Advanced Analysis Centre

Biohazard Spill Standard Operating Procedure

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Author: A. Holliss

Purpose

This SOP provides instruction on the proper response to a spill of a hazardous biological agent.

Scope

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.

Definitions/Acronyms

Biohazardous Material - Any pathogenic, infectious or hazardous biological material, that presents a risk or potential risk to the health of humans, animals, plants, or the environment. The risk can be directly through infection or indirectly through damage to the environment. The material may cause disease in other living organisms or cause significant impact to the environment.

Disinfectant – A chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects.

HBV - Hepatitis B Virus that causes liver inflammation (hepatitis); there is no cure for disease from HBV.

Requirements/Policies/Regulations

- Human Pathogens & Toxins Act S.C. 2009, c. 24
- Occupational Health and Safety Act R.S.O. 1990, c. O.1
- Environmental Protection Act, R.S.O. 1990
- University of Guelph Safety Policy 851.04.04 Spills to the Environment and Reporting
- University of Guelph Safety Policy 851.11.01 Medical Surveillance - Biosafety

Training

Training and competency:

- WHMIS, Lab Safety, Biosafety training
- Completion of the Agreement on Biosafety form

Postings:

In the event of a spill, block access to the area and post a Biohazard Spill Notice sign to keep others away from the spill area until it has been cleaned.

Maintenance

Keep adequate spill cleaning materials in the lab – paper towels, personal protective equipment, biohazard waste bag, bucket, appropriate disinfectant, and readily available instructions for spill clean-up. Also helpful is signage to keep others away from a spill area.

Eyewash stations in laboratories are to be activated weekly to flush the lines and verify operation.

Safety Precautions

⚠ **If you need to transport a biohazardous material through public areas** – place biohazardous material into a durable, well-sealed primary container, and a leak proof, durable secondary container labeled with a biohazard symbol. If a spill occurs in a public space, evacuate the immediate area and call EHS at x53282 for assistance.

Description of the Task

Preparation:

- Each laboratory should have its own spill prevention and response plan specific to the biohazardous materials used in that laboratory. The plan should include the rationale for selection of the disinfecting agent, the approach to its application, contact time and other parameters.
- 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.
- If it is a major spill - evacuate the lab and call x52000 from a safe location.

Procedure:

If a biohazardous material spills on you – move to a safe area, remove any contaminated clothing, and thoroughly wash any exposed body parts. If it gets in

your eye, flush at the eyewash for at least 15 minutes. If it gets on shoes, soak the shoes in a tray with disinfectant.

If you are going to clean a spill - you must be wearing a lab coat, gloves and eye protection. You must use an effective chemical disinfectant, absorbent material i.e. paper towels, and a biohazard waste bag to contain the waste. You may also need forceps, a dustpan and broom, and/or a sharps container.

When choosing a chemical disinfectant – a 1:10 dilution of standard bleach is typically suitable, however:

- Be aware that some agents are resistant to bleach. If you are unsure of chemical susceptibility, review the SDS with the University's online SDS subscription service.
- Some bacteria (e.g., *Bacillus anthracis, Bacillus cereus, Clostridium difficile*) are resistant to chemical disinfection, as are some protozoa (e.g., *Cryptosporidium parvum, Giardia lambia).* Prolonged contact times, higher concentrations and alternate disinfectants may be required.
- Areas being disinfected should be well soaked with the selected disinfectant to avoid drying before the end of the optimum contact time.
- 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 if there are significant amounts of organic materials. You may need to use additional quantities or select an alternative disinfectant from Table 1.
- See Appendix A for a description of the various types of liquid chemical disinfectants; household and commercially available products.

Table 1 Laboratory Surface Disinfectants

Common Liquid Disinfectant		Usage Requirements		Active Against (positive effect +, no effect -, variable effect v)				
Category	**Disruption Mechanism**	**Dilution**	**Contact Time(min)**	**Vegetative Bacteria**	**Lipo-viruses**	**Nonlipid Viruses**	**Bacterial Spores**	**HBV**
Chlorine	Oxidizing agent denatures proteins	1-5%	10-30	+	+	+	-	+
Ethanol and Isopropanol	Membrane damage, denaturing protein, solubilizes lipids	70-85%	10-30	+	v	v	-	v
Formaldehyde	Crosslinking of proteins, DNA and RNA	0.2-8%	10-30	+	+	+	+	+
Glutaraldehyde		2%	10-30	+	+	+	+	+
Iodophors	Attacks surface proteins and destabilizes fatty acids and NA's	0.5-10%	10-30	+	+	+	-	v
Phenolic	Disrupts membrane, denatures proteins	1-5%	10-30	+	+	v	-	v
Quaternary Ammonium Compounds	Membrane damage, denaturing protein, disrupts lipids	0.1-2%	10-30	+	+	-	+	-

NOTE: The hepatitis B virus (HBV) can survive in a dried state on surfaces at room temperature for over a week. It can be inactivated with a 1:10 solution of bleach and water.

