

1.0 Introduction

This Biosafety Standard Operating Procedure (BSOP) outlines procedures that should be followed for the decontamination of biohazardous materials on surfaces and equipment and in the event of a spill involving biohazardous materials. A copy of this BSOP should be posted prominently in the laboratory to allow for quick reference by workers.

2.0 Scope

This BSOP applies to all staff and students in all locations authorized by Memorial University of Newfoundland to work with biohazardous materials.

3.0 Responsibilities

This section outlines responsibilities within the university for the implementation of this SOP.

a. Environmental Health and Safety (EHS)

- Review and amend this BSOP as necessary.

b. Department Heads

- Ensure that this BSOP is available and followed by staff/students within the department.

c. Laboratory Supervisors/Principal Investigators

- Ensure that a fully stocked biohazard spill kit (see Appendix) is available within the laboratory.
- Ensure that all workers are aware of this BSOP as well as the location of the biohazard spill kit within the laboratory.
- Ensure that all spills involving biohazardous materials are appropriately cleaned and decontaminated, as outlined in this BSOP.
- Ensure that a Biohazard Spill Report (Appendix B) is completed for any biohazardous spill within their authorized location(s) and submitted to the BSO.

d. Authorized Workers

- Ensure that this BSOP is followed for biohazard decontamination and for any biohazardous spill.
- Ensure that their supervisor is notified of any biohazardous spill as soon as is practical.
- Aid their supervisor in the completion of a Biohazard Spill Report (Appendix B).

4.0 Definitions

Minor spills – minor spills involve small volumes (i.e. less than 100 ml) that are easily contained with absorbent materials (eg paper towels) and do not contain highly pathogenic/infectious pathogens or highly toxic toxins [as determined by a Local Risk Assessment (LRA)].

Major spills - major spills involve large volumes (i.e. more than 100 ml) that are not easily contained with absorbent materials (eg paper towels) and/or contain highly pathogenic/infectious pathogens or highly toxic toxins [as determined by a Local Risk Assessment (LRA)]. The presence of highly pathogenic/infectious pathogens or highly toxic toxins results in an automatic “major” classification.

5.0 Biohazard Decontamination

Decontamination is the process by which materials and surfaces are rendered reasonably free of microorganisms or toxins and safe to handle. The primary objective of decontamination is to protect containment zone personnel and the community from exposure to pathogens that may cause disease. Decontamination is fundamental to preventing the spread of pathogens within and outside of the lab. Depending on the situation, decontamination may require sterilization (the complete destruction of all microorganisms, including bacterial spores) or disinfection (the destruction and removal of specific types of microorganisms). Refer to BSOP01: Management of Biohazardous Waste for details on sterilization. Effective decontamination of areas and/or equipment represents a critical containment barrier whereby failure in the decontamination procedure can result in occupational exposure to infectious agents and/or the unintentional release of agents from a containment facility.

A. Selection of chemical disinfectant.

The selection of an appropriate disinfectant will depend upon the organism in use. The application of the disinfectant requires consideration of factors such as organic load, concentration and contact time, among others. A thorough risk assessment must be done when any new pathogens are introduced to the lab, and this protocol revised accordingly. Details on the selection of appropriate disinfectants can be found in chapter 15 of the Canadian Biosafety Handbook, 2nd Ed.

Presently, two general disinfectants are used in labs at MUN: chlorine (bleach 10 %) and alcohol (70%). Used properly, these disinfectants are effective against most fungal spores, vegetative Gram-positive and Gram-negative bacteria, enveloped viruses and *Mycoplasma spp*. If your materials potentially contain infectious prions, protozoal oocysts, bacterial endospores, Mycobacteria or non-enveloped viruses, other disinfectants may need to be used. Remember, the selection of an appropriate disinfectant will depend on its effectiveness against the particular

biohazardous agents used and the effectiveness of a disinfectant for a given biohazard must be demonstrated prior to its use in the lab.

B. Preparation of chemical disinfectants.

1. Before beginning work, make sure appropriate disinfectant is available.
2. Stock solutions should be diluted with water.
3. Prepare 70% ethanol solution with water. Ethanol has long term stability, so long as it is in a closed container that does not allow evaporation.
4. Prepare working bleach solutions (0.5% free Cl⁻) by diluting household bleach 1:10 with ultra-pure water. Working bleach solutions are unstable, especially if exposed to light. They should be kept in closed, dark containers and made fresh (i.e. weekly). Do not keep for longer than a month.
5. Label all solutions with contents and preparation date.

