University of Massachusetts
UMASS Lowell

ENVIRONMENTAL AND EMERGENCY MANAGEMENT

ENVIRONMENTAL HEALTH AND SAFETY

III. BIOSAFETY STANDARD OPERATING PROCEDURES (SOPs)

University of Massachusetts Lowell
UMASS

ENVIRONMENTAL AND EMERGENCY MANAGEMENT
Environmental Health and Safety
University Crossing Suite 140
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http://www.uml.edu/eem/

BIOSAFETY STANDARD OPERATING PROCEDURES (SOPs) LIST

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SOP BIO-001 LAB GLASSWARE USE AND DISPOSAL

SCOPE

The procedures described in this policy refer to the disposal of lab glassware, used and empty glass and broken glass that is produced during work in the laboratory.

This policy **does not apply** to any glassware that has been previously contaminated with:

1. Biohazardous Materials;
2. Acutely Hazardous Substances according to the Code of Mass Regulations 310.30, and Policies of UMass Lowell EEM-EHS Department.
    a. **Acutely Hazardous Substances** are **"P-List"** and **"U-List"** chemicals (listed in the Chemical Hygiene Plan pages 31-45) and Code **F027** substances (clorophenol and tri-, tetra-, and penta- derivatives). The glass contaminated with those chemicals should be disposed as Hazardous Waste Materials.

DEFINITIONS

Lab glassware is any item that could puncture regular trash bags and potentially cause injuries to someone handling the trash bag. It also means any intact glassware that could potentially break during waste handling activities.

The following items are considered Lab Glassware under this policy:

- Glass pipettes used or broken
- Glass Pasteur pipettes
- Empty glass bottles
- Flasks and beakers
- Vials and test tubes
- Empty broken glassware


Glass vials


Glass containers

DISPOSAL

All glassware and/or broken glass must be disposed in cardboard boxes lined with a clear plastic bag similar to those in the pictures below:

- The boxes should be used until ¾ full;
- Use tongs or a brush and dustpan to handle broken glass;
- Call EEM-EHS at 4-2618 for full container pick up or request new supplies.

IMPORTANT: Never dispose of the following items in these boxes:

- Any glassware used previously with **biohazardous or infectious materials** of any kind;
- Glassware (bottles, pipettes, etc.) used previously with **Acutely Hazardous Substances;**
- Liquid waste (any amount);
- Sharps (needles, syringes, blades, lancets, scalpels);
- Plastic petri dishes or culture plates;
- Plastic vials and conical tubes;
- Regular Trash.

For any questions on glass disposal and/or biosafety issues, “P-List”, “U-List” or Code F027 chemicals, contact EEM-EHS at biosafety@uml.edu or Ext. 4-2618.

ADDENDUM TO POLICY AND PROCEDURES FOR GLASS DISPOSAL

LIST OF ACUTELY HAZARDOUS SUBSTANCE OR "P-LIST" CHEMICALS

The following list of Acutely Hazardous Substances is known as "P-List" chemicals (listed in the Chemical Hygiene Plan pages 31-45). Any glass or material contaminated with any of the following chemical substances should be disposed as Hazardous Waste Materials.

DO NOT dispose glass contaminated with any of these "P-List" substances in the regular glass disposal cardboard box.

The current list of "P-List" Hazardous Substances may be found on the EPA Website[1]. The current regulations for Hazardous Waste in Massachusetts may be found on the Energy and Environmental Affairs Website[2].

P###	Chemical Name
P026	1-(o-Chlorophenyl)thiourea
P081	1,2,3-Propanetriol, trinitrate (R)
P042	1,2-Benzenediol, 4-[1-hydroxy-2-(methylamino)ethyl]-, (R)-
P067	1,2-Propylenimine
P185	1,3-Dithiolane-2-carboxaldehyde, 2,4-dimethyl-, O- [(methylamino)- carbonyl]oxime
P004	1,4,5,8-Dimethanonaphthalene, 1,2,3,4,10,10-hexa- chloro-1,4,4a,5,8,8a,-hexahydro-, (1alpha,4alpha, 4abeta,5alpha,8alpha,8abeta)
P060	1,4,5,8-Dimethanonaphthalene, 1,2,3,4,10,10-hexa- chloro-1,4,4a,5,8,8a-hexahydro-, (1alpha,4alpha, 4abeta,5beta,8beta,8abeta)-
P002	1-Acetyl-2-thiourea
P048	2,4-Dinitrophenol
P051	2,7:3,6-Dimethanonaphth [2,3-b]oxirene, 3,4,5,6,9,9 -hexachloro-1a,2,2a,3,6,6a,7,7aoctahydro-, (1aalpha,2beta,2abeta,3alpha,6alpha,6abeta,7 beta, 7aalpha)-, & metabolites
P037	2,7:3,6-Dimethanonaphth[2,3-b]oxirene, 3,4,5,6,9,9- hexachloro-1a,2,2a,3,6,6a,7,7aoctahydro-, (1aalpha,2beta,2aalpha,3beta,6beta,6aalpha,7 beta, 7aalpha)-
P045	2-Butanone, 3,3-dimethyl-1-(methylthio)-, O-[methylamino)carbonyl] oxime
P034	2-Cyclohexyl-4,6-dinitrophenol
P001	2H-1-Benzopyran-2-one, 4-hydroxy-3-(3-oxo-1- phenylbutyl)-, & salts, when present at concentrations greater than 0.3%
P069	2-Methyllactonitrile
P017	2-Propanone, 1-bromo-
P005	2-Propen-1-ol
P003	2-Propenal
P102	2-Propyn-1-ol

[1] http://www.epa.gov/osw/hazard/wastetypes/listed.htm
[2] http://www.mass.gov/eea/agencies/massdep/recycle/regulations/310-cmr-30-000.html.

P007 3(2H)-Isoxazolone, 5-(aminomethyl)-
P027 3-Chloropropionitrile
P047 4,6-Dinitro-o-cresol, & salts
P059 4,7-Methano-1H-indene, 1,4,5,6,7,8,8-heptachloro- 3a,4,7,7a-tetrahydro-
P008 4-Aminopyridine
P008 4-Pyridinamine
P007 5-(Aminomethyl)-3-isoxazolol
P050 6,9-Methano-2,4,3-benzodioxathiepin, 6,7,8,9,10,10- hexachloro-1,5,5a,6,9,9a-hexahydro-, 3-oxide
P127 7-Benzofuranol, 2,3-dihydro-2,2-dimethyl-, methylcarbamate
P088 7-Oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid
P023 Acetaldehyde, chloro-
P057 Acetamide, 2-fluoro-
P002 Acetamide, N-(aminothioxomethyl)-
P058 Acetic acid, fluoro-, sodium salt
P003 Acrolein
P070 Aldicarb
P203 Aldicarb sulfone
P004 Aldrin
P005 Allyl alcohol
P046 alpha,alpha-Dimethylphenethylamine
P072 alpha-Naphthylthiourea
P006 Aluminum phosphide (R,T)
P009 Ammonium picrate (R)
P119 Ammonium vanadate
P099 Argentate(1-), bis(cyano-C)-, potassium
P010 Arsenic acid H3AsO4
P012 Arsenic oxide As2O3
P011 Arsenic oxide As2O5
P011 Arsenic pentoxide
P012 Arsenic trioxide
P038 Arsine, diethyl-
P036 Arsonous dichloride, phenyl-
P054 Aziridine
P067 Aziridine, 2-methyl-
P013 Barium cyanide
P024 Benzenamine, 4-chloro-
P077 Benzenamine, 4-nitro-
P028 Benzene, (chloromethyl)-
P046 Benzeneethanamine, alpha,alpha-dimethyl-
P014 Benzenethiol
P188 Benzoic acid, 2-hydroxy-, compd with (3aS-cis)- 1,2,3,3a,8,8a-hexahydro-1,3a,8 trimethylpyrrolo [2,3-b]indol-5-yl methylcarbamate ester (1:1)
P028 Benzyl chloride

