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	Laboratory Checklist - Biological Safety Level 2 (BSL-2)			FM-0089	15-072
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Laboratory Checklist Biological Safety Level 2 (BSL-2)

PI Name (Print): _____


Biosafety Protocol (FM-0088) # _____

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," which ensures the PI's facilities meet BSL-2 standards for their project. The IBC cannot approve a project if the PI does not provide evidence of meeting these standards. The remaining pages provide a brief explanation of each item and links to more information. After completing the form, send the first two pages only to the BioSafety Officer at atdjhurley@northcarolina.edu. If you mark "N/A" to a requirement, you must indicate why in the comments. For help with your risk assessment or biosafety containment facilities, equipment, or practices, contact the DHMRI Biosafety Officer at 704-250-5056.

Building Name: _____

Room number(s): _____

	Yes	No	N/A	Item	Comments
1.0	Forms, Documents, and Training				
1.1	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Biosafety Protocol (FM-0088) submitted	
1.2	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	A Biosafety manual has been adopted and is available	
1.3	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	BSL-2 and rDNA training requirements met	
1.4	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Bloodborne pathogens requirements met (including human cell lines)	
2.0	Laboratory Facilities				
2.1	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Proper doors in place, closed while working	
2.2	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Entryway signs posted	
2.3	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Handwashing sink, soap, and paper towels	
2.4	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Easily cleanable surfaces	
2.5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Proper benchtops	
2.6	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Proper chairs located at biological safety cabinets	
2.7	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Proper windows	
2.8	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Biological Safety Cabinet location	
2.9	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Filter in place on vacuum-lines	
2.10	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Eyewash station meets requirements	
2.11	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Emergency shower	
2.12	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Proper ventilation	
2.13	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Biological Safety Cabinet exhaust	
2.14	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Autoclave is available for waste	
2.15	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Properly validating and documenting autoclave waste treatment	
2.16	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Proper collection and disposal of liquid (culture) waste	

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	Yes	No	N/A	Item	Comments
3.0	Safety Equipment				
3.1	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Biohazard labels posted on equipment	
3.2	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Biological safety cabinet maintenance	
3.3	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Biological safety cabinet properly used	
3.4	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Centrifuge safety precautions taken	
3.5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Lab coats are worn and not taken home	
3.6	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Eye & face protection is available and worn	
3.7	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Gloves are available and worn	
3.8	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Respiratory protection in place with infected animals	
3.9	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Sharps precautions are observed	
4.0	Special Practices				
4.1	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Entry/exit requirements are established	
4.2	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Need for medical surveillance/serum samples evaluated	
4.3	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Need for immunizations has been evaluated	
4.4	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Worker proficiency has been demonstrated to PI	
4.5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Proper containerization will be followed	
4.6	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Routine surface disinfection and equipment decontamination followed	
4.7	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Spill kit & clean-up procedures are in place	
4.8	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Exposure incidents will be properly reported	
4.9	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Pets & house plants excluded from BSL-2 area	
4.10	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Aerosol generation precautions are taken	

Signature, Principal Investigator or designee

Date

Who may the Biosafety Officer contact with questions about this form?

Print name

Contact phone

Email address

Biosafety Officer Action

Approved


Approved pending IBC approval

Not approved

Not approved, comments attached

Biosafety Officer

Date

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1.0 FORMS, DOCUMENTS, AND TRAINING

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

1.1 Biosafety protocol submitted

The first step in designating space at DHMRI for research with biological materials is notifying the Biosafety Officer of your intent to do so, which is done by submitting a Biosafety Protocol (FM-0088). The Biosafety Protocol is reviewed in a separate procedure from the Safety Plan by the IBC.

1.2 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.

The NCRC Laboratory Biosafety Manual can be paired with an approved Biosafety Protocol (FM-0088) to meet this requirement. However, laboratories with work practices alternative to the NCRC Biosafety Manual must include SOPs for such practices in their Biosafety Protocol submission.

1.3 BSL-2 and rDNA training requirements met

The PI is responsible for ensuring all training requirements are met and documented. The DHMRI IBC will accept documentation that the research team has completed from the home institution as evidence of training.

1.4 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)


The DHMRI IBC will accept documentation that the research team has completed from the home institution as evidence of training.

All workers to which the OSHA BBP standard applies should have, or officially decline, the Hepatitis B vaccination series. PI's submitting protocols to the DHMRI IBC are responsible for obtaining this vaccine for their research staff working on the submitted protocol. DHMRI supplies this vaccine for DHMRI employees only.