C. Protocol for surface decontamination.

Laboratory surfaces and equipment should be decontaminated before and after use with biohazardous materials.

1. For highly contaminated surfaces or where cross-contamination is a particular concern, use bleach first followed by 70% EtOH.
2. If the surface you are decontaminating is visibly dirty, first clean with detergent to remove excess dirt. All cleaning materials must be treated as biohazardous waste (Refer to BSOP01: Management of Biohazardous Waste for details).
3. Thoroughly spray the surface with the disinfectant and allow for an appropriate contact time.

This is short, about 1 min, for susceptible organisms or long, about 10 min, for resistant organisms. Contact time requirements must be determined prior to work with biohazards.

- a) 70% ethanol: Good for general use.

Evaporates quickly, so is not suitable for long contact times. Avoid using 100% ethanol, which evaporates too quickly and does not effectively penetrate cells. Dilute in pure water.

- b) Chlorine (bleach): Should contain 0.5% free chlorine. Remove after contact by wiping down with 70% EtOH or sterile water. Must be kept in dark.

Chlorine is corrosive to metals, so must be cleaned off after use. Can be made from commercial bleaches, such as Clorox, which contains 5.84% available chlorine, so a 10X dilution in water is used. Should be kept in opaque bottles and refreshed frequently. Is generally more effective than EtOH, including for the decontamination of DNA.

4. Wipe clean with paper towel and dispose in regular garbage.

6.0 Biohazard Spill Response Procedures

For an itemized list of the contents recommended for a Biological Spill Kit, see Appendix A of this BSOP. Spill kits should be customized to meet the needs of the laboratory (i.e. appropriate for the biohazards utilized in the lab). Print and laminate a copy of these protocols and include it with your Biological Spill Kit.

A. General Spill Clean-up Procedure [outside Biosafety Cabinet (BSC)].

1. Immediately secure all other biohazardous materials in the vicinity and notify others (including your supervisor) that there has been a biological spill.
2. Bring Biological Spill Kit to the site of the spill. If possible, contain the spill by covering with absorbent materials (i.e. paper towels).
3. Remove any contaminated clothing and place in labeled biohazard waste disposal bag. For major spills, wash hands and evacuate the laboratory. Proceed to step 4. For minor spills, proceed to step 5.
4. Prevent access to the laboratory (post signs, warning tape, etc.). Allow 20-30 minutes, during which aerosols should have settled, and re-enter the laboratory.
5. Don required PPE (i.e. gloves, lab coat, safety goggles, etc.).
6. If freshly prepared disinfectant is not available, prepare a fresh solution of appropriate disinfectant (i.e. 10% v/v household bleach). Cover an area twice the size of the spill with absorbent materials and, working from the outer edge, gently pour or spray the disinfectant over the absorbent materials working towards the centre of the spill. Avoid splashes.
7. Allow at least 30 min of contact time, and place the materials in regular garbage. DO NOT autoclave items previously treated with bleach. Use a dustpan and/or forceps if there is glass or other sharps involved.
8. Repeat Steps 6 and 7 if the spill contained a high organic load (e.g. blood).
9. Perform a final cleaning/disinfection by lightly dampening some paper towel with disinfectant and wiping down the entire spill area.
10. Ensure that any contaminated clothing is autoclaved prior to laundering. Dispose of disinfected materials via the regular waste stream.
11. Remove Biohazard Spill signage from laboratory door.
12. Report the spill to your immediate supervisor and together complete the Biohazard Spill Report and forward to the BSO.
13. Restock Biological Spill Kit.

B. Spill inside a BSC.

1. Leave the BSC fan turned on and secure all other biohazardous materials in the vicinity.
2. Immediately notify others that there has been a biological spill.
3. Remove outer layer of gloves and any contaminated clothing and place in an autoclavable biohazardous waste bag inside the BSC.
4. Bring Biological Spill Kit to the site of the spill. Don new PPE if necessary.
5. Cover the spill with paper towels.
6. Apply a disinfectant that is effective against the agents (e.g. 10% household bleach). Pour or spray disinfectant, from the edge of the spill toward the centre. A gentle flooding action will avoid creating aerosols. If spilled material has gone through the grill, pour disinfectant through the perforated grill onto catch tray beneath.
7. Allow at least 30 min of contact time, and place the materials in regular garbage. DO NOT autoclave items previously treated with bleach. Use a dustpan and/or forceps if there is glass or other sharps involved.
8. Repeat Steps 6 and 7 if the spill contained a high organic load (e.g. blood).
9. Surface decontaminate all objects in the cabinet with disinfectant.
10. Allow BSC to run for at least 10 minutes (with UV on if available) before resuming work.
11. Report the spill to your immediate supervisor and together complete the Biohazard Spill Report and forward to the BSO.
12. Restock Biological Spill Kit.

