

BIOLOGICAL SPILLS



Spill Inside a biological safety cabinet

1. LEAVE THE CABINET ON.
2. While wearing gloves, spray or wipe cabinet walls, work surfaces, and equipment with disinfectant. If necessary, flood the work surface, as well as drain pans and catch basins below the work surface, with disinfectant (usually 10% bleach solution) for at least 20 minutes contact time.
3. Soak up the disinfectant and spill with paper towels. Drain the catch basin into a container. Lift front exhaust grill and tray, and wipe all surfaces. Ensure that no paper towels or solid debris are blown into the area beneath the grill.
4. Autoclave all clean-up materials and protective clothing. Wash hands and exposed skin areas with disinfectant.
5. EHS should be notified if the spill overflows into the interior of the cabinet. It may be necessary to do a more extensive cabinet decontamination.

Small spill of BSL-2 material outside a biological safety cabinet

1. Wearing gloves and a labcoat, cover the spill with paper towels and disinfectant (usually a 1/10 dilution of bleach).
2. Allow sufficient contact time with disinfectant (usually >20 minutes).
3. Pick up towels and discard into biohazard waste container.
4. Pick up broken glass with forceps and place in sharps container.
5. Re-wipe the spill area with disinfectant and wash your hands with soap or hand-washing disinfectant.

Large spill of BSL-2 material outside a biological safety cabinet (>500 ml)

- GET HELP!
- The methods are the same as for small BSL-2 spills, only on a larger scale.

Summary of Disinfectants and Their Uses				
Disinfectant	Dilution**	Effective On:	Ineffective On:	Comments
Phenolics: eg. Lysol™**	1/20 (5%)	Bacteria, Most Viruses, TB, HIV	Spores, Polio, Coxsackie Viruses.	Relatively insensitive to high protein concentrations. Cor- rosive.
Chlorine Bleaches: eg. Clorox™**	1/10 (10%)	Bacteria, Some Spores, Viruses, TB, HIV	Some Spores	Prepare once a week. It takes ~20 minutes to disinfect. Corrosive.
Iodophors: eg. Wescodyne™**	1/100 (1%)	Bacteria, Most Viruses, TB	Spores	A surface disinfectant. Iodine is insoluble, so it's not good in solutions. Corrosive.
Alcohols (Ethanol, Isopropanol)	70%	Bacteria, Most Viruses	Spores, TB	100% alcohol is a preservative! Flammable.

* The use of brand names does not imply a recommendation.
** Concentration of named brands.
† Use 1/5 dilution

TRAINING

For additional information regarding biosafety at UNH or to request BSL-2 training, please contact EHS at 862-4041.

RESOURCES

**University of New Hampshire
Institutional Biosafety Committee**
Perpetuity Hall, 11 Leavitt Lane
Durham, NH 03824
603.862.4041 (Telephone)
603.862.0047 (Facsimile)

<http://www.unh.edu/ehs>

Centers for Disease Control and Prevention
1600 Clifton Road
Atlanta, GA 30333
404.639.3534 (Telephone)
800.311.3435 (Toll-free)

<http://www.cdc.gov>

**National Institutes of Health
Office of Biotechnology Activities**
6705 Rockledge Drive, Suite 750, MSC 7985
Bethesda, MD 20892-7985
301.496.9838 (Telephone)
301.496.9839 (Facsimile)

<http://www4.od.nih.gov/oba>

American Biological Safety Association
1202 Allanson Road
Mundelein, IL 60060
847.949.1517 (Telephone)
847.566.4580 (Facsimile)

<http://www.absa.org/>



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BSL-2

Biosafety Level Two

The UNH Biosafety Program is concerned with protecting laboratory personnel and the environment from biohazardous substances. This is primarily accomplished by utilizing the Department of Health and Human Service publication titled, "Biosafety in Microbiological and Biochemical Laboratories" (HHS Publication No. CDC 93-8395).

This pamphlet explains Biosafety Level Two (BSL-2) protocols. It describes proper work practices, what to do in case of a biological spill, and how to properly dispose of biological waste. It also contains a listing of additional resources.