SPILL ON FLOOR OR BENCH

If there is potential for aerosolization, 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 other appropriate chemical disinfectant – see Table 1) over the area. Apply disinfectant concentrically beginning at the outer margin of the spill area, working toward the center, soaking the covering paper towels.
- 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. Alternatively, if there is broken glass or other sharps involved, use a dustpan or a piece of stiff cardboard to collect the material and deposit it into a puncture-resistant sharps container for disposal.
- Pick up the soaked paper towels and other absorbent materials and place in a biohazard waste bag.
- Clean the area again with the disinfectant and paper towels. 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 autoclaving.

- Notify your supervisor and report the spill and successful clean-up using EHS Incident Report form.

SPILL IN 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.

Contingency Plan and Reporting

Incident Response:

Complete the Incident Report Form to report the spill to your supervisor and EHS.

Waste Management and Environmental Responsibility

- Both the spilled material and the absorbent may be considered hazardous waste and must be disposed of in compliance with environmental regulations.
- Refer to the SDS of the chemical disinfectant for proper disposal procedures.

References/Material/Resources

- Safety Data Sheets
- Pathogen Safety Data Sheets (PSDS)
- PHAC's database for biological agents ePATHogen
- equipment manuals from manufacturers
- AAC Fume Hood SOP

Distribution of Copies

Document accessible on AAC website.

SOP History

16-Jan-2008	Biohazard Spill SOP	A. Doane
06-Dec-2018	CBS-SOP-010-18 Chemical Spill SOP	A. Holliss
23-Apr-2019	AAC Chemical Spill SOP	A. Holliss

Reason for Changes:

- Adaptation of CBS document for AAC

Review Frequency:

All AAC SOP's are to be reviewed every two years or as changes in legislation or procedures necessitate.

Appendix A: Common Liquid Disinfectants

When choosing a disinfectant, the following factors should be considered:

- type and level of microbial contamination
- concentration of active ingredient
- duration of contact between disinfectant and item to be disinfected
- pH
- temperature
- humidity
- presence of organic matter or soil load

Chlorine

Chlorine is a fast-acting oxidant and a broad-spectrum chemical germicide. It is normally sold as bleach, an aqueous solution of sodium hypochlorite (NaOCl), which can be diluted with water to provide various concentrations of available chlorine. A general all-purpose laboratory disinfectant should have a concentration of 1 g/l available chlorine. A stronger solution, containing 5 g/l available chlorine, is recommended for dealing with biohazardous spillage and in the presence of large amounts of organic matter. Domestic bleach, contains 50 g/l available chlorine and should therefore be diluted 1:50 or 1:10 to obtain final concentrations of 1 g/l and 5 g/l, respectively. Bleach is not recommended as an antiseptic but may be used as a general-purpose disinfectant and for soaking contaminated metal-free materials. Chlorine is highly alkaline and can be corrosive to metal. Bleach solutions can become non-active if too old. Generally, the shelf life of household bleach is one year. Bleach test strips are available from Fisher Scientific (**Catalog No.** 14-412-60) to indicate if bleach solution meets the 0.525% minimum requirement for disinfecting hard surfaces and equipment.

- Chemistry Stockroom has 4 litre bottles of bleach available for purchase.

Formaldehyde

5% formalin in water may be used as a liquid disinfectant. It is active against vegetative bacteria, spores, fungi and lipid- and nonlipid-containing viruses. Formaldehyde's drawbacks are its pungent, irritating odor, reduction in efficacy at refrigeration temperature and several safety concerns including its suspect as a carcinogen.

Glutaraldehyde

Glutaraldehyde (OHC(CH2)3CHO) is generally supplied as a solution with a concentration of about 20 g/l (2%). It is also active against vegetative bacteria, spores, fungi and lipid- and nonlipid-containing viruses. It is non-corrosive, faster acting, ten times more effective than formaldehyde and less toxic. However, it takes several hours to kill bacterial spores. Glutaraldehyde is toxic and an irritant to skin and mucous membranes and contact with it must be avoided. Glutaraldehyde is also an inhalation hazard. It must be used in a fume-hood or in well-ventilated areas. It is not recommended as a spray or solution for the

decontamination of environmental surfaces.

- Cidex a commercially prepared glutaraldehyde disinfectant is used routinely for cold surface sterilization of clinical instruments. Fisher Scientific stocks a 25% glutaraldehyde solution in various volumes.

Phenolic Compounds

This group encompasses a broad group of agents that are active against vegetative bacteria and lipid-containing viruses and, when properly formulated, also show activity against mycobacteria. They are not active against spores and their activity against nonlipid viruses is variable. They act to denature and coagulate proteins and are general protoplasmic poisons. Some phenolic compounds are sensitive to and may be inactivated by water hardness and therefore must be diluted with distilled or deionized water. They may be absorbed by latex gloves and can also penetrate the skin. Phenolic compounds can be irritating to the skin and eyes and may have an associated odor.

- Chloroxylenol is the active ingredient in Dettol (4.8%), a household disinfectant and antiseptic.
- Pine-Sol is another household disinfectant which uses 1-5% sodium petroleum sulfonate as the surfactant.

