**Laboratory Checklist for**

**Biological Safety Level 2 (BSL-2) Containment at NC State University**

Print PI Name:       PI Box#       BUA#

Each item on this checklist is a separate requirement under either federal, state, local or University regulations or requirements for work requiring containment at biosafety level 2 (BSL-2). The first two pages of this document serve as the “checklist.” The remaining pages provide a brief explanation of each item and links to more information. Upon request from Environmental Health and Safety or the Institutional Biosafety Committee, print and complete, sign, and return the first two pages to Box 8007. If you mark “NA” to a requirement, you must indicate why in the space provided. For help with your risk assessment or biosafety containment facilities, equipment, or practices, contact the EHS Biosafety Section at 515-6858, or 515-4190.

**Building Name:**       **Room number(s):**

 **(Complete/NA)**

 (For each line, if NA indicate why)

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Signature, Principal Investigator or designee Date

Who may EHS contact with questions about this form?

|  |  |  |
| --- | --- | --- |
|       | **\_**   **-**   **-**    **\_** |       |

Print name Contact phone Email address

*EHS Action*

\_\_\_\_\_\_\_Approved

\_\_\_\_\_\_\_Approved pending IBC approval

\_\_\_\_\_\_\_Not approved

\_\_\_\_\_\_\_Not approved, comments attached

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

EHS Biological Safety Officer Date

 **Forms, Documents, and Training**

This checklist is adopted from the CDC/NIH publication Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th edition located at <http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>, state and local medical waste requirements, and NC State University policies.

***Safety Plan & Biological Use Authorization (BUA) submitted***

The first step in designating space at NC State University for research with biological materials is notifying EHS of your intent to do so. This is done by submitting a Biological Use Authorization (BUA) form. The BUA is reviewed as a separate procedure from the Safety Plan. The BUA is reviewed by the Institutional Biosafety Committee. For general guidance on this registration and procurement process, refer to Chapter 1, Procedures Governing the Use of Biohazardous Agents in the [NCSU Laboratory Biosafety Manual](https://drive.google.com/open?id=0Bwfv9WVwZC73R3lRclA1RTlPSjQ) on the EHS website.

Access all current Safety Plan documents here: <https://ehs.ncsu.edu/safety-plan/> . Safety Plans are renewed annually.

***A Biosafety Manual has been adopted and is available***

Regulatory and funding agencies expect a laboratory-specific biosafety manual be prepared and adopted as policy. The manual must be available to workers at all times.

EHS provides a [Laboratory Biosafety Manual](https://drive.google.com/open?id=0Bwfv9WVwZC73R3lRclA1RTlPSjQ) on the EHS website that can be paired with an approved Biological Use Authorization (BUA) form to meet this requirement. Laboratories with work practices alternative to the Laboratory Biosafety Manual to minimize or eliminate exposures should include SOPs for such practices with their registration form (BUA) to the IBC.

***The new biosafety training requirements are met***

Biosafety training requirements have changed! If you haven’t already, review [this notification](https://drive.google.com/open?id=1kkJYmL6ON33lOiYJWyd1IUKgvt6n2qck). You may use the notification to forward the training links to your workers.

Online training sets standards across campus. Use the Statement of Informed Consent page in the Biological Use Authorization (BUA) form as a tool to document your review of biohazards with all laboratory workers including those handling and/or treating biohazard waste. This review can occur on an annual basis when the Safety Plan is updated for all hazards.

The PI is responsible for ensuring all training requirements are met and for managing records of safety training completion.

The EHS safety training website is <https://ehs.ncsu.edu/training/> .

***Bloodborne pathogens requirements met (including human cell lines)***

All workers exposed to human derived materials 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 <https://ehs.ncsu.edu/home-page-info/biological/bloodborne-pathogens/> ).

EHS has posted a general online Bloodborne Pathogens training to help PI’s manage this annual training requirement. After a worker completes the online self-study BBP training annually, they should retain the certificate of completion along with the current Exposure Control Plan which must remain accessible to workers at all times.

All workers to which the OSHA Bloodborne Pathogen standard applies should have, or officially decline, the Hepatitis B vaccination series. For questions regarding this and other vaccination series, refer to the “Access to Medical Surveillance Services and Consultation” section of the EHS website at <https://ehs.ncsu.edu/health-safety/occupational-health/medical-surveillance/> .

**Laboratory Facilities**

***Proper doors in place, closed while working***

Ensure laboratory doors are closable and have locks in accordance with the institutional policies. Self-closing doors are preferred. The Principal Investigator is ultimately responsible for the control of, and access to, laboratories where risk group 2 agents (refer to your BUA) are stored or manipulated.

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.

***Entryway signs posted***

Entryway signs for BSL-2 and BSL-3 are handled at NC State University through EHS. BSL-1 labs do not require biohazard sign/notification. Refer to Chapter 7 of the Laboratory Biosafety Manual for procedures on updating the door placards.

Although EHS supplies the approved laboratory entryway sign, the Principal Investigator has the final responsibility for assessing each circumstance of entry to the laboratory
and determining who may enter or work in the laboratory.

 ***Handwashing sink, soap, and paper towels***

The lab space designated at BSL-2 has a sink available for hand washing. The sink may be manually, 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 so that hands are washed after removing gloves and before leaving the BSL-2 area.

***Easily cleanable surfaces***

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 benchtops or flooring that may harbor microbes in the event of a spill.

To have repairs initiated in the work area, contact your Facility Liaison (for contact information by building refer to <https://facilities.ofa.ncsu.edu/facilities-liaisons/> )

***Proper benchtops***

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.

For repairs, contact Facility Liaison (see link above).

***Proper chairs located 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 openings to the exterior of the building fitted or repaired, contact your Facility Liaison (building contact information: <https://facilities.ofa.ncsu.edu/facilities-liaisons/> )

***Biological Safety Cabinet location***

Biological safety cabinets (BSC), (a.k.a. “tissue culture hoods”) must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. Refer to Chapter 6 of the Laboratory Biosafety Manual regarding placement considerations for your BSC and tips for effective use. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions. For questions or to arrange an appointment, contact the EHS Biosafety section.

Having a BSC moved to a new location requires the cabinet first be decontaminated so the movers can safely handle it. After placement in the new location, the BSC will need to be re-certified. Refer to the Laboratory Biosafety Manual and contact the Biosafety Officer.

***Filter in place on vacuum lines***

Vacuum lines located at biosafety cabinets 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 also applies to aspirating liquid at the benchtop.

To order, access your Marketplace account on your MyPack Portal (at <https://portal.acs.ncsu.edu/> ) and [www.fishersci.com](http://www.fishersci.com) ; search for part # 09-744-75 or # 09-744-76.

***Eyewash station meets University requirements***

To be compliant with BSL-2 standards at NC State University, eyewash stations must be plumbed units that meet the ANSI Standard Z358.1-2014 or latest edition. For information about the ANSI Standard and flushing your eyewash regularly, refer to the EHS Fact Sheet at

<https://drive.google.com/file/d/0B0zH_IZG0_wvQjNEakh5TE5CWGM/view> .

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.

***Emergency shower***

A shower facility, other than emergency drench hoses, must be located in the building. Refer to the EHS Safety Shower Fact Sheet at <https://drive.google.com/file/d/0B0zH_IZG0_wvVGN5R1pFVlk0ckE/view> . Ensure yours is checked/flushed annually (contact Facility Liaison).

 ***Proper ventilation***

There are no specific requirements on ventilation systems in BSL-2 labs at NC State University. 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.
 ***Biological Safety Cabinet exhaust***

Because most biological safety cabinets (BSC) at NC State University 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. More information about biological safety cabinets is available in the university Laboratory Biosafety Manual.

***Autoclave is available for biowaste***

An autoclave must be accessible to decontaminate all biowaste before disposal. If an autoclave is not accessible to your lab, contact EHS. Refer to the [EHS Biological Waste website](https://ehs.ncsu.edu/home-page-info/environmental-affairs/biological-waste/) and the Laboratory Biosafety Manual for waste collection requirements (use clear autoclavable bags and [red receptacles](https://drive.google.com/open?id=0B36FhbUXVzebNE5rd3BaOUh0WGM)) and decontamination requirements. Ensure autoclave operators are properly trained.

***Properly validating and documenting autoclave waste treatment***

When autoclaving waste, validating your autoclave’s performance is required under state law. Each load is to be tested and the test documented. 3M SteriGage Test Packs #41360 are the system accepted for this test at NC State University and are available through vendors on the MarketPlace. Logs should be kept for 3 years. For a sample log sheet and available Test Pack vendors, visit the EHS website at <https://drive.google.com/file/d/0Bwfv9WVwZC73UmVPa0t3dFlCZFk/view>

***Proper collection and disposal of liquid (culture) waste***

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 indicate this on your Biological Use Authorization. Select an appropriate disinfectant and ensure users are trained on the proper use and contact time of the product. For more disinfection information, refer to the Laboratory Biosafety Manual.

 **Safety Equipment**

***Biohazard labels posted on equipment***

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 biohazard on or in freezers, incubators, centrifuges, biological safety cabinets, etc. which are used with potential pathogen or human derived material. Biohazard warning labels may be
ordered through vendors on the MarketPlace. An example is [www.fishersci.com](http://www.fishersci.com), order # 18-999-934.

***Biological Safety Cabinet maintenance***

Certification is required annually for Biosafety Cabinets at NC State University. To arrange for certification of your biological safety cabinet contact the EHS Biosafety section.

***Biological Safety Cabinet properly used***

Biological safety cabinets (BSC) (a.k.a. “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 intranasally, 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 taken***

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 are worn and not taken home***

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 as arranged
by department. Laboratory clothing may not be taken home (see Lab Coat Cleaning and Disposal Guideline at <https://drive.google.com/open?id=0B36FhbUXVzebbHdCZzZjS2ZVMGM> ).

***Eye & face protection is available and worn***

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.

***Gloves are available and worn***

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 EHS [Hand Protection Guide](https://drive.google.com/open?id=0B36FhbUXVzebV2FHSEk1MDlISHM) details requirements for the use of gloves at NC State University. 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 in place with infected animals***

Eye, face and respiratory protection is used in rooms containing infected animals as determined by the risk assessment and EHS. (<https://drive.google.com/file/d/0B36FhbUXVzebbHdQZWtJYUxVVTA/view>

For more information on respiratory protection at NCSU, visit <https://ehs.ncsu.edu/health-safety/occupational-safety/respiratory-protection-programs/>

***Sharps precautions are observed***

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 biohazard sharps containers. The landfill should not receive plastic sharps containers from NC State. To request that your plastic sharps containers are collected, complete a collection request via EHSA online at [go.ncsu.edu/ehsa](http://go.ncsu.edu/ehsa).

Vendor contact information is available through MarketPlace to help you find disposal containers to meet your needs. Here is more information on [general sharps precautions from EHS website](https://drive.google.com/file/d/0B0zH_IZG0_wveGcwTHpmc3JHVlU/view).

**Special practices**

***Entry/exit requirements are established***

All persons entering the designated BSL-2 area are advised of the potential hazards and they meet any and all specific entry/exit requirements when manipulations involving materials from the BUA are taking place. These requirements are reviewed with new staff and the BUA is reviewed annually as part of the Supervisor’s Safety Self Assessment Checklist located within the Safety Plan.

Before exiting the BSL-2 laboratory, 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.

***Need for Medical surveillance/serum samples evaluated***

The laboratory has assessed the need for, and established necessary 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 BUA. For more information, refer to the EHS website for Occupational Health at <https://ehs.ncsu.edu/health-safety/occupational-health/medical-surveillance/>.

Procedures for animal surveillance are discussed at the “Animal Contact” section of the EHS website: <https://drive.google.com/file/d/0B36FhbUXVzebbHdQZWtJYUxVVTA/view> .

***Need for immunizations has been evaluated***

Laboratory personnel are provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory. If applicable, this is listed on the BUA.

If the lab group is working with a known pathogen for which there is an effective vaccine, the vaccine has been made available to all workers. For more information, refer to the EHS website for Occupational Health at <https://ehs.ncsu.edu/health-safety/occupational-health/medical-surveillance/>.

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, refer to the EHS website for Occupational Health at <https://ehs.ncsu.edu/health-safety/occupational-health/medical-surveillance/>. .

For more information about the risk of human tissue and the Bloodborne Pathogen Standard please refer to the OSHA interpretation letter titled Applicability of 1910.1030 to established human cell lines (06/21/1994) at <http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=INTERPRETATIONS&p_id=21519> .

Procedures for animal contact are available from the EHS website at <https://drive.google.com/file/d/0B36FhbUXVzebbHdQZWtJYUxVVTA/view> .

***Worker proficiency has been demonstrated to PI***

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 used for this purpose only. These requirements are reviewed with new staff and the BUA is reviewed annually upon completion of the Supervisor’s Safety Self Assessment Checklist for self-audit is to be completed annually and is located within the Safety Plan.

For more information about animal handling technical proficiency, refer to the EHS website at <http://www.ncsu.edu/ehs/www99/right/handsMan/animal/animal.html> .

***Proper containerization will be followed***

Materials listed on the BUA as requiring BSL-2 containment will be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within the building.

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. An example is moving samples between the lab and animal procedure areas. Containers such as igloo coolers or Rubbermaid containers will suffice provided they are lined with a Ziploc or plastic bag, have enough absorbent material placed inside, and are posted with a biohazard warning label on the outermost container.

***Routine surface disinfection and equipment decontamination is in place***

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 BUA.

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 the BUA. Workers are referred to the Laboratory Biosafety Manual for waste decontamination requirements at NC State University.

Prior to repair, maintenance, or removal from the laboratory, equipment will be decontaminated for the required contact time of an appropriate disinfectant.

***Spill kit & clean-up procedures are in place***

Workers are properly trained and equipped to contain, decontaminate, and clean up spills involving material that requires BSL-2 containment. The procedures to be followed if an accident contaminates personnel or the environment are listed in the Laboratory Biosafety Manual. At a minimum, bleach is provided in the lab space and plenty of paper towels are available for spill clean up purposes.

***Exposure incidents will be reported properly***

Incidents that may result in overt exposures to materials handled at BSL-2 will be immediately evaluated and treated according to procedures described at <https://drive.google.com/file/d/0Bwfv9WVwZC73OUlWSjhkeGJLNmM/view> . This document should be posted at or near locations where potentially infectious material is handled such as at the biosafety cabinet. All such incidents including any incidents involving recombinant DNA must be reported to the Biosafety Officer and Institutional Biosafety Committee in addition to completing the First Report of Injury.
 ***Pets & house plants are excluded from BSL-2 area***

Animals and plants not associated with the work being performed are not permitted in the BSL-2 laboratory.

***Aerosol generation precautions are taken***

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