BSL-2 designated work is suitable for agents of moderate hazard to personnel and the environment. It differs from BSL-1 in that:

- Laboratory personnel need to have specific training in handling pathogenic agents and are directed by competent scientists;
- Access to the laboratory is limited when work is being conducted;
- Precautions are taken with contaminated sharp items;
- Procedures in which infectious aerosols or splashes may be created must be conducted in biological safety cabinets or other physical containment equipment.

Examples of agents that must be used in a BSL-2 laboratory include:

- Hepatitis B virus;
- Salmonellae spp.;
- Toxoplasma spp. (non-pathogenic); and
- Human-derived blood and body fluids.

All work with recombinant organisms or select infectious agents must be registered with the UNH Institutional Biosafety Committee (see back of pamphlet). Vaccine strains that have undergone multiple *in vivo* passages should not be considered avirulent simply because they are vaccine strains.

Additionally, many agents not ordinarily associated with disease processes in humans are opportunistic pathogens that may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals.

LABORATORY DESIGN




- Laboratories should be away from public areas.
- Rooms that house restricted agents (i.e., select infectious agents) should be locked.
- BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other potentially disruptive equipment so as to maintain proper air flow parameters for containment.
- A sink for handwashing must be available.
- A plumbed eyewash station and shower must be available.
- The laboratory must be designed so that it can be easily cleaned.
- Bench tops must be impervious to water and resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
- Spaces between benches, cabinets, and equipment must be easily accessible for cleaning.
- A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate information to be posted includes the agent(s) in use, the biosafety level, the required immunizations, the investigator's name and telephone number, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.
- If the laboratory has windows that open to the exterior they must be fitted with fly screens.

WORK PRACTICES

- Properly maintained Biological Safety Cabinets (BSCs), preferably Class II, or other appropriate PPE or physical containment devices are used whenever:
 - a. 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, and harvesting infected tissues from animals or embryonic eggs.
 - b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the 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.
- Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative of-



fices). All protective clothing is either discarded with bio-hazardous waste or laundered by the institution; it should never be taken home by personnel.

- Face protection (goggles, mask, face shield or other splatter guard) is used for splashes or sprays of infectious or other hazardous materials (to the face) that may occur. 
- Gloves are worn when hands may contact potentially infectious materials. Wearing two pairs of gloves may be appropriate. Gloves are discarded when they become overly contaminated or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Powdered latex gloves are forbidden. Hands are washed following removal of gloves and before leaving the laboratory. 
- Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.
- A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
- Laboratory equipment and work surfaces should be decontaminated with a disinfectant on a routine basis, after work with infectious materials is finished, and especially after spills, splashes, or other contamination by infectious materials.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the laboratory. 
- Mouth pipetting is prohibited; mechanical pipetting devices are used.

POLICIES & PROCEDURES

- Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress.
- Persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.
- Only individuals advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
- Immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or

TB skin testing) must be offered to laboratory and support personnel.

- Laboratory and support personnel receive training on the potential hazards associated with the work involved, precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.
- Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director.
- Contaminated equipment must be decontaminated before it is sent for repair or maintenance and properly packaged for transport before removal from the facility.
- Spills and accidents that result in exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided and written records are maintained.
- An insect and rodent control program is in effect (call EHS for more information).
- Animals not involved in the work being performed are not permitted in the lab.

SHARPS

- Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative. Plasticware should be substituted for glassware whenever possible.
- Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials.
- Used disposable needles must not be manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
- Syringes, which re-sheath the needle, needleless systems, and other safety devices, are used.
- Broken glassware must not be handled by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps.

BIOLOGICAL WASTE

- All cultures, stocks, and other regulated wastes are decontaminated before disposal.
- Materials to be decontaminated outside the laboratory are placed in a durable, leakproof container and closed for transport.
- Materials to be decontaminated off-site from the facility are packaged in accordance with regulations, before removal from the facility.