Designating Biological Safety Level 2 at UNC

Forms, Documents, and Training

Lab Safety Plan & Schedule F (Biological Hazards)

The first step in designating BSL-2 space at UNC is notifying EHS of your desire to do so. This is done by submitting an up-to-date copy of your Laboratory Safety Plan (LSP).


The Lab Safety Plan can be daunting; to complete the requirements for each section properly, refer to the general UNC Laboratory Safety Manual, Chapter 2. The latest copy of the Lab Safety Manual is available here: http://ehs.unc.edu/manuals/index.shtml.

For BSL-2 consideration, the Schedule F (Biological Hazards) of the Lab Safety Plan is completed and submitted to EHS for approval. The Lab Safety Plan is to be updated annually with EHS.

Schedule G (Recombinant DNA)

Recombinant DNA research on campus probably falls into one of the three following groups that may require registration with the UNC Institutional Biosafety Committee (IBC):

• Exempt Experiments
• Experiments Requiring Prior Approval
• Experiments Requiring IBC Notice Simultaneous with Initiation

Examples of each are available online at http://ehs.unc.edu/ih/biological/recombinant.shtml.

Forms and other information may be accessed at http://ehs.unc.edu/ih/biological/dna.shtml.

Annual in-house Training

All biological hazards listed on the Schedule F will be reviewed annually with all laboratory workers including those handling and/or treating biohazard waste. The annual in-house training form at http://ehs.unc.edu/forms/LSM_Appendix_1_D_15.pdf (or an equivalent) will be used to document this training and kept in the lab safety binder. These requirements are reviewed with new staff and the Schedule F is reviewed annually. Accessing this website allows lab workers to view all lab members' training requirements: https://itsapps.unc.edu/EHS
Bloodborne Pathogens Requirements (including human cell lines)

All workers exposed to human source materials will complete Bloodborne Pathogens (BBP) training annually. OSHA defines workers as exposed to BBP if they work with human blood or other potentially infectious material (including human cell lines—continuous or primary see http://ehs.unc.edu/ih/biological/bbp.shtml)

Complete the online self-study BBP training at: http://ehs.unc.edu/training/self_study/bbp/index.shtml. You may want to forward this link along to any lab workers who will require this training.

All workers to which the OSHA Bloodborne Pathogen standard applies should have, or officially decline, the Hepatitis B vaccination series at the University Employee Occupational Health Clinic http://ehs.unc.edu/ueohc.

For more information about the risk of human tissue and the Bloodborne Pathogen Standard please refer to the EHS website at http://ehs.unc.edu/ih/biological/bbp.shtml.

For more information about the Hepatitis B vaccination series, view the CDC fact sheet at: http://www.cdc.gov/ncidod/diseases/hepatitis/b/factvax.htm

Laboratory Facilities

Proper Doors

Laboratory doors are self-closing and have locks in accordance with the institutional policies. The Principal Investigator is ultimately responsible for the control of, and access to, laboratories where risk group 2 agents are stored or manipulated. (see http://oba.od.nih.gov/oba/rac/Guidelines/APPENDIX_B.htm)

Laboratory doors are kept closed while experiments are in progress. This practice not only protects persons who might otherwise enter the laboratory, it reduces interruptions to laboratory staff that could lead to accidents. Biological safety cabinets and laboratory hoods work best, and offer the most worker protection, when the doors to the laboratory are closed. For general information regarding access control of UNC labs, review chapter 6-4 of the UNC Laboratory Safety Manual.

Entryway signs

BSL-2 entryway signs are posted for lab spaces in which large volumes or high concentrations of risk group 2 agents are present. Also, posting entryway signs for all other BSL-2 lab space is
required (e.g. for areas where human blood or other potentially infectious material including human cell lines is present). EHS has supplied an approved BSL-2 door placard for the designated BSL-2 space at http://ehs.unc.edu/ehs/forms.shtml#bio. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

**Hand washing sink**

The lab space designated at BSL-2 has a sink available for hand washing. The sink may be manual, hands-free, or automatically operated and should have soap and disposable paper towels readily available at all times for washing hands at the sink. Ideally, the sink is located near the exit door.

**Easily cleanable**

The laboratory should be designed so that it can be easily cleaned and decontaminated. This can be difficult in older buildings that were designed without present day biosafety precautions in mind. Carpets and rugs in laboratories are not permitted. Check areas for worn and damaged bench tops or flooring that may harbor microbes in the event of a spill.

To have Facilities Services repair the area, speak with your department’s business manager and/or submit an online service request (at: http://www.fac.unc.edu/Services/ServiceRequests/tabid/60/Default.aspx).

**Proper bench tops**

Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning. To have Facilities Services repair an area, speak with your department’s business manager and/or submit an online service request.

**Proper chairs at biological safety cabinets**

Chairs used at the biological safety cabinet must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant. Cloth covered chairs should not be used at the biosafety cabinet. The chairs must be capable of supporting anticipated loads and uses. This practice should be applied to chairs at lab benches too.

**Proper Windows**

Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens. To have
Biological Safety Cabinet location

EHS must be consulted regarding the placement and use of your biological safety cabinet. Contact us (see http://ehs.unc.edu/ih/biological/) to arrange an appointment. Biological safety cabinets (BSC), (aka “tissue culture hoods”) must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions. To have Facilities Services install deflectors at room air vents, speak with your department’s business manager and/or submit an online service request. Technically, if a BSC has passed certification in place with the vents running, deflectors are not necessary however, EHS recommends not leaving it to chance...remember, that air barrier is pretty delicate on BSCs and seasonal airflow variations in laboratory buildings can be significant.

In-line HEPA filters

Vacuum lines located at biosafety cabinets (aka: “tissue culture hoods”) must have protection via an absorbent or liquid disinfectant trap and a High Efficiency Particulate Air (HEPA) filter, or its equivalent to prevent contamination of the vacuum system. Filters must be replaced as needed. This practice should also apply to aspirating liquid at the BSL-2 benchtop. Order info is available at www.fishersci.com; search for part # 09-744-75 or # 09-744-76.

Eyewash station

To be compliant with BSL-2 standards at UNC, eyewash stations must be plumbed units that meet the ANSI Standard Z358.1-2004 such as: Personal eye flush squeeze bottles do not meet ANSI requirements, because they cannot deliver the required minimum flow rate and duration. EHS discourages the presence of these bottles particularly in BSL-2 labs because they have a limited shelf life, are prone to contamination, and are ineffective at dual-eye or eye-face irrigation. Facilities Services can install a deck-mounted unit at an existing sink (place work orders online). EHS recommends the following unit to compensate for the distance and obstructions to the nearest safety shower from some labs: Fisher Scientific deck mount eye wash #S47711 https://www1.fishersci.com/Coupon?gid=2374690&cid=1341 For more information about eyewash facilities in UNC labs, see Chapter 3 of the UNC Laboratory Safety Manual.

Emergency shower

A shower facility, other than emergency drench hoses, must be located in the building. To compensate for the distance and obstructions to the nearest safety shower from some designated BSL-2 space, EHS recommends the following unit: Fisher Scientific deck mount eye wash#S47711 https://www1.fishersci.com/Coupon?gid=2374690&cid=1341
Proper ventilation

There are no specific requirements on ventilation systems in BSL-2 labs at UNC. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory. Chapter 7-4 (Facility Requirements) of the general UNC Laboratory Safety Manual indicates the following:

“Exhaust ventilation systems are designed to maintain an inflow of air from the corridor into the work area. The exhaust air from the work area must discharge directly to the outdoors, and clear of occupied buildings and air intakes. Exhaust air from the work area must not recirculate. The exhaust air from glove boxes must filter through high-efficiency particulate air (HEPA) and charcoal filters. EHS shall determine the need for and type of treatment for other primary containment equipment. Exhaust air treatment systems that remove toxic chemicals from the exhaust air by collection mechanism such as filtration or absorption must operate in a manner that permits maintenance, to avoid direct contact with the collection medium. All exhaust air from primary containment equipment must discharge directly to the outdoors and disperse clear of occupied buildings and intakes.”

Biological Safety Cabinet exhaust

Because most biological safety cabinets (BSC) at UNC re-circulate HEPA-filtered exhaust air into the laboratory environment, the cabinet should be tested and certified at least annually and operated according to manufacturer’s recommendations. Never use hazardous chemicals in these cabinets, the vapors bypass the HEPA (particulate) filters and enter your breathing zone.

Some BSCs on campus may be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation should be verified annually. Only minute amounts of hazardous chemicals should be used in an exhausted cabinet. More information about biological safety cabinets is available in the UNC Biological Safety Manual.

Autoclave

In UNC laboratories, an autoclave must be accessible to decontaminate all biohazard waste before disposal. This varies from the UNC Hospitals requirement for biohazard waste because UNC Hospitals incinerates all biohazard waste. If an autoclave is not accessible to your lab, contact EHS.

Refer to the EHS Biohazard Waste Management website for waste decontamination requirements. (http://ehs.unc.edu/ih/biological/infectious_waste.shtml)
**Chemical disinfection of liquid microbiological waste**

Refer to the “Liquids” section of the Biohazard Waste Disposal Chart at [http://ehs.unc.edu/ih/biological/docs/disposal_chart.pdf](http://ehs.unc.edu/ih/biological/docs/disposal_chart.pdf). If your liquid waste was used for propagating microbes/viral vectors/toxins AND you are unable to autoclave your liquid biohazard waste, you will need to make application to the North Carolina Medical Waste Division to dispose of this chemically disinfected liquid microbiological waste down the drain. For more information, refer to the EHS Chemical Treatment of Liquid Microbiological Waste website at [http://ehs.unc.edu/ih/biological/microbio.shtml](http://ehs.unc.edu/ih/biological/microbio.shtml).


**Safety Equipment**

**Biohazard Labels**

Laboratory equipment used for BSL-2 containment is posted with the universal biohazard warning symbol (to communicate hazard to maintenance workers, visitors, etc.). This symbol is used to identify the actual or potential presence of a biological hazard on or in freezers, incubators, centrifuges, biological safety cabinets, etc. which are used with agents listed on Schedule F of the Laboratory Safety Plan. Biohazard warning labels may be printed on a color printer from the EHS website at: [http://ehs.unc.edu/ih/lab/labels/](http://ehs.unc.edu/ih/lab/labels/) or they may be ordered from [www.fishersci.com](http://www.fishersci.com), search for reorder # 18-999-934.

**Biological Safety Cabinet maintenance**

Because most biological safety cabinets (BSC) (aka “tissue culture hoods”) on campus recirculate HEPA filtered exhaust air into the laboratory environment, the cabinet should be tested and certified at least annually and operated according to manufacturer’s recommendations. Never use hazardous chemicals in these cabinets, the vapors bypass the HEPA filter to enter your breathing zone. Some BSCs on campus can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation should be verified annually. Only minute amounts of hazardous chemicals should be used in an exhausted cabinet.

To arrange for certification of your biological safety cabinet or to inquire about UNC covering the cost, [contact EHS](http://ehs.unc.edu/ih/biological/microbio.shtml).
**Biological Safety Cabinet use**

Biological safety cabinets (BSC) (aka “tissue culture hoods”) must be used whenever procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intra-nasally, and harvesting infected tissues from animals or eggs.

BSCs must be used whenever procedures with high concentrations or large volumes of infectious agents are conducted. Such materials should only be centrifuged in the open laboratory when sealed rotor heads or centrifuge safety cups are used.

**Centrifuge safety precautions**

Many activities associated with centrifuges may create significant amounts of infectious aerosol, including: filling centrifuge tubes; removing plugs or caps from tubes after centrifugation; removing supernatant; re-suspending sedimented pellets; breakage of tubes during centrifugation; and centrifugation itself. Follow these steps to prevent the generation of aerosols in centrifuges:

1. Routinely inspect the centrifuge to ensure there is no leakage.
2. Do not overfill centrifuge tubes.
3. Wipe the outside of the tubes with an appropriate disinfectant after they are filled and sealed.
4. Centrifuge inside a biological safety cabinet. If a biological safety cabinet is not available, internal aerosol containment devices (e.g., sealed canisters, safety cups or buckets with covers, heat sealed tubes or sealed rotors) should be used.
5. Remove aerosol containment devices and open them in a biological safety cabinet. If the biological safety cabinet is in use, a minimum of 10 minutes settling time should be allowed before opening.

**Lab coats**

Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use are worn while working in designated BSL-2 space. Protective clothing is removed before leaving for non-
laboratory areas (e.g., cafeteria, library, administrative offices). Protective clothing is disposed of appropriately, or deposited for laundering by the institution (see http://ehs.unc.edu/ih/lab/ppe.shtml). Laboratory clothing may not be taken home.

Chapter 5-7 of the general UNC Laboratory Safety Manual has more information regarding laboratory clothing, protective apparel and laundering and UNC.

**Eye & face protection**

Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection is disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories also wear eye protection.

Chapter 4 of the campus safety manual details UNC’s laboratory eye and face protection policy (http://ehs.unc.edu/manuals/ehsmanual/5-12.shtml)

**Gloves**

Special care is taken to avoid skin contamination at BSL-2. Gloves are worn to protect hands when handling experimental animals and when skin contact with the agent is unavoidable. Glove selection is based on an appropriate risk assessment. Alternatives to latex gloves are available. Gloves are not worn outside the laboratory. The general UNC Laboratory Safety Manual (Chapter 5-5) details requirements for the use of gloves in laboratories. In addition, BSL-2 laboratory workers should:

a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.

b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

**Respiratory protection with infected animals**

Eye, face and respiratory protection is used in rooms containing infected animals as determined by the risk assessment and EHS.
See the general UNC Laboratory Safety Manual Chapter 14 for safe handling of laboratory animals.

**Sharps precautions**

Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from lab animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used at BSL-2. Extreme caution is used when handling needles and syringes to avoid auto inoculation and the generation of aerosols during use and disposal. Needles are not to be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use.

Needles and syringes are promptly placed into appropriately labeled plastic sharps containers. Red containers are clearly marked as “Biohazardous Sharps” and autoclaved (remember to mark an “X” with autoclave tape directly over the biohazard warning label) prior to disposal or, if the sharps are not biohazardous, white or clear plastic containers are labeled "Nonhazardous Sharps".

When ordering the plastic sharps containers online from www.fishersci.com, please use catalog # 1482664B and/or 14830124. Labels for these containers can be obtained from http://ehs.unc.edu/labels/index.shtml scroll down to the heading, “Disposal of Laboratory Materials.”

For other biohazardous waste collection and disposal methods, a Disposal Chart is available at http://ehs.unc.edu/ih/biological/docs/disposal_chart.pdf.

Fisher Scientific is currently contracted by UNC to supply storerooms on campus. A Fisher representative can also help you find a product to meet your needs.

**Contact info:** Jeremy Crosson, Sales Representative
Fisher Scientific
919-673-6801
919-843-5604 UNC customer service
919-843-5605 fax
919-962-2160 Fisher chemistry storeroom
jeremy.crosson@thermofisher.com
www.fishersci.com
Special practices

Entry/exit requirements

All persons entering the designated BSL-2 area are advised of the potential hazards and they meet specific entry/exit requirements when manipulations involving materials from the Lab Safety Plan’s Schedule F (biological hazards) are taking place. These requirements are reviewed with new staff and the Schedule F is reviewed annually as part of the lab’s annual in-house training.

Before exiting the BSL-2 laboratory for non-laboratory areas, lab coats, gowns, smocks, and gloves are removed and left in the laboratory. All workers wash their hands after they de-glove and prior to exiting the lab after they handle materials involving viable material.

Medical surveillance/serum samples

Each laboratory must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel as appropriate. If applicable, this will be listed on the Lab Safety Plan’s Schedule F (biological hazards) under “Medical Surveillance.” For more information, contact EHS biological safety or the University Employee Occupational Health Clinic (http://ehs.unc.edu/ueohc/). Procedures for animal surveillance are discussed in Chapter 14-9 of the general UNC Laboratory Safety Manual.

Immunizations

Laboratory personnel are provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory. If applicable, this should be listed on the Lab Safety Plan’s Schedule F (biological hazards) under “Medical Surveillance.”

If your group is working with a known pathogen for which there is an effective vaccine, the vaccine should be made available to all workers. For more information, contact EHS biological safety or the University Employee Occupational Health Clinic (http://ehs.unc.edu/ueohc/).

Anyone in the lab working with (including treating waste) human blood or other potentially infectious material (including human cell lines, established, or primary) is required by the OSHA Bloodborne Pathogen standard to have, seek, or officially decline the Hepatitis B vaccination series. For questions regarding this and other vaccination series, please contact the University Employee Occupational Health Clinic. CDC Hepatitis B vaccination information is available at http://www.cdc.gov/ncidod/diseases/hepatitis/b/factvax.htm.

For more information about the risk of human tissue and the Bloodborne Pathogen Standard please refer to the EHS website at http://ehs.unc.edu/ih/biological/bbp.shtml.
Procedures for animal bites and immunizations are discussed in Chapter 14-6 of the general **UNC Laboratory Safety Manual**.

**Biosafety Manual**

The UNC Biosafety Manual and Exposure Control Plan has been adopted as laboratory policy and the Schedule F (Biological hazards) is reviewed with each worker at least annually during annual in-house training. A paper copy of the Biosafety Manual and Schedule F must be available and accessible to workers at all times and documentation of the annual in-house training is available in the lab safety binder.

The latest copy of the UNC Laboratory Exposure Control Plan is available on line at [http://ehs.unc.edu/ih/biological/bbp.shtml](http://ehs.unc.edu/ih/biological/bbp.shtml) (for now, print the General Version) and keep in your safety binder.

**Worker proficiency**

The Principal Investigator has the final responsibility for determining who may enter or work in the BSL-2 space and for advising persons of the potential hazard and entry requirements (e.g. immunization) for entry. The Principal Investigator has ensured that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents. At a minimum, this includes training in aseptic techniques and in the biology of the organisms used in the experiment so that the potential biohazards can be understood and appreciated. All procedures are performed carefully to minimize the creation of aerosols. Eating, drinking, smoking and applying cosmetics are not permitted in the work area. Food will be stored and consumed in designated areas (labels here) used for this purpose only. These requirements are reviewed with new staff and the Schedule F is reviewed annually as part of the lab’s annual in-house training.

For more information about animal handling technical proficiency, see the general **UNC Laboratory Safety Manual**, Chapter 14-3.

**Proper containerization**

Potentially infectious materials will be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility. A leak proof box, preferably equipped with a gasket seal lid, is used for transport of potentially infectious materials from one location to another on campus. This is particularly important when moving samples from patient care areas in UNC Hospitals to the lab. Containers such as igloo coolers or Rubbermaid containers will suffice provided they have enough absorbent material placed inside and a biohazard warning label on the outermost container.
**Routine decontamination**

Laboratory equipment and work surfaces are decontaminated once a day and after any spill of viable material. The appropriate disinfectant and recommended contact time is listed on the Lab Safety Plan’s Schedule F (biological hazards) under “Safety Precautions.”

The lab has determined how and where the decontamination of all cultures, stocks, and other potentially infectious materials will be performed before disposal. This is listed on your Lab Safety Plan’s Schedule F (biological hazards) under “Safety Precautions.” Refer to the EHS Biohazard Waste Management website for waste decontamination requirements. ([http://ehs.unc.edu/ih/biological/infectious_waste.shtml](http://ehs.unc.edu/ih/biological/infectious_waste.shtml))

Prior to repair, maintenance, or removal from the laboratory, equipment will be decontaminated.

**Spill kit & clean-up**

Workers are properly trained and equipped to contain, decontaminate, and clean up spills involving infectious material. The emergency plan that describes the procedures to be followed if an accident contaminates personnel or the environment is listed on the Lab Safety Plan’s Schedule F (biological hazards) under #8 “Emergency Procedures.” At a minimum, bleach is provided in the lab space and plenty of paper towels are available for spill clean-up purposes.

Refer to the EHS Biohazard Waste Management website for waste decontamination requirements. ([http://ehs.unc.edu/ih/biological/infectious_waste.shtml](http://ehs.unc.edu/ih/biological/infectious_waste.shtml)).

**Exposure incidents reported**

Incidents that may result in overt exposures to materials handled at BSL-2 will be immediately evaluated and treated according to procedures described in this manual. All such incidents must be reported to the laboratory supervisor, the University Employee Occupational Health Clinic (contact info: [http://ehs.unc.edu/ueohc/](http://ehs.unc.edu/ueohc/), and the Institutional Biosafety Committee.

**Pets & house plants excluded**

Animals and plants not associated with the work being performed are not permitted in the laboratory. This is also consistent with the Campus Safety Manual, Chapter 2.

**Aerosol generation precautions**

All procedures involving the manipulation of infectious materials that may generate an aerosol will be conducted within a biological safety cabinet (aka “tissue culture hood”) or other physical containment device.