2.0 LABORATORY FACILITIES

2.1 Proper doors in place, closed while working

Ensure laboratory doors are closable and have locks; self-closing doors are preferred. The PI is responsible for the control of, and access to, laboratories where Biological Agents

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requiring BSL2 facilities and practices (refer to your Biosafety Protocol) 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.

2.2 Entryway signs posted

Entryway signs must be posted for all BSL-2 labs. BSL-1 labs do not require biohazard sign/notification.

Although the Biosafety Officer supplies the approved laboratory signage, the PI has the final responsibility for assessing each circumstance of entry to the laboratory and determining who may enter or work in the laboratory.

2.3 Handwashing sink, soap, and paper towels

Any BLS-2 designated lab space must have 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. Ideally, the sink is located near the exit door so that hands are washed after removing gloves and before leaving the BSL-2 area.

2.4 Easily cleanable surfaces

The laboratory should be designed so that it can be easily cleaned and decontaminated. 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.

2.5 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.


2.6 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 an appropriate disinfectant. Cloth covered chairs are not permitted. The chairs must be capable of supporting anticipated loads and uses. This practice should be applied to chairs at lab benches too.

2.7 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.

2.8 Biological Safety Cabinet location

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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. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions. Biological safety cabinets and work best, and offer the most worker protection, when the doors to the laboratory are closed.

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. For questions regarding BSC placement or recertification, contact the Biosafety Officer.

2.9 Filter in place on vacuum lines

Vacuum lines located at BSCs 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 applies to aspirating liquid at the BSL-2 benchtop.

The following Fisher Scientific products are examples: part # 09-744-75 or # 09-744-76.

2.10 Eyewash station meets OSHA requirements

All BSL-2 laboratory eyewash stations must be plumbed units that meet the ANSI Standard Z358.1-2004 or latest edition. (Personal eye flush squeeze bottles do not meet ANSI requirements)

A deck-mounted unit installed at an existing sink is also an option. The following Fisher Scientific deck mount swivel eyewash stations are examples: part # S67330 and # S67331.

2.11 Emergency shower


A shower facility, other than emergency drench hoses, must be located in the building and must be checked/flushed monthly.

2.12 Proper ventilation

There are no specific requirements on ventilation systems in BSL-2 labs at DHMRI. 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.

2.13 Biological Safety Cabinet exhaust

If the lab’s BSCs re-circulates 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.

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Some BSCs 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 must be verified annually.

2.14 Autoclave is available for waste

An autoclave must be accessible to decontaminate all biohazard waste before disposal. If an autoclave is not accessible to your lab, contact the Biosafety Officer. Ensure autoclave operators are properly trained and documentation of training is available.

2.15 Properly validating and documenting autoclave waste treatment

Validating autoclave performance is required under state law when autoclaving biohazardous waste. You are required to submit your laboratory SOP for autoclave validation as part of the Biosafety Protocol.

2.16 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 in the Biosafety Protocol submission. Select an appropriate disinfectant and ensure users are trained on the proper use and contact time of the product.

3.0 SAFETY EQUIPMENT

3.1 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 Fisher Scientific (part # 18-999-934).

3.2 Biological Safety Cabinet maintenance


Annual certification is required for Biosafety Cabinets.

3.3 Biological Safety Cabinet properly used

BSCs 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, intranasal inoculation of animals, 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.


3.4 Centrifuge safety precautions taken

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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 recommended steps to prevent the generation of aerosols in centrifuges:

- 3.4.1 Routinely inspect the centrifuge to ensure there is no leakage.
 - 3.4.2 Do not overfill centrifuge tubes.
 - 3.4.3 Wipe the outside of the tubes with an appropriate disinfectant after they are filled and sealed.
 - 3.4.4 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.
 - 3.4.5 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.
- 3.5 Lab coats are worn and not taken home
- Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working in designated BSL-2 space. Protective clothing must be removed before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Protective clothing is disposed of appropriately, or deposited for laundering. Laboratory clothing may not be taken home.
- 3.6 Eye & face protection is available and worn
- Eye and face protection (goggles, mask, face shield or other splatter guard) are 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. Anyone wearing contact lenses in laboratories must also wear eye protection.
- 3.7 Gloves are available and worn
- Gloves must be worn at BSL-2 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 must be made available. Gloves cannot be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
- 3.7.1 Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.

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- 3.7.2 Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
- 3.7.3 Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

3.8 Respiratory protection in place with infected animals

Eye, face and respiratory protection are used in rooms containing infected animals as determined by the Biosafety Officer.

The PI is responsible for:

- 3.8.1 Providing respirators when required.
- 3.8.2 Ensuring that research staff using respirators are medically evaluated and fit tested annually.

Your servicing EHS department can provide assistance in identifying respirators appropriate for the exposure anticipated, in identifying medical providers to review respirