Can cause skin and eye irritation	
Precautions	Flammable	Carcinogenic		Never mix with acids; toxic chlorine gas will be released			May be toxic to animals, especially cats and pigs	
Vegetative Bacteria	Effective	Effective	Effective	Effective	Effective	Effective	Effective	YES—Gram Positive Limited—Gram Negative
Mycobacteria	Effective	Effective	Variable	Effective	Limited	Effective	Variable	Variable
Enveloped Viruses	Effective	Effective	Limited	Effective	Effective	Effective	Effective	Variable
Non-enveloped Viruses	Variable	Effective	Limited	Effective	Limited	Effective	Variable	Not Effective
Spores	Not Effective	Effective	Not Effective	Variable	Limited	Variable	Not Effective	Not Effective
Fungi	Effective	Effective	Limited	Effective	Effective	Variable	Variable	Variable
Efficacy with Organic Matter	Reduced	Reduced	?	Rapidly reduced	Rapidly reduced	Variable	Effective	Inactivated
Efficacy with Hard Water	?	Reduced	?	Effective	?	?	Effective	Inactivated
Efficacy with Soap/Detergents	?	Reduced	Inactivated	Inactivated	Effective	?	Effective	Inactivated

? Information not found

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For additional product names, please consult the most recent Compendium of Veterinary Products.

REFERENCES: Linton AH, Hugo WB, Russel AD. Disinfection in Veterinary and Farm Practice. 1987. Blackwell Scientific Publications; Oxford, England; Quinn PJ, Markey BK. Disinfection and Disease Prevention in Veterinary Medicine, In: Block SS, ed., Disinfection, Sterilization and Preservation. 5th edition. 2001. Lippincott, Williams and Wilkins: Philadelphia.

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