C. Spill inside a centrifuge

- I. In a sealed safety cup or sealed rotor.
 - All sealed centrifuge buckets should be loaded and unloaded in a BSC. If breakage is suspected within the safety cup, the safety cap should be loosened and the bucket autoclaved. Alternatively, the safety cup may be chemically disinfected.
- II. Infectious material visible upon opening the centrifuge.
 1. If a breakage occurs or is suspected while the machine is running, the motor should be switched off and the machine left closed (for 30 minutes) to allow settling.
 2. If a breakage is discovered after the machine has stopped, the lid should be replaced immediately and left closed (for 30 minutes).
 3. Inform the laboratory supervisor or principal investigator, and others in the vicinity of the spill.

4. Don appropriate PPE prior to opening the centrifuge. All personnel not involved in the spill clean-up, must exit the area of the spill.
5. Cover the spill with absorbent towels.
6. Apply a disinfectant that is effective against the agents (i.e. 10% household bleach). Pour or spray disinfectant, from the edge of the spill toward the centre. A gentle flooding action will avoid creating aerosols.
7. All broken tubes, glass fragments, buckets, trunnions and the rotor should be placed in a non-corrosive disinfectant (forceps must be used to handle and retrieve glass debris).
8. Unbroken sealed safety cups may be placed in disinfectant and carried to a BSC to be unloaded. The centrifuge bowl should be swabbed with the same disinfectant, at the appropriate dilution, and then swabbed again, washed with water and dried.

References:

1. Public Health Agency of Canada Spill procedures.
2. University of Alberta – Biological Spill Remediation Protocol. Oct 31 2013.
3. WHO Laboratory Biosafety Manual, 3rd Ed. 2004.
4. Cold-Ocean Deep-Sea Research Facility CDRF.B.4010 General decontamination and disinfection.
5. Canadian Biosafety Handbook, 2nd Ed. 2016. Public Health Agency of Canada.

Version History:

Version	Date	Author(s)	Notes
1.0	2015-09-10	Rod Hobbs	First writing.
1.1	2015-10-01	Rod Hobbs	Addition of IBC comments.
1.2	2017-04-10	Rod Hobbs	Correction of hyperlinks and web links

Appendix A: Biological Spill Kit Recommended Contents

Note: Biological spill kits must be customized to suit the needs of the laboratory. For example, disinfectants must be effective against the specific biohazardous materials used in the laboratory.

Contents:

- A copy of these spill response procedures
- Absorbent materials (paper towels)
- Disposable gloves
- Lab coat
- Safety goggles
- N95 dust mask
- Disposable shoe covers
- Marking pencil to mark the spill area
- All-purpose disinfectants (appropriate for the biohazards used, e.g. 70% ethanol or bleach)
 - Note, if bleach is the disinfectant of choice, it should be prepared fresh from an undiluted stock stored within the kit.
- Clear autoclavable biohazardous waste bags and tags
- Tongs and/or forceps
- Dust pan
- Biological spill warning (Keep Out) signs
- Tape

Any non-disposable items used in a biological spill must be appropriately decontaminated.



Biohazard Decontamination and Spill Response Standard Operating Procedure

BSOP-03

Approved by: IBC
Date: Nov 6, 2015

Appendix B: Biohazard Spill Report Log. Double click to open PDF.



Biohazard Spill Report Log 2015

This report is to be completed by the Permit Holder or Senior Laboratory Supervisor in the event of a spill involving biohazardous materials. This is a fillable PDF form.

LOCATION

The spill occurred at (time) _____ a.m. / p.m. on (date) _____ in room #/area _____ of building _____
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WORKERS INVOLVED IN SPILL

Name of Worker	Personal Contamination (yes/no)

ADDITIONAL DECONTAMINATION STEPS TAKEN

<p>Document any additional decontamination, monitoring or care provided to exposed workers. Select "yes" or "no" from the drop-down.</p> <ul style="list-style-type: none"> • Hand/face/hair washed: • Shower: • Contaminated street clothes bagged/decontaminated : • First aid: • Worker sent to hospital: • Incident report filed with MIMS:

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