

 <p>750 Chase Parkway • Waterbury, CT 06708</p>	Safe Operating Procedure	
	Biosafety Manual	
	Working with BSL-1 and BSL-2 Agents	
	Issued: October 2011	Revised:

Purpose

The purpose of this manual is to provide NVCC personnel (faculty and laboratory staff) who work with microbial agents (e.g. bacteria, viruses, fungi, etc.) information on the safe handling of these agents. All laboratory exercises at NVCC are conducted as learning experiences for undergraduate students. Therefore, experiments are normally conducted with BSL-1 agents. However, it may be possible that a BSL-2 agent will be used on occasion. The risk of doing so will be evaluated prior to any lab experiments.

A. Guidelines for Good Laboratory Practices at BSL-1 and BSL-2¹

NOTE: Indented and bulleted items in this section indicate additional requirements for work at BSL-2.

1. Immediately notify the laboratory supervisor or responsible faculty member of any accident, injury, illness, or overt exposure associated with laboratory activities. Seek medical treatment if necessary. Complete an incident report form and send it to the Office of EAOH&S. Injuries requiring more than simple first aid must be reported to the NVCC Public Safety Department at 203-575-8112.
2. For those intending to work with blood or body fluids, the training module for Bloodborne Pathogens must be completed and submitted to the Office of EAOH&S.
3. Be aware that access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures or specimens is in progress.
4. The lab supervisor must ensure that all laboratory personnel receive appropriate initial training, necessary on-going training, and supervision regarding hazards associated with the agents involved; the necessary precautions to prevent exposures; and exposure evaluation procedures.
5. An individual's personal health status may impact an individual's susceptibility to infection or necessary medical surveillance. Any conditions in this regard (i.e. immune-compromised individuals) should be discussed with lab supervisor as appropriate.
 - Only personnel advised of the special hazards and meeting any specific entry requirements, i.e., appropriate immunizations, serum sampling, are permitted in the laboratory. Understand and follow all biosafety procedures provided by the supervisor.

¹ Excerpted from the *CDC/NIH Biosafety in Microbiological and Biomedical Laboratories* and the *NIH Guidelines for Research Involving Recombinant DNA Molecules*)

- Be aware that possession or use of select biological agents or toxins requires special federal government registration and inspection; restricted lab access; written and strictly followed safety and security plans; personnel background checks and training; accurate records and/or reporting of agent use, transfer, loss, or destruction. Any plans for obtaining such materials must be discussed with the Biosafety Officer and approved by the IBC.
 - Ensure that when infectious agents are in use in the laboratory, a biohazard sign is posted on the lab access door. This sign identifies the agent(s) in use, the biosafety level, any required immunizations, the responsible faculty member's name and telephone number, and any PPE that must be worn in the laboratory.
6. Wash hands frequently and always after handling viable material, after removing gloves, and before leaving the laboratory. A sink, located near exit doors, for hand washing is present in each laboratory.
 - Consider foot, knee, or automatically operated hand washing sinks.
 - Know the location of a readily accessible eyewash station.
 7. Do not eat, drink, smoke, chew gum, handle contact lenses, or apply cosmetics in the laboratory. Persons wearing contact lenses in the laboratory should also wear goggles or a face shield.
 8. Do not bring any food, medications, or cosmetics, into the laboratory for storage or later use. All food for consumption is stored outside the work area in cabinets or refrigerators designated specifically for that purpose.
 - Do not bring animals or plants unrelated to experimental work into the laboratory.
 9. Mouth-pipetting is not allowed; only mechanical pipetting devices are permitted.
 10. Perform all procedures carefully to minimize the creation of splashes or aerosols.
 11. Follow policies for safe handling of sharps and always use Universal Precautions. Use a high degree of caution when handling any contaminated sharp item, such as needles and syringes, slides, pipettes, capillary tubes, and scalpels. Substitute plasticware for glass whenever possible. Handle broken glassware with brush and dustpan, tongs, or forceps - not directly with hands.
 12. Do not bend, shear, break, recap, or remove used needles from disposable syringes or otherwise manipulate such units by hand before disposal. Dispose of needles and syringes in the puncture resistant container provided in the laboratory for this purpose. Place full containers in an autoclave bag and sterilize before disposal in medical waste boxes.
 - Restrict needles and syringes or other sharp instruments in the laboratory for use only when there is no alternative, such as for parenteral injection,

phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles.

- Use only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) for injection or aspiration of infectious material.

13. Use of lab coats, gowns, or other designated laboratory uniform is recommended to prevent contamination or soiling of street clothing.

- Wear lab coats, gowns, smocks, or other provided protective garments while working with hazardous materials. When leaving the lab, remove and leave coats and other protective clothing in the lab for either disposal or laundering.

14. Wear gloves if the skin on the hands is broken or if a rash is present. Protective eyewear should be worn for procedures that have a risk of splashes of microorganisms or other hazardous materials to the face.

- Wear gloves when manipulating infectious materials or agents or when hands must otherwise contact contaminated surfaces. Remove and change gloves when overtly contaminated or when torn or punctured. Do not wear contaminated gloves outside the lab. Do not wash or reuse disposable gloves. Consider alternatives to latex gloves to prevent allergic response.
- Wear appropriate face protection (goggles, mask, face shield or other splatter guard) for anticipated splashes or sprays of infectious materials to the face when agents **must** be handled outside the BSC. Persons wearing contact lenses should also wear eye protection.

15. Decontaminate equipment and work surfaces at completion of work, at the end of the day, and following spills of viable materials. If a spill occurs, cover the spill with paper towels and soak the towels with a 1 to 10 dilution of chlorine bleach or other suitable disinfectant at the appropriate dilution. Allow the material to soak for approximately 20 minutes before wiping up. Clean up materials must be collected in biohazard bag.

16. Bench tops are impervious to water and resistant to solvents, acids, alkalis, and chemicals used for surface decontamination.

17. Laboratory surfaces and spaces between fixtures are designed to be easily cleaned; no carpets or rugs.

18. Work on open bench tops is permitted. Special containment equipment such as a biological safety cabinet (BSC) is not generally required for agents assigned to BSL-1.

- Work in the open laboratory is permitted, except that a properly maintained biological safety cabinet is required whenever:
 - Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from

ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonate eggs.

- High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.

Be aware that air sampling studies have shown that most of the common manipulations of bacterial and viral cultures in research laboratories release aerosols of viable organisms. This must be considered when evaluating need for use of the biological safety cabinet or other physical containment device.

19. Dispose of all regulated medical wastes (potentially biohazardous) and associated wastes as described in the Biomedical Waste Policies and Procedures Manual.
20. Cover containers of all cultures, tissues, specimens of body fluids, or other potentially infectious waste to prevent leakage during collection, handling, processing, storage, transport, or shipping.
21. Have an insect and rodent control program in place. Ensure screens are fitted on exterior windows that open into the lab.

B. Decontamination

1. Definitions

Decontamination is a process or treatment that renders an instrument or environmental surface safe to handle. A decontamination procedure can be as simple as clean-up with detergent and water or as thorough as sterilization. Sterilization, disinfection, and antisepsis are all forms of decontamination.

Sterilization is the use of physical or chemical processes to destroy all microbial life, including highly resistant forms, such as bacterial spores.

Disinfection is the elimination of essentially all pathogenic non-spore forming microorganisms but not necessarily all microbial forms from work surfaces and equipment. Effectiveness is influenced by a number of factors, including: types and numbers organisms; amount of organic matter; the object being disinfected; the disinfectant being used; exposure time, temperature and concentration.

Antisepsis is the application of a liquid antimicrobial to skin or other living tissue to inhibit or destroy microorganisms. Examples include hand washing with germicidal solutions or swabbing skin before an injection.

2. When to Decontaminate

All material and equipment contaminated with or containing potentially infectious agents should be decontaminated:

- Upon completion of procedures involving the use of biologically-active materials
- In the event of spills of such materials
- At least daily
- Before being washed, stored, or discarded

Decontamination is accomplished by steam heat sterilization in an autoclave, or by surface application of or placement in a chemical disinfectant solution, such as 1:10 bleach solution or its equivalent.

3. Autoclave Use

Autoclaving (saturated steam under pressure of approximately 15 psi to achieve a chamber temperature of at least 250°F for a designated time) is the preferred and most convenient method to rapidly destroy all forms of microbial life. However, to do this the autoclave process must reach proper temperature and time and also prevent the entrapment of air in the bag or container of treated material.

- Material to be sterilized must come into contact with live steam.
- Bags or containers should be left open during autoclaving or water (~200ml) should be added to sealed bags to generate steam.
- Heat indicator tape should be used with each autoclave load to indicate that sterilization has been completed.
- Autoclave sterility monitoring should be conducted on a regular basis using biological indicators (such as *B. stearothermophilus* spore strips) placed among treated materials and at locations throughout the autoclave. The spores, which are more resistant to heat than most microbials, provide validation of general microbial destruction when they are effectively inactivated (250°F for 13 minutes) by autoclave operation.

4. Chemical Disinfectant Use

Chemical disinfectants are used for decontamination of surfaces and liquid wastes prior to final disposal down the drain. It is important to use the recommended concentration.

GENERAL RECOMMENDATIONS:

Liquid Decontamination

- Add liquid chlorine bleach to provide a final 1:10 dilution
- Let stand at least 20 minutes
- Discard down the drain

Surface Decontamination (allow to air dry)

- Wipe with 1:10 dilution of chlorine bleach (made fresh), or
- Wipe with Vesphene disinfectant (per label concentration), or
- Wipe with 70% alcohol

C. Exposure to Infectious Agents

In the event of an exposure to an infectious agent or material, the following guidelines should be used:

1. Intact skin
 - Remove contaminated clothing
 - Vigorously wash contaminated skin for 1 minute with soap and water
2. Broken, cut or damaged skin or puncture wound
 - Remove contaminated clothing
 - Vigorously wash contaminated skin for 5 minutes with soap and water
 - Seek medical attention
3. Eye
 - Immediately flush eyes for at least 15 minutes with water, preferably using an eyewash, rinsing from the nose outward to avoid contamination of the unaffected eye.
 - Hold eyelids away from your eyeball and rotate your eyes so that all surfaces may be washed thoroughly.
 - Seek medical attention
4. Ingestion or Inhalation
 - Seek medical attention
 - Do not induce vomiting unless advised to do so by a health care provider

D. Biological Material Spills

In the event of a spill of biological material, the individual(s) who caused the spill is responsible for the clean-up. NVCC does not have a spill response team.

- Consider using plastic-backed liner to cover work surfaces where work is being performed. This will minimize the consequences of any spill of biological material.
 - Have the following items on hand to serve as a simple spill kit:
 - Chlorine bleach or some other concentrated disinfectant
 - A package or roll of paper towels
 - Autoclavable bags
 - Rubber gloves
 - Forceps for pick-up of broken glass
1. Small Spill (spill that can be covered by a few paper towels):
 - Put on personal protective equipment (gloves, eye protection, and lab coat).
 - Cover the spill with paper towels and gently apply disinfectant at proper concentration, proceeding from the outer edge of the spill to its center. Leave in place for 20 minutes.

- Pick up the towels and discard into a biohazard container. Pick up any pieces of broken glass with forceps and place in sharps container.
 - Re-wipe the spill area with disinfectant and thoroughly wash hands after glove removal.
2. Large Spill of BSL-2 Material (>500ml) Outside of a Biological Safety Cabinet:
- Hold your breath and leave the room immediately.
 - Warn others to stay out of the spill area to prevent spread of contamination; post a sign stating: "**DO NOT ENTER, BIOHAZARD SPILL**", contact (name and phone #) for information".
 - Remove any contaminated clothing and put into a biohazard bag for later autoclaving.
 - Wash hands and exposed skin and inform your supervisor about the spill
 - Put on protective clothing (lab coat, gloves and, if indicated, surgical mask, eye protection, shoe covers) and assemble clean-up materials.
 - Wait 30 minutes before re-entering the contaminated area to allow dissipation of aerosols.
 - Cover the spill with paper towels and gently apply disinfectant, proceeding from the outer edge of the spill to its center. Leave in place for 20 minutes
 - Collect all treated material and discard in a biohazard container. Pick up any broken glass with forceps and place them into a sharps container.
 - Re-wipe the spill area with disinfectant and wash hands thoroughly at completion of clean-up.

E. Biological Waste Handling

For further information, consult the Biomedical Waste Policy and Procedures Manual.