P015 Beryllium powder
P017 Bromoacetone
P018 Brucine
P021 Calcium cyanide Ca(CN)2
P189 Carbamic acid, [(dibutylamino)- thio]methyl-, 2,3-dihydro-2,2-dimethyl- 7-benzofuranyl ester
P191 Carbamic acid, dimethyl-, 1-[(dimethyl-amino) carbonyl]- 5-methyl-1H- pyrazol-3-yl ester
P192 Carbamic acid, dimethyl-, 3-methyl-1- (1-methylethyl)- 1H-pyrazol-5-yl ester
P190 Carbamic acid, methyl-, 3-methylphenyl ester
P127 Carbofuran
P022 Carbon disulfide
P095 Carbonic dichloride
P189 Carbosulfan
P023 Chloroacetaldehyde
P029 Copper cyanide
P029 Copper cyanide Cu(CN)
P030 Cyanides (soluble cyanide salts), not otherwise specified
P031 Cyanogen
P033 Cyanogen chloride
P033 Cyanogen chloride (CN)Cl
P016 Dichloromethyl ether
P036 Dichlorophenylarsine
P037 Dieldrin
P038 Diethylarsine
P041 Diethyl-p-nitrophenyl phosphate
P043 Diisopropylfluorophosphate (DFP)
P044 Dimethoate
P191 Dimetilan
P020 Dinoseb
P085 Diphosphoramide, octamethyl-
P111 Diphosphoric acid, tetraethyl ester
P039 Disulfoton
P049 Dithiobiuret
P050 Endosulfan
P088 Endothall
P051 Endrin
P051 Endrin & metabolites
P042 Epinephrine
P031 Ethanedinitrile
P194 Ethanimidothioc acid, 2-(dimethylamino)-N- [[(methylamino)carbonyl]oxy]-2-oxo-, methyl ester
P066 Ethanimidothioic acid, N-[[(methylamino)carbonyl]oxy]-, methyl ester
P101 Ethyl cyanide
P054 Ethyleneimine

P097 Famphur
P056 Fluorine
P057 Fluoroacetamide
P058 Fluoroacetic acid, sodium salt
P198 Formetanate hydrochloride
P197 Formparanate
P065 Fulminic acid, mercury(2+) salt (R,T)
P059 Heptachlor
P062 Hexaethyl tetraphosphate
P068 Hydrazine, methyl-
P116 Hydrazinecarbothioamide
P063 Hydrocyanic acid
P063 Hydrogen cyanide
P096 Hydrogen phosphide
P060 Isodrin
P192 Isolan
P196 Manganese dimethyldithiocarbamate
P196 Manganese, bis(dimethylcarbamodithioato-S,S')-,
P202 m-Cumenyl methylcarbamate
P065 Mercury fulminate (R,T)
P092 Mercury, (acetato-O)phenyl-
P082 Methanamine, N-methyl-N-nitroso-
P064 Methane, isocyanato-
P016 Methane, oxybis[chloro-
P112 Methane, tetranitro- (R)
P118 Methanethiol, trichloro-
P198 Methanimidamide, N,N-dimethyl-N'-[2-methyl-4- [[(methylamino) carbonyl]oxy]phenyl]-
P199 Methiocarb
P066 Methomyl
P068 Methyl hydrazine
P064 Methyl isocyanate
P071 Methyl parathion
P190 Metolcarb
P128 Mexacarbate
P073 Nickel carbonyl
P073 Nickel carbonyl Ni (CO)4, (T-4)-
P074 Nickel cyanide
P074 Nickel cynaide Ni(CN)2
P075 Nicotine & salts
P076 Nitric oxide
P078 Nitrogen dioxide
P076 Nitrogen oxide NO
P078 Nitrogen oxide NO2
P081 Nitroglycerine (R)

P082 N-Nitrosodimethylamine
P084 N-Nitrosomethylvinylamine
P040 O,O-Diethyl O-pyrazinyl phosphorothioate
P085 Octamethylpyrophosphoramide
P087 Osmium oxide OsO4, (T-4)-
P087 Osmium tetroxide
P194 Oxamyl
P089 Parathion
P024 p-Chloroaniline
P199 Phenol, (3,5-dimethyl-4-(methylthio)-, methylcarbamate
P020 Phenol, 2-(1-methylpropyl)-4,6-dinitro-
P009 Phenol, 2,4,6-trinitro-, ammonium salt (R)
P048 Phenol, 2,4-dinitro-
P034 Phenol, 2-cyclohexyl-4,6-dinitro-
P047 Phenol, 2-methyl-4,6-dinitro-, & salts
P202 Phenol, 3-(1-methylethyl)-, methyl carbamate
P201 Phenol, 3-methyl-5-(1-methylethyl)-, methyl carbamate
P128 Phenol, 4-(dimethylamino)-3,5-dimethyl-, methylcarbamate (ester)
P092 Phenylmercury acetate
P093 Phenylthiourea
P094 Phorate
P095 Phosgene
P096 Phosphine
P041 Phosphoric acid, diethyl 4-nitrophenyl ester
P094 Phosphorodithioic acid, O,O-diethyl S-[(ethylthio)methyl] ester
P039 Phosphorodithioic acid, O,O-diethyl S-[2-(ethylthio)ethyl] ester
P044 Phosphorodithioic acid, O,O-dimethyl S-[2- (methylamino)-2-oxoethyl] ester
P043 Phosphorofluoridic acid, bis(1-methylethyl) ester
P071 Phosphorothioic acid, O,O,-dimethyl O-(4- nitrophenyl) ester
P089 Phosphorothioic acid, O,O-diethyl O-(4-nitrophenyl) ester
P040 Phosphorothioic acid, O,O-diethyl O-pyrazinyl ester 3
P097 Phosphorothioic acid, O-[4-[(dimethylamino)sulfonyl]phenyl] O,O-dimethyl ester
P204 Physostigmine
P188 Physostigmine salicylate
P110 Plumbane, tetraethyl-
P077 p-Nitroaniline
P098 Potassium cyanide
P098 Potassium cyanide K(CN)
P099 Potassium silver cyanide
P201 Promecarb
P203 Propanal, 2-methyl-2-(methyl-sulfonyl)-, O-[(methylamino)carbonyl] oxime
P070 Propanal, 2-methyl-2-(methylthio)-, O-[(methylamino)carbonyl]oxime
P101 Propanenitrile
P069 Propanenitrile, 2-hydroxy-2-methyl-

P027 Propanenitrile, 3-chloro-
P102 Propargyl alcohol
P075 Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-, & salts
P204 Pyrrolo[2,3-b]indol-5-ol, 1,2,3,3a,8,8a-hexahydro- 1,3a,8-trimethyl-, methylcarbamate (ester), (3aS-cis)-
P114 Selenious acid, dithallium(1+) salt
P103 Selenourea
P104 Silver cyanide
P104 Silver cyanide Ag(CN)
P105 Sodium azide
P106 Sodium cyanide
P106 Sodium cyanide Na(CN)
P108 Strychnidin-10-one & salts
P018 Strychnidin-10-one, 2,3-dimethoxy-
P108 Strychnine & salts
P115 Sulfuric acid, dithallium(1+) salt
P110 Tetraethyl lead
P111 Tetraethyl pyrophosphate
P109 Tetraethyldithiopyrophosphate
P112 Tetranitromethane (R)
P062 Tetraphosphoric acid, hexaethyl ester
P113 Thallic oxide
P113 Thallium oxide Tl2O3
P114 Thallium(I) selenite
P115 Thallium(I) sulfate
P109 Thiodiphosphoric acid, tetraethyl ester
P045 Thiofanox
P049 Thioimidodicarbonic diamide [(H2N)C(S)]2NH
P014 Thiophenol
P116 Thiosemicarbazide
P026 Thiourea, (2-chlorophenyl)-
P072 Thiourea, 1-naphthalenyl-
P093 Thiourea, phenyl-
P185 Tirpate
P123 Toxaphene
P118 Trichloromethanethiol
P119 Vanadic acid, ammonium salt
P120 Vanadium oxide V2O5
P120 Vanadium pentoxide
P084 Vinylamine, N-Methyl-N-nitroso-
P001 Warfarin, & salts, when present at concentrations greater than 0.3%
P121 Zinc cyanide
P121 Zinc cyanide Zn(CN)2
P122 Zinc phosphide Zn3P2, when present at concentrations greater than 10% (R,T)

P205 Zinc, bis(dimethylcarbamodithioato-S,S')-,
P205 Ziram

For any questions on biosafety issues, “P-List”, “U-List” or Code F027 chemicals, contact EEM-EHS at biosafety@uml.edu or Ext. 4-2618.

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SOP BIO-002 FOR SHARPS USAGE AND DISPOSAL

SCOPE

This policy describes the disposal of **sharp waste** as part of the UMass Lowell Biohazardous Waste Program, in compliance with 105 Code of Massachusetts Regulation 480.200 (E) with the fundamental purpose to protect staff, faculty and students that could be at risk when handling any waste sharps.

DEFINITIONS

Sharps are any items having corners, edges, or projections capable of cutting or piercing the skin. The following items are considered sharps **WHETHER OR NOT CONTAMINATED** with biohazardous materials:

- Syringes, with or without needles (syringe components, suture needles, pen needles);
- Needles with attached tubing;
- Scalpels, razor blades, lancets.





The following items are considered sharps **ONLY WHEN CONTAMINATED** with biohazardous materials

- Empty blood vials
- Broken glassware
- Splintered plastic pipettes
- Glass Pasteur pipettes
- Glass slides and covers.

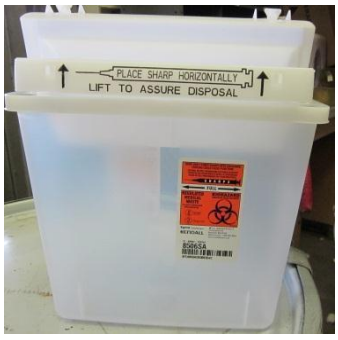



IMPORTANT

- Use personal protective equipment (glasses gloves) when working with or disposal of sharps
- NEVER recap a needle before disposal;
- DO NOT pickup contaminate or broken glass with bare hands, use forceps or other mechanical device.

SHARP CONTAINERS

Sharp containers are devices for the specific purpose of sharps disposal and in most cases are labeled with the biohazard sign. Immediate disposal of used needles into a sharps container is a required standard procedure.




Sharps containers are for sharps ONLY. The containers should be used until there are ¾ full. Call EEM-EHS at 4-2618 for pick up for disposal or to request new supplies.

Disposal of non-sharp biohazard waste in sharps container adds significant costs to waste management. The following items **SHOULD NOT BE DISPOSED** in sharps containers:

- Gloves;
- Paper towels;
- Lab glassware;
- Plastic pipettes and pipette tips;
- Petri dishes or culture plates;
- Plastic vials and conical tubes.

USAGE OF SHARPS AND NEEDLES

Syringes and hypodermic needles are dangerous instruments. The use of needles and syringes should be restricted to procedures for which there is no alternative.

Blunt cannulas should be used as alternatives to needles wherever possible (i.e., procedures such as oral or intranasal animal inoculations). Needles and syringes should never be used as a substitute for pipettes.

PROCEDURES AND RECOMMENDATIONS WHEN WORKING WITH SHARPS AND BIOLOGICAL MATERIALS

Follow these recommendations when using syringes and needles with biohazardous or potentially infectious agents:

1. Before using any sharp, be sure to have a convenient size sharp container;
2. Minimize the use of reusable syringes and needles;
3. Use disposable needle locking syringe units whenever possible;
4. Work in a biosafety cabinet whenever possible;
5. Wear appropriate PPE: gloves, safety glasses;
6. Fill the syringe carefully to minimize air bubbles;
7. Expel air, liquid and bubbles from the syringe vertically into a cotton moistened with alcohol 70%;
8. Wrap the needle and stopper in cotton moistened with disinfectant when removing a needle from a rubber-stoppered bottle;

9. Use a separate pan of disinfectant for reusable syringes and needles;
10. If it is essential that a contaminated needle be recapped or removed from a syringe, the use of a mechanical device or the one handed scoop method (See Addendum) must be used;

11. The use of needle nipping devices is prohibited and the devices must be discarded as infectious waste;
12. Do not use a syringe to mix infectious fluid forcefully;
13. Do not contaminate the needle hub when filling the syringe in order to avoid transfer of infectious material to fingers;
14. Bending, recapping, clipping, or removal of needles from syringes is prohibited;
15. Do not place syringes in pans containing pipettes or other glassware in order to eliminate sorting them later;
16. Used disposable needles and syringes must be placed in appropriate sharps disposal containers and discarded as infectious waste;
17. Never overfill a sharps container.

For any questions on sharps disposal and/or biosafety issues, contact EEM-EHS at biosafety@uml.edu or Ext. 4-2618.

ADDENDUM TO POLICY AND PROCEDURES FOR SHARPS USAGE AND DISPOSAL

DISPOSAL OF CONTAMINATED SYRINGES

POLICY

In compliance with the OSHA BBP Standard, this SOP applies to any worker who needs to pick up and or dispose potentially contaminated needles and syringes.

KIT CONTENTS

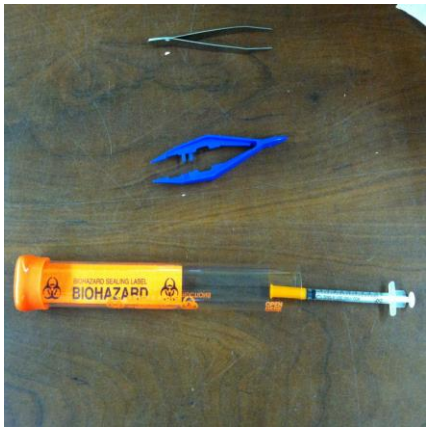
1. Biohazard sharps tube
2. Pair of gloves
3. Tweezers/Forceps

PROCEDURE

1. Open the bag and take out the contents.

2. Put on the gloves.
3. Open the biohazard tube and place it near the syringe.
4. Using ONLY one hand, pick up the contaminated syringe with the tweezers. Needle should point down towards the bottom of the tube.

5. Place the syringe in the biohazard tube carefully.



6. Tap the tube for the needle to stay in place.
7. Cap the tube with both hands.

8. Place the biohazard tube and the tweezers back in the bag.
9. Remove the gloves in a sterile manner and discard them.
10. Reseal the bag and label it.

For additional information on sharp disposal, supplies and Biosafety issues, contact EEM-EHS at biosafety@uml.edu or Ext. 4-2618.

SOP BIO-003 FOR THE DISPOSAL OF SOLID BIOHAZARDOUS WASTE

SCOPE

This policy describes the management and disposal of biological and medical waste, as part of the UMass Lowell Biohazardous Waste Program, with the fundamental purpose to protect staff, faculty and students that could be at risk when working with biohazardous material.

This policy is in compliance with Massachusetts Department of Public Health regulations (such as the State Sanitary Code Title VIII and 105 CMR 480.00), and Mass Department of Environmental Protection regulations 310 CMR 19.000.

DEFINITIONS

Biohazardous Agents are any agents that are biological in nature, and have the capacity to produce harmful effects upon other biological organism. Biohazardous agents include, but are not limited to:

- Bacteria
- Fungi
- Viruses
- Rickettsia
- Chlamydia
- Parasites
- Recombinant products
- Allergens
- Human and non-human primate cell lines and the potentially biohazardous agents these cells may contain
- Clinical specimens
- Tissue from experimental animals
- Toxins of biological origin
- Other biohazardous agents like prions or as defined by State and Federal regulations.

Regulated Biological/Medical Waste is defined as any material such as sharps; blood and blood products; pathological wastes; cultures and stocks of infectious agents and associated biologicals; animal carcasses, body parts and bedding **that contains or has been contaminated with a biohazardous agent**.

Solid Biohazardous Waste is any material, lab plastic ware and general lab ware, **contaminated with any biohazardous agent** (*see list above*). Solid biohazardous waste includes:

- Pipette tips
- Petri dishes
- Tissue culture plates
- Flasks and tubes
- Blood vials
- Surgical wraps
- Absorbent material
- Gowns, gloves, and any other labware contaminated with biohazards.

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DISPOSAL CONTAINERS FOR BIOLOGICAL WASTE

All contaminated lab ware (see above), must be disposed of in cardboard boxes lined with Red Biohazard plastic bags that can be decontaminated by autoclaving or by incineration.

IMPORTANT: Never over fill card boxes. They only should be filled until ¾ of the total volume.

USE OF THE AUTOCLAVE TO DECONTAMINATE AND DISPOSE OF SOLID WASTE

At UMass Lowell, only those autoclaves in laboratories that comply with the specifications of the 105 CMR 480.000 may be used to decontaminate biological or medical waste according to Bio-006 SOP. For any questions about autoclaving biological waste, contact EEM-EHS at biosafety@uml.edu or Ext. 4-2618.

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DISPOSAL OF THE AUTOCLAVED WASTE

1. Wait until the **Red Biohazard Plastic Bag** has cooled completely;
2. Transfer the cold red autoclaved bag to a regular **Black Plastic Bag;**
3. Close tightly and dispose of the black bag in the regular trash;
4. **Never** dispose of the autoclaved red bag directly in the regular trash.

AUTOCLAVE RECORDS

In compliance with the 105 CMR 480.000, when biological/or medical waste is decontaminated by autoclaving it, specific records should be kept in a log.

Date	Time	User Name	Location of Waste Generation	Amount of Waste	Cycle Used	Dates of Bio-Test Runs

EEM-EHS coordinates and performs the bio-test to validate the equipment.

INCINERATION OF SOLID WASTE - USE OF THE BURNING BOX

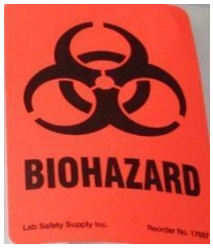
The incineration box or burning box has different shapes and sizes, but should always be lined with a red biohazard plastic bag. These boxes can be used when a certified autoclave is not available for decontamination of any biological waste.

EEM-EHS recommends that the following materials should not be autoclaved but disposed in the burning box:

- Personal protective equipment (gowns, gloves) soaked with large amount of human blood, body fluids or any infectious agents;
- Absorbent material (pads) and surgical wraps contaminated with infectious agents;
- Human blood vials;
- Human bodily fluid vials
- Small animal parts

DISPOSAL OF ANIMAL AND HUMAN PARTS

All animal and human parts should be disposed directly in a plastic pail or any sturdy container, with a lid that fits tightly when closed and is marked with the Biohazard sign. Refer SOP Bio-013 for more information



For pick up of full containers, and to request new supplies, contact EEM-EHS at biosafety@uml.edu or Ext. 4-2618.

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SOP BIO-004 FOR DECONTAMINATION AND DISPOSAL OF LIQUID BIOHAZARD WASTE

SCOPE

This SOP applies to the disposal of any liquid or culture media that has been in contact with cells, viable organisms, or any other of their parts.

DECONTAMINATION PROCEDURES AND DISPOSAL

1. All liquid biological waste should be collected in bottles or plastic containers that contain 100 ml of household bleach for each liter of liquid waste. The bottles should be labeled as hazardous waste and stored in the Satellite Accumulation Area (SAA);

2. Small volumes generated from sample preparation can be inactivated by adding bleach at a final concentration of 10%, and then collected in labeled liquid-waste containers;

3. For laboratories or facilities that **continuously generate** large volumes of liquid-waste, the EEM-EHS Department recommends the following:
    a) Decontaminate collected liquid with fresh bleach at final concentration of 10% (100 ml of house bleach for each liter of liquid waste);
    b) Fill out a Non-Hazardous Waste Determination Form (EEM-EHS website)[1]. An EEM-EHS/ Biosafety Officer will determine if the liquid is non-hazardous. Store the containers of decontaminated liquid waste in the SAA until EEM-EHS makes the final determination;
    c) If **no hazardous** chemicals are found, the liquid can be poured down the sink with abundant water;
    d) If hazardous chemicals are found, containers should be kept in the SAA. EEM-EHS will pick-up the bottles containing the hazardous liquid waste;
    e) Bottles with liquid waste should be no more than ¾ full.
    f) Call EEM-EHS at 4-2618 for pickup of full containers of request new bottles.

To get the Non-Hazardous Waste Determination Form, go to the EEM-EHS web site[1]. If you have any questions related to this SOP or any biosafety issue, contact EEM-EHS at biosafety@uml.edu or Ext. 4-2618.

[1] https://www.uml.edu/eem/

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SOP BIO-005 FOR THE DECONTAMINATION OF REUSABLE LABWARE, WORK-SURFACES AND EQUIPMENT

SCOPE

This SOP applies to the decontamination, cleaning, and disinfection of reusable labware, equipment, and all work-surfaces (benches, BSC work-surface) before and after using any biohazard or potential infectious material.

DEFINITIONS

Cleaning is the removal of visible soil (e.g. organic and inorganic material) from objects and surfaces. It is accomplished mechanically using water with detergents or enzymatic products. Cleaning is essential before disinfection and sterilization, because inorganic and organic materials that remain on the surfaces of instruments can interfere with the effectiveness of these processes.

Disinfection is a process that eliminates or kills many or all-pathogenic microorganisms, except bacterial spores, that are present of surfaces or inanimate objects like equipment. Specific chemical agents called sporicides can eliminate bacterial spores.

Decontamination removes pathogenic microorganisms from objects rendering them safe to handle, use, or discard.

Biosafety Cabinet (BSC) is a piece of equipment designed to protect the operator, the laboratory environment, and work materials from exposure to infectious aerosols and splashes that may be generated when manipulating substances containing infectious agents such as viruses, bacteria, and primary tissue

PROCEDURES FOR DECONTAMINATION AND CLEANING

Reusable Labware

1. All reusable plasticware or glass labware such as cylinders, flasks, beakers, and others that cannot be autoclaved for practical reasons, can be decontaminated by soaking the labware in 10% fresh bleach solution or as recommended by the manufacturer;

2. Immerse completely all labware in a pail with a 10% fresh bleach solution and soak the material for at least one hour;
3. Rinse with abundant water and a final rinse with distilled water;
4. The equipment can be air-dried.

Work-Surface or Bench Work

1. **Before Work** clean the bench- work surface with soap and water if it is soiled or wipe the surface with 10% fresh bleach solution followed with a water wipe down to remove all bleach residual.
2. **After Work c**lean, decontaminate and remove all equipment and supplies from the work area. Work surfaces should be wiped with a disinfectant that would kill the infectious agent that has been used.

Equipment

1. All equipment shall be cleaned and decontaminated before and after working with any biologically potential infectious material or blood;
2. The use of 10% bleach can be corrosive for some equipment that has metal surfaces;
3. Several commercial EPA approved disinfectants, which are not corrosive to metals, can be used on equipment with metal parts;
4. Clean and decontaminate the equipment by following the instructions of the equipment-manufacturer or vendor;
5. Plastic parts can be submerged in 10% bleach solution for 30 minutes, rinsed with abundant water and a final rinse with distilled water;
6. Dry plastic parts with paper towels;
7. Choose the appropriate disinfectant for the agent(s) that you are working with;

Biosafety Cabinet (See SOP Bio-010)

1. The BSC's work surface should be kept in pristine condition;
2. Disinfect by spraying the surface with 70% ethanol or isopropanol before and after each use;
3. Corrosive chemicals such as 10% bleach should be avoided. In case of small spill, bleach 5-10% should be used and followed with a wipe down of abundant sterile water and 70% ethanol. See SOP Bio-010 for cleaning spills inside BSC;
4. To avoid cross-contamination it is recommended to keep a cleaning/ decontamination log after disinfection of the BSC.

For any advice in choosing a germicide agent or sporicides, contact EEM-EHS at biosafety@uml.edu or Ext. 4-2618.

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SOP BIO-006 USE OF AUTOCLAVE FOR STERILIZATION OF MATERIALS AND BIOLOGICAL WASTE

SCOPE

This policy describes the use of autoclave/steam sterilization for general sterilization of labware, liquid and solid materials, and biological waste. The policy is part of the Biohazardous Waste Disposal Program in compliance with Massachusetts Department of Public Health regulations State Sanitary Code Title VIII 105 CMR 480.00, Massachusetts Department of Environmental Protection regulations 310 CMR 19.00, and UMASS Lowell policies

This standard operating procedure (SOP) applies to all laboratories that generate regulated biological waste. This waste includes material such as labware contaminated with blood, blood products, non-fixed pathological waste, cultures and stocks of infectious agents and associated biological material, animal carcasses, animal bedding, and sharps.

This SOP also applies to the sterilization by autoclaving of different materials and items that need to be sterile for biological work in the laboratory.

DEFINITION

Autoclave is an airtight vessel utilized for sterilization of objects by using steam under pressure. During the autoclaving process, each item is exposed to direct steam at the required temperature and pressure for a specified time.

Biohazardous Agent is any agent that is biological in nature, capable of self-replication, and has the capacity to produce harmful effects upon biological organisms. Biohazardous agents include, but are not limited to: bacteria, fungi, viruses, rickettsia, chlamydia, parasites, recombinant products, allergens, cultured human and animal cells and the potentially biohazardous agents these cells may contain, clinical specimens, tissue from experimental animals, toxins of biological origin, other biohazardous agents like prions or as defined by State and Federal regulations.

Decontamination is a procedure that eliminates or reduces microbial contamination to a safe level with respect to transmission of infection.

Disinfection is a procedure that kills pathogenic microorganisms, but not necessarily their spores. Chemical germicides formulated as disinfectants are used on inanimate surfaces (i.e., medical devices) and not used on skin or any body parts.

Infectious Waste is waste containing, or potentially containing, pathogens of sufficient virulence and quantity so that exposure to the waste by a susceptible host could result in the development of a communicable disease.

Pathological Waste includes all animal and human non-fixed organs, tissues, body parts other than teeth; products of conception; fluids removed by trauma, during surgery, autopsy, or other medical procedure; and infected animal carcasses.

Regulated Biohazardous/Medical Waste includes any material such as sharps; blood and blood products; pathological waste; cultures and stocks of infectious agents and associated biologicals; and animal bedding that contains or has been contaminated by a biohazardous agent. Biohazardous waste can be separated into sharps (See Policies and Procedures for Disposal of Sharps), liquid waste, and solid waste.

Steam is the vapor created by heating water to 212°F (100°C).

Steam Sterilization is moist heat in the form of saturated steam under pressure. This is the most widely used and dependable methods available for sterilization. The exposure of any item to moist heat at 250°F or 121°C under pressure (at least 15 psi) for 15 to 30 minutes allows the destruction of all forms of microbial life on any item.

Sharps include objects that can cause a puncture or laceration. Sharp waste includes needles, scalpels, lancets, and shards of glass or plastic contaminated with biological agents.

Universal Precautions is an infection control method where all human blood and any other potentially infectious materials are treated as if known to be positive for bloodborne pathogens such as Hepatitis B Virus, Hepatitis C Virus, and Human Immunodeficiency Virus.

RESPONSIBILITIES

Employees

1. Familiarize themselves with this SOP and associated work instructions;
2. Abide by this SOP and associated SOP requirements;
3. Immediately report injuries, accidents, unsafe conditions, and unsafe acts to their supervisor and/or Environmental Health and Safety (EEM-EHS);
4. Attend applicable training classes.

Supervisors

1. Assure that permanent and temporary workers are trained in and follow the requirements of this SOP;
2. Assure that employees attend appropriate training sessions;
3. Investigate and report accidents and unsafe conditions to Environmental Health and Safety.

Environmental and Emergency Management- Environmental Health and Safety (EEM-EHS)

1. Provide professional guidance and resources related to management of biohazardous waste;
2. Conduct training for employees;
3. Monitor the implementation of this SOP in various labs;
4. Assure that accidents and other hazardous situations, which may unnecessarily expose employees to biological hazards, are properly reported, evaluated, and corrected;
5. Oversee all aspects of contracts with outside biological waste vendors;
6. Act as site-wide liaison with other departments and institutions sharing common facilities and resources pertaining to biological waste management.

Principal Investigator/Laboratory Director/Laboratory Manager

1. Review and approve the implementation of this SOP;
2. Support managers and supervisors in their efforts to implement the SOP;
3. Investigate and report accidents and unsafe conditions to Environmental Health and Safety.

PERSONAL PROTECTION EQUIPMENT (PPE)

1. Before handling any type of biological waste to be put in or taken out of the autoclave, employees should wear proper PPE: lab coat, safety glasses, heat resistant gloves and closed-toe shoes;
2. When handling biological waste, all employees should follow universal precautions;
3. Employees should never reach into a biohazardous waste container to retrieve materials.

EQUIPMENT AND SUPPLIES

1. Supplies as biohazard autoclave bags should be purchased by each department;
2. Occasionally and or in a case of emergency, you can get supplies from EEM-EHS Stock Room by calling EEM-EHS at extension 4-2618;
3. Supplies as biohazard signs or those for monitoring the autoclaves as chemical monitoring–strips and biological test indicators will be supplied by EEM-EHS Autoclave Program;
4. If the autoclave has been repaired and or any service has been done to it, a biological test needs to be performed. Contact the EEM-EHS at biosafety@uml.edu.

RECORDKEEPING

1. Record all data from any run in the Daily Autoclave log as date; time-in of treatment; the type of load (clean material or waste); quantity of waste treated; printed name and signature of the person responsible for treatment; and any relevant information when applicable;
2. The person in charge of the autoclave will be responsible to maintain all records and logs;
3. Biological testing results (growth/no growth) is coordinated by the BSO and all records from "Biological Test Logs" should be kept in the EEM-EHS office;
4. All records collected in the "Autoclave user log" and "Biological Test log must be maintained for 3 years.
5. The autoclave operator (or person in charge) should notify their supervisor and record any incident or problems when working or monitoring the autoclave.

MONITORING THE AUTOCLAVE - GUIDELINES RECOMMENDED BY THE CDC

The CDC recommends monitoring the autoclave mechanical and chemically every time that a run is performed. A monitoring biological test should be performed monthly.

At UMass Lowell, EEM-EHS will monitor the autoclave using the *Geobacillus stearothermophilus* once a month. Autoclaves that run more than 10 runs per week (40 loads per month) are recommended to perform a biological test more often. The Biosafety Officer (BSO) will make recommendations for each particular case.

Daily Monitoring Mechanical Procedures

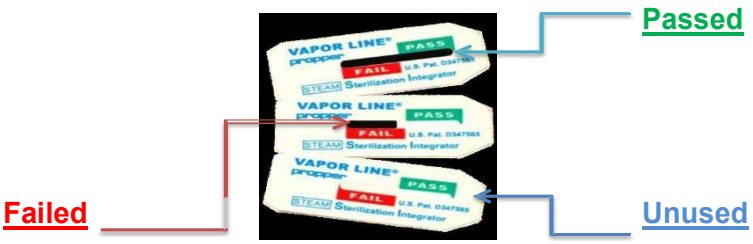
All users must follow the CDC recommendations for mechanical monitoring of steam sterilization. These include:

1. The daily assessment of cycle time and temperature by examining the temperature record chart (or computer printout;
2. An assessment of pressure checking the pressure gauge.

Chemical Testing Guidelines

1. The Center for Disease Control (CDC) requires that a chemical indicator be placed on the inside of each waste package to verify steam penetration;
2. Every run/load will carry a **Class 5 Vapor Line Steam Integrator;** in order to corroborate the correct time, steam, and temperature of each autoclave run;
3. The Class 5 Vapor Line Steam Integrator strips clearly indicate Pass/Fail for the run by changing color. The sterilization cycle has achieved spore death when the indicator reaches the "Pass" area;
    - If the indicator only reaches the "Fail" zone, the batch needs to be autoclaved again using a fresh indicator;

IMPORTANT If Fail results continue, report the condition to the Autoclave Room Manager in charge;



4. All runs results should be noted in the Daily Autoclave Use log;
5. The Class 5 Vapor-line Steam Integrators or similar will be provided by EEM-EHS Department and can be requested by calling the EEM-EHS extension 4-2618

Biological Testing Guidelines

1. The CDC recommends that all autoclaves should be biologically monitored at least weekly with the appropriate commercial preparation of spores;
2. At UMass Lowell sterilization is done **only** by steam in autoclaves monitored by the EEM-EHS;
3. The biological testing is done once a month by the Biosafety Officer (biosafety@uml.edu);
4. On an established monthly schedule, the Biosafety Officer will perform biological monitoring using a Bio-Test indicator from Autoclave Testing Service, Inc.;
5. The Bio-Test indicator contains spores from the *Geobacillus thermophiles*, and will be used in each autoclave at standard conditions for the use of each autoclave.
6. After the regular run, the Bio-Test indicators will be incubated for 24-48 hours at 65^0C together with a control Bio-Test indicator that has being maintained at room temperature.

Positive for growth, color change indicates that Autoclave has FAILED

Negative for growth or NO change of color indicates Autoclaved PASS the

7. Results of the biological indicator tests (indicating date and Pass or Fail) must be documented on the **"Biological Test Log"**

STEAM STERILIZATION PROCEDURES FOR CLEAN MATERIAL AND BIOHAZARDOUS WASTE

The following procedures apply for steam sterilization of clean material and for sterilization of biohazardous waste.

IMPORTANT: To Prevent Burns or Spills

Observe the following safety procedures during loading and unloading the autoclave**:**

1. Loosen screw caps on bottles and tubes of liquids before autoclaving;
2. Be sure to wear a face shield when you open the autoclave. Steam can burn your face;
3. Check that chamber pressure has returned to zero before opening door;
4. Stand behind door when opening it;
5. Slowly open autoclave door only a crack;
6. Beware rush of steam. Make sure that the door to the autoclave room is closed in order to prevent steam from escaping into corridor;
7. Wait 5 minutes after opening door before removing liquids. Keep face away from door as it opens. Escaping steam may burn face;
8. Do not put solvents, volatile or corrosive chemicals (such as phenol, chloroform, bleach, etc.), or radioactive materials in an autoclave;
9. Load the bags into the autoclave and operate the autoclave according to the manufacturer's operating instructions.

Packing and Loading the Autoclave

1. DO NOT sterilize clean material together with biohazardous waste in the same load and run;
2. All infectious waste must be placed in biohazard autoclave bags, obtained from the EEM-EHS (extension 4-2618), and be loosely sealed with autoclave tape to allow steam to penetrate;
3. Sealed autoclave bags must be brought directly to the autoclave room in a secondary container (cart with sides, tote, etc.);
4. Ensure sufficient water in load to allow steam penetration or add 250 mL water to bags containing solids to ensure steam penetration;
5. In order to corroborate the correct time, steam, and temperature of each run, every load will carry a **Class 5 Vapor Line Steam Integrator.** The Class 5 Vapor-line Steam Integrators will be provided by EEM-EHS Department and can be requested by contacting EEM-EHS at biosafety@uml.edu or Ext. 4-2618.
6. Biohazard waste bags to be autoclaved later should be located in specific area of the autoclave room and clearly marked with biohazard symbol;
7. Biohazard waste bags CANNOT be held in the autoclave room for more than 24 hours without being autoclaved;
8. Bags to be autoclaved must be placed in secondary containers or decontamination pans;

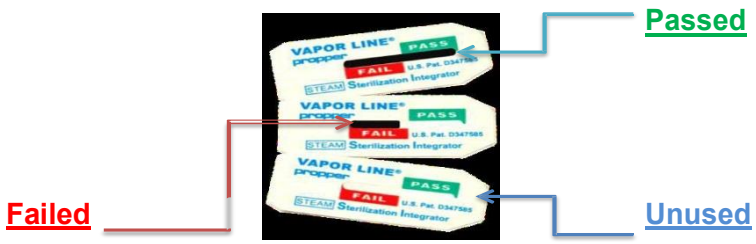
9. There should only be one bag per pan or enough space between bags to allow adequate steam circulation;
10. Do not overfill containers (prevent spill and boil over);
11. Do not allow bags to touch or strap sides of autoclave;
12. Complete “Daily Autoclave Log”.

Loading the Autoclave

1. Ensure the autoclave is operating properly before commencing;
2. Determine the appropriate exposure time, temperature and pressure for the load to autoclave based on spore testing;
3. Ensure the autoclave attains the desired temperature (normally 121^{0}C) and pressure (minimum 15 psi) for the desired time (minimum 30 min.);
4. Record the information in “Autoclave Use Log”;

Unloading the Autoclave

1. Wait until the chamber pressure gauge reads zero before opening;
2. Open slightly to allow steam to escape (protect yourself from the steam);
3. Wait 20-30 minutes, more if necessary, for the contents of the autoclave to cool;
4. Carefully remove the secondary container with the waste bag to reduce the risk of spillage;
5. Wait until the autoclaved plastic bag has cooled completely;
6. Verify temperature and duration of exposure has been met;
7. Verify that each chemical monitor-strip has changed color. Proper sterilizing conditions turn the indicator on the monitor strip black;

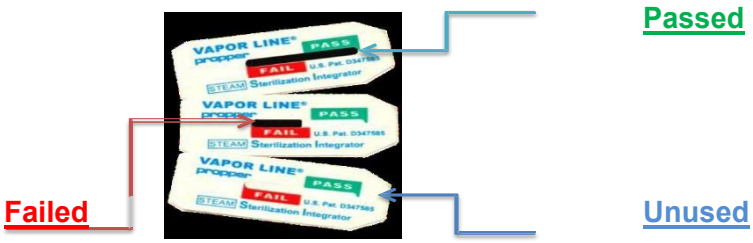


8. If any of the chemical monitor-strips did not turn black, then the entire load must be re-autoclaved.

Disposal of Autoclaved Biological Waste

Autoclaved bags of biological waste are noninfectious and can be disposed of in the same manner as non-infectious waste in the regular trash. Regular trash is not regulated by 105 CMR 480.000.

1. After verification that each chemical monitor-strip changed color by turning black, the operator should begin to unload the bags;



2. Transfer the cold autoclaved bag to a regular **black plastic trash bag;**
3. Close bag tightly and dispose of it in the regular trash;
4. **DO NOT** dispose the autoclaved red bag directly into the regular trash.

RELATED DOCUMENTS

The following SOPs are related to this document:

1. **SOP Bio-002** Sharp Usage and Disposal;
2. **SOP Bio-003** Disposal of Solid Biohazardous Waste;
3. **SOP Bio-004** Decontamination and Disposal of Liquid Biohazard Waste;
4. Exposure Control Plan (UMass Lowell Bloodborne Pathogens Program).

REFERENCES

1. 105 CMR, Department of Public Health 480.000
   http://www.mass.gov/eohhs/docs/dph/regs/105cmr480.pdf
2. CDC Biological and Infectious Waste
   https://www.cdc.gov/nceh/ehs/etp/biological.htm
3. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, Dec 2009
   http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
4. Environmental Health and Safety web site
   http://www.uml.edu/eem/

For additional information on autoclaving, Biological testing, biological waste disposal and/or any biosafety issues; contact EEM-EHS at biosafety@uml.edu or Ext. 4-2618.

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SOP BIO-007 CLEANING A BIOLOGICAL SPILL INSIDE THE CENTRIFUGE

SCOPE

The SOP applies to the situation in which a tube(s) containing human blood or other biological fluids is broken or spilled inside a centrifuge. Biological fluids such as cell suspension, bacteria culture, blood, or any other human bodily fluid of human origin are considered potential infectious and should be handled following appropriated practices with PPE.

MATERIALS

Before beginning the decontamination and cleaning process, gather the following materials:

1. Forceps or tweezers;
2. Paper towels;
3. Container to soak contaminated parts of the centrifuge;
4. Approved EPA disinfectant or freshly prepared bleach 10% solution
5. Spray bottle with 70% Ethanol or 70% isopropanol;
6. Sharps disposal container;
7. Biohazard waste container.

Disinfectant Solutions

Choose the appropriate EPA approved disinfectant that will not damage the centrifuge but is effective against bloodborne pathogens (HepB, HIV, Mycobacterium, etc.).

EPA recommended disinfectants and CDC disinfection can be found at EPA[1] and CDC[2] websites:

Household bleach is 5.25% to 6.15% sodium hypochlorite, (approx. 60,000ppm of Chlorine) depending on manufacturer, and is commonly used diluted in water at 1:10. Recommended contact time of 20 to 30 minutes is effective to disinfect items contaminated with blood. Bleach should be wiped off with water to avoid corrosion in equipment like centrifuge parts or surfaces of BSC. Plastic parts can be soaked in 10% household bleach and rinse with abundant water before final rinse in distilled water and air dried.

[1] https://www.epa.gov/pesticide-registration/list-e-epas-registered-antimicrobial-products-effective-against-mycobacterium
[2] http://www.cdc.gov/hicpac/Disinfection_Sterilization/17_00Recommendations.html

Before Cleaning

1. Be sure to wear double gloves, lab coat, and safety glasses or a face shield;
2. Unplug the centrifuge and allow the rotor to completely stop;
3. Allow 20-30 minutes for any aerosols that were generated to settle before opening the centrifuge;
4. Put a visible sign to inform others about the spill in the centrifuge;
5. Be sure to choose the right disinfectant that will not damage the centrifuge and parts.

PROCEDURES FOR CLEANING THE SPILL

1. Use a mechanical device, like forceps or tweezers, to remove the broken tube and any pieces and dispose them in a sharps container;
2. Remove the tube adapter from the rotor to a container that can fit all pieces of the centrifuge;
3. Soak all pieces in a disinfectant solution for 20 minutes;
4. After soaking, use forceps to retrieve all parts from the disinfectant solutions to be washed and rinsed;
5. Before cleaning the interior of the centrifuge, be sure that all visible pieces of glass have been removed;
6. Use paper towels to soak all the liquid or blood that is spilled in the bottom of the centrifuge. Repeat this step until all liquid has been absorbed;
7. Dispose all contaminated paper towels in the Biohazard Waste Container;
8. Spray disinfectant in the interior of the centrifuge and let it stand for at least 10 minutes;
9. Wipe the surface of the centrifuge twice with paper towels wet with distilled water, and twice with 70% ethanol or isopropanol;
10. Place material in the Biohazard Container;
11. Soak the tube adapter in disinfectant for 20 minutes. Rinse, let it dry, and spray with 70% ethanol or isopropanol;
12. Remove gloves and dispose of them in the Biohazard Waste Container;
13. Remove lab coat if contaminated, and place in Biohazard Container or Contaminated Laundry Container;
14. Wash hands thoroughly with soap and water.

For additional information on any biosafety issues; contact EEM-EHS at biosafety@uml.edu or Ext. 4-2618.

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SOP BIO-008 CLEANING AND DECONTAMINATION AFTER SMALL SPILLS IN THE LAB OR INSIDE THE BSC

SCOPE

This SOP applies to all laboratories that are using biohazard materials including infectious agent, recombinant microorganisms, and materials like blood or any body fluid of human origin. It applies to the cleaning and disinfection of small liquid spills (20 ml or less) in the lab and the cleaning inside the biological safety cabinet (BSC).

DEFINITIONS

Cleaning for the purpose of this SOP is the removal of any visible material from surfaces as bench work, floor or the interior work-surface of the biosafety cabinet.

Disinfection is a process that eliminates or kills many or all pathogenic microorganisms, except bacterial spores that can be present in the material spilled. It requires the use of a disinfectant like house bleach freshly prepared. In some cases a CDC approved disinfectant with sporicidal properties will be needed.

Decontamination removes pathogenic microorganisms from objects rendering them safe to handle, use, or discard.

Biosafety Cabinet (BSC) Is a piece of equipment designed to protect the operator, the laboratory environment, and work materials from exposure to infectious aerosols and splashes that may be generated when manipulating substances containing infectious agents, such as viruses, bacteria, and primary tissue

SMALL SPILL IN THE LABORATORY

Initial Response

1. Notify ALL people and restrict access to the area as appropriate;
2. Notify your Supervisor;
3. Confine the area as appropriate to prevent spread of the spill. Do not put yourself at risk;
4. If the clean-up is out of your control, **CALL Extension 44-911** from a landline or **978-934-4911** from a cellphone;

5. Have on hand all information (MSDS of the pathogen or a copy of the IBC registration) about the organism or bio-hazard spilled: bacteria, cell, cultured material etc.

Spill Clean-up Materials

All laboratories using biohazard materials should have the following materials available, located in a designated space.

1. Personal protective equipment (PPE): safety glasses, goggles, or face shield, utility gloves, wrap-around lab coat, shoe covers (optional);
2. Forceps, tongs, broom, dust pan;
3. Sharps container;
4. Disinfectant solution;
5. Paper towels or other absorbent;
6. Red/Orange Biohazardous Waste Bag.

Spill Clean-Up Procedure

1. Wear appropriate PPE: gloves, lab-coat and glasses;
2. Allow aerosol to settle for 20-30 minutes;
3. Remove sharp objects mechanically using forceps, tongs, or any other available method. Dispose of sharps in a sharps container;
4. Disinfect perimeter with 10% bleach or any other EPA approved disinfectant. EPA recommended disinfectants and CDC disinfection can be found at EPA[1] and CDC[2] websites
5. Cover the spill with absorbent material;
6. Apply disinfectant on top of the absorbent material;
7. Wait for 20-30 minutes;
8. Blot up material until no sign of material is present;
9. Dispose clean-up material into a Red/Orange Biohazardous Waste Bag;
10. Fill out an incident report

SMALL SPILL INSIDE A BIOSAFETY CABINET

Additional information is found in SOP Bio-010 Use and cleaning of the BSC.

When cleaning any spill inside the BSC, gather the materials mentioned above and follow the next steps:

1. The blower of the cabinet should be switched on always;
2. Clean-up as per directions above, making sure to wipe down back and side walls of cabinet;

[1] https://www.epa.gov/pesticide-registration/list-e-epas-registered-antimicrobial-products-effective-against-mycobacterium

[2] http://www.cdc.gov/hicpac/Disinfection_Sterilization/17_00Recommendations.html

3. If material has spilled into the catch basin beneath the work surface, add a volume of disinfectant equal to the quantity in the basin, wait 20 minutes, and absorb with paper towels and dispose;
4. After completion, allow cabinet to run for ten minutes before resuming work.

For additional information on any biosafety issues; contact EEM-EHS at Ext. 4-2618.
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SOP BIO-010 USE AND DECONTAMINATION OF THE BIOSAFETY CABINET

SCOPE

The purpose of this SOP is to demonstrate how to safely use and maintain the biological safety cabinets (BSC); and to ensure the proper containment of manipulated biological material as well as the safety of anyone operating the BSC.

DEFINITIONS

Biosafety cabinets (BSCs) are hoods with high efficiency particulate air *(HEPA) filters* that provide personnel, environmental, and product protection when appropriate practices and procedures are followed. Safety equipment including BSCs, PPE, or other physical containment devices (e.g. safety centrifuge cups) must be used whenever procedures with a potential to create infectious aerosols or splashes are conducted, or whenever high concentrations or large volumes of infectious agents are used. Examples of such procedures include pipetting, centrifuging, grinding, blending, shaking, mixing, vortexing, sonicating, opening containers with pressure differentials, or harvesting infected tissues. The BSC is the principal Biosafety level 2 (BL-2) device used in laboratories to provide such containment.

Three types of BSCs (Class I, II, and III) are used in microbiological laboratories. Open-fronted Class I and Class II BSCs are partial containment devices, which provide a primary barrier offering significant levels of protection to laboratory personnel and to the environment when used in combination with good microbiological techniques.

The **Class I BSC** is suitable for work involving low to moderate risk agents, where there is a need for containment but not for product protection. It provides protection to personnel and the environment from contaminants within the cabinet. The Class I BSC does not protect the product from "dirty" room air.

The **Class II BSC** meets requirements to protect personnel, the environment and the product since it protects the material inside the cabinet (e.g., cell cultures, microbiological stocks) from external contamination.

There are different types of Class II BSCs: Type A (A1, A2), Type B1 ant Type B2. The major differences between these types are in the percent of air that is exhausted or recirculated, and the manner in which exhaust air is removed from the work area. Type B1 and B2 are BSCs ducted that can exhaust the air removed outside the laboratory area, outside the facility.

Although B1 is ducted, 40% of the air is recirculated and 60% removed or exhausted. BSCs Class II Type B2 are mostly ducted with 100% of the air exhausted
The gas-tight **Class III BSC** or glove box provides the highest attainable level of protection to personnel, the environment and the product. It is the only cabinet that provides a total physical barrier between the product and personnel. It is for use with high-risk biological agents and is used when absolute containment of highly infectious or hazardous material is required.

Additional information on the proper use and selection of a BSC can be found on 5th Ed Biosafety in Microbiological and Biomedical Laboratories (BMBL)[1].

Decontamination is the destruction of microorganisms to some safe level (but not necessarily zero).

Disinfection is the chemical or physical treatment that destroys most vegetative microbes, but not spores.

High Efficiency Particulate Air (HEPA) Filter traps 99.97% of particles of 0.3 μm in diameter and 99.99% of particles of greater or smaller size. The filter captures all infectious agents and ensuring that only clean air, free of microbes, is exhausted from the cabinet or directed to the work-surface.

Sanitization is the reduction of a microbial load on a surface to a safe public health level.

Sterilization is the total destruction of all microorganisms.

RESPONSIBILITIES:

Principal Investigator or Designated Person in Charge is responsible for providing all lab members adequate training to operate the BSC in a safety manner and ensures that the BSC properly maintained and annually re-certified.

Users, prior to operating the BSC, need to have training and demonstrate understanding of the proper work with BSC. All users must report any spills or accidents to EEM-EHS.

PROCEDURES:

Start of BSC Operation:

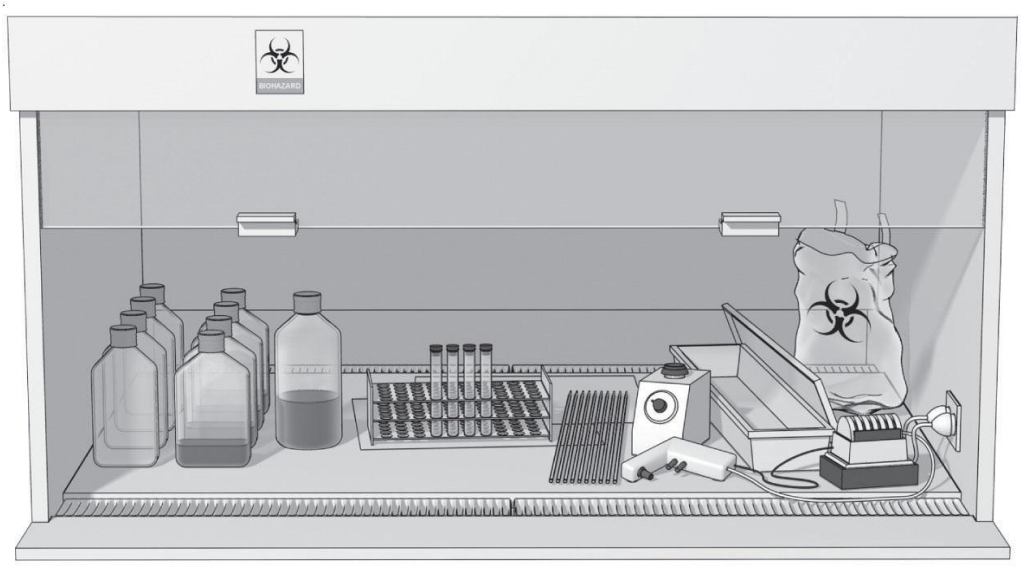
1. Wear appropriate PPE (lab coat, gloves with long cuffs, eye safety glasses if necessary and no open toed shoes in the lab; appropriate PPE will vary according to the type of work being performed);
2. Have all your supplies ready;
3. Turn on the BSC for at least 10 minutes (this allows adequate aeration of the cabinet);