Quaternary Ammonium Compounds

QAC's are used as mixtures and often in combination with other germicides, such as alcohols. They have good activity against some vegetative bacteria and lipid-containing viruses. Certain types (e.g. benzalkonium chloride) are used as antiseptics. The germicidal activity of certain types of quaternary ammonium compounds is considerably reduced by organic matter, water hardness and anionic detergents. Care is therefore needed in selecting agents for precleaning when quaternary ammonium compounds are to be used for disinfection. Potentially harmful bacteria can grow in quaternary ammonium compound solutions. Owing to low biodegradability, these compounds may also accumulate in the environment.

- Lysol® products (cleaners and disinfectant wipes) contain dimethyl ethylbenzyl ammonium chloride as their active ingredient. The liquid cleaners may also contain sodium bicarbonate to soften the diluting water and enhance the efficacy of the product.
- Control III contains a combination of two quaternary compounds, (alkyl dimethyl benzyl ammonium chloride and alkyl dimethyl ethylbenzyl ammonium chloride) which makes it a highly effective, broad-spectrum disinfectant. This environmentally friendly ready to use solution can be disposed of down the drain.
- Decon™ Conflikt™ Detergent Disinfectant is a quaternary ammonium disinfectant (same quat compounds as Control III) in a prediluted ready-to-use spray format available from Fisher Scientific, and VWR.

- Bacdown™ Detergent Disinfectant, BDD™ is a concentrated, phosphate-free, disinfectant containing surfactants and quaternary ammonium compounds that will safely clean and disinfect surfaces in labs, production areas and process equipment. Formulated for cleaning and sanitizing inanimate hard, non-porous surfaces and provides effective biological decontamination against HIV-1, HBV and more when used as instructed. Fisher Scientific product number is 18-800-101.

Alcohols

Ethanol (C2H5OH) and isopropyl alcohol, (C_3H_8O) have similar disinfectant properties and are active against vegetative bacteria, fungi and lipid-containing viruses but not against spores. Their action on nonlipid viruses is variable. A 70% solution of ethanol can be used on skin, work surfaces of laboratory benches and to soak small pieces of surgical instruments. A major advantage of aqueous solutions of alcohols is that they do not leave any residue on treated items. Alcohols may harden rubber and dissolve certain types of glue. Alcohols are volatile and flammable and must not be used near open flames. Working solutions should be stored in proper containers to avoid the evaporation of alcohols. Alcohols evaporate rapidly, which makes extended contact times difficult to achieve unless the items are immersed.

NOTE: Do not use 70% ethanol to clean a Class II, Type A recirculating biosafety cabinet. The vapors from ethanol are flammable and the lower explosive limit for ethanol is easily attained.

- CiDehol® a sterile, ready-to-use 70% isopropyl alcohol solution is available from VWR and Fisher Scientific in various volumes.

Iodine and Iodophors

The action of these disinfectants is similar to chlorine, although they may be slightly less inhibited by organic matter. Iodine can stain fabrics and environmental surfaces and is generally unsuitable for use as a disinfectant. Iodine can be toxic and should not be used on aluminum or copper. Organic iodine-based products must be stored at 4 to 10°C to avoid the growth of potentially harmful bacteria in them. **Iodophors should be made up in cold water to prevent breakdown of the disinfectant.**

- Wescodyne is a general-purpose detergent/germicide *tamed iodine* available from VWR, Catalogue # CA11007-340 (4 x 1l bottles). This product is not recommended for use as it has been found to react with the black bench surfaces in the Science Complex making them sticky, and stains surfaces, floors, lab coats and clothing.

Hydrogen Peroxide and Peracetic Acids

Like chlorine, hydrogen peroxide (H_2O_2) and peracetic acids (CH_3CO_3H) are strong oxidants and can be potent broad-spectrum germicides inactivating germs

by disrupting cell membranes and deactivating enzymes. They are also safer than chlorine to humans and the environment. Hydrogen peroxide can be used for the decontamination of work surfaces of laboratory benches and biosafety cabinets. Both solutions can be corrosive to metals such as aluminum, copper, brass and zinc, and can also decolorize fabrics, hair, skin and mucous membranes. Do not mix with bleach as it would produce the toxic gas chloramine.

- Actril Surface Disinfectant, is a ready to use sterilant for hard surfaces. It is a powerful disinfectant containing acetic acid (5.2%), hydrogen peroxide (1%) and peracetic acid (0.008%). VWR product number is CA11006-040.
- PREempt* Ready to Use Disinfectant Wipes, Contec Inc, CA (VWR Cat# CA10836-018) These 6 by 7 inch sheets are ideal for cleaning and disinfecting environmental surfaces in laboratory areas with fast contact times and broad-spectrum efficacy.

References:

- Guidance on Regulations for the Transport of Infectious Substances 2015–2016
- WHO Laboratory Biosafety Manual Third edition 2004
- Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition 2009, Appendix B: Decontamination and Disinfection