[1] http://www.cdc.gov/biosafety/publications/bmbl5/

4. Disinfect cabinet surfaces using an approved disinfectant such as 70% ethanol;
5. Do not rely upon ultraviolet (UV) light as the sole decontaminating agent. UV light loses its effectiveness over time and too often is not replaced before its intensity drops below optimal level. Even when the UV light is operating correctly, surface decontamination should be performed before and after every cabinet use
6. Start organizing your cabinet area, make sure to surface disinfect anything that you are bringing in and out of the cabinet;
7. Waste containers, suction collecting flasks, and dirty supplies beakers must be placed on one side of the cabinet. Avoid clutter as it will impede the laminar air flow;
8. Only bring in the necessary instruments and supplies;
9. Heat sources such as Bunsen burners are **not allowed** within the BSC;
10. Do not block the grill/grid area of the cabinet, this will allow adequate airflow and filtration. If you block the grid area, air will penetrate the lab space instead of being drawn towards the HEPA filter;
11. Your work should be done about one full palm-in (5-6 inches) away from the front grid.

Clean to Dirty Layout

The following graphic shows a typical layout "clean to dirty"[2] to work in a class II BSC. Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded in the shallow pan and other contaminated materials (as pipettes-tips and small plastic tubes) can be placed in the small biohazard bag. This arrangement can be reversed for left-handed persons.



Left Side: Clean Cultures **Right Side:** Waste and Dirty Supply Collection

[2] The clean to dirty layout was obtained from BMBL 5th Ed. Appendix A, figure 11 (2009) at http://www.cdc.gov/biosafety/publications/bmbl5/

End of BSC Operation

1. Take everything out accordingly, while disinfecting once again;
2. Once empty (do not store anything in BSC) disinfect the interior completely: that includes back and side walls, ceiling, inside bottom, and the inside of glass door shaft;
3. Let the BSC run for an additional 10-15 min after cleaning;
4. Check below the grid area to ensure no visible particles or spills were trapped;
5. If a UV light is being used it must be checked on a weekly basis (cleaned with lint free material soaked in alcohol), and certified on an annual basis to ensure that the proper wavelength for decontamination is being attained.

BSC Decontamination when working with Group Risk 2 Infectious Agents

In case of spills involving large amounts of infectious microorganism that overflow below the grid area, it will be necessary to have a complete decontamination that could involve the use of chemical or gases.

The decontamination by any chemical (Formaldehyde, Hydrogen Peroxide, and others) method requires the intervention of personnel trained for this procedure. The EEM-EHS department uses authorized vendors, and the Biosafety Officer coordinates the process with them. In some cases, decontamination with chemical gases can take 12-15 hours.

Certification/Recertification of BSC:

At UMass Lowell, the EEM-EHS Department oversees and manages the maintenance of biosafety cabinets and offers testing and certification for all of them. This service is done yearly during spring and it is offered free of charge to investigators or Departments.

To ensure proper work Biosafety cabinets, authorized technical personnel must test and recertify BSCs. Certification/re-certification should be done at least once a year in the following situations:

- Before initial use;
- After being moved from one location to another;
- After changing the HEPA filter;
- After cleaning/decontamination of a serious spill inside the cabinet.

To coordinate the disinfection by an authorized vendor or for additional information on decontamination, re-certification, spills, biological waste disposal and/or any biosafety issues, contact the Biosafety Officer at biosafety@uml.edu or call EEM-EHS at 978-934-2618.

ENVIRONMENTAL AND EMERGENCY MANAGEMENT
Environmental Health and Safety
University Crossing Suite 140
Lowell MA 01854
http://www.uml.edu/eem/

SOP BIO-012
BIOSAFETY LEVEL 2 PRACTICES (*NIH Guidelines)*
http://osp.od.nih.gov/sites/default/files/nih_guidelines.html#_toc351276355

The following information is obtained from the NIH Guidelines[1] for research involving recombinant or synthetic nucleic acid molecules:

Appendix G-II-B. Biosafety Level 2 (BL2)
See Appendix G-III-N, *Footnotes and References of Appendix G*

Appendix G-II-B-1. Standard Microbiological Practices (BL2)

- **Appendix G-II-B-1-a.** Access to the laboratory is limited or restricted by the Principal Investigator when work with organisms containing recombinant or synthetic nucleic acid molecules is in progress.
- **Appendix G-II-B-1-b.** Work surfaces are decontaminated at least once a day and after any spill of viable material.
- **Appendix G-II-B-1-c.** All contaminated liquid or solid wastes are decontaminated before disposal.
- **Appendix G-II-B-1-d.** Mechanical pipetting devices are used; mouth pipetting is prohibited.
- **Appendix G-II-B-1-e.** Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.
- **Appendix G-II-B-1-f.** Persons wash their hands: (i) after handling materials involving organisms containing recombinant or synthetic nucleic acid molecules and animals, and (ii) when exiting the laboratory.
- **Appendix G-II-B-1-g.** All procedures are performed carefully to minimize the creation of aerosols.
- **Appendix G-II-B-1-h.** Experiments of lesser biohazard potential can be conducted concurrently in carefully demarcated areas of the same laboratory.

[1] http://osp.od.nih.gov/sites/default/files/nih_guidelines.html#_toc351276355

Appendix G-II-B-2. Special Practices (BL2)

- **Appendix G-II-B-2-a.** Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

- **Appendix G-II-B-2-b.** The Principal Investigator limits access to the laboratory. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

- **Appendix G-II-B-2-c.** The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g. immunization) may enter the laboratory or animal rooms.

- **Appendix G-II-B-2-d.** When the organisms containing recombinant or synthetic nucleic acid molecules in use in the laboratory require special provisions for entry (e.g. vaccination), a hazard warning sign incorporating the universal biosafety symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

- **Appendix G-II-B-2-e.** An insect and rodent control program is in effect.

- **Appendix G-II-B-2-f.** Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before exiting the laboratory for non-laboratory areas (e.g. cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.

- **Appendix G-II-B-2-g.** Animals not involved in the work being performed are not permitted in the laboratory.

- **Appendix G-II-B-2-h.** Special care is taken to avoid skin contamination with organisms containing recombinant or synthetic nucleic acid molecules; gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable.

- **Appendix G-II-B-2-i.** All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

- **Appendix G-II-B-2-j.** Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant or synthetic nucleic acid molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably autoclaved, before discard or reuse.

- **Appendix G-II-B-2-k.** Spills and accidents which result in overt exposures to organisms containing recombinant or synthetic nucleic acid molecules are immediately reported to the Institutional Biosafety Committee and NIH/OBA. Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

- **Appendix G-II-B-2-l.** When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

- **Appendix G-II-B-2-m.** A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

Appendix G-II-B-3. Containment Equipment (BL2)

- **Appendix G-II-B-3-a.** Biological safety cabinets (Class I or II) (see Appendix G-III-L, *Footnotes and References of Appendix G*) or other appropriate personal protective or physical containment devices are used whenever:

- **Appendix G-II-B-3-a-(1).** Procedures with a high potential for creating aerosols are conducted (see Appendix G-III-O, *Footnotes and References of Appendix G*). These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs.

- **Appendix G-II-B-3-a-(2).** High concentrations or large volumes of organisms containing recombinant or synthetic nucleic acid molecules are used. Such materials may be centrifuged in the open laboratory if sealed beads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

Appendix G-II-B-4. Laboratory Facilities (BL2)

- **Appendix G-II-B-4-a.** The laboratory is designed so that it can be easily cleaned.

- **Appendix G-II-B-4-b.** Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

- **Appendix G-II-B-4-c.** Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

- **Appendix G-II-B-4-d.** Each laboratory contains a sink for hand washing.

- **Appendix G-II-B-4-e.** If the laboratory has windows that open, they are fitted with fly screens.

- **Appendix G-II-B-4-f.** An autoclave for decontaminating laboratory wastes is available.

For questions or additional information, contact the Biosafety Officer at biosafety@uml.edu or call ext. 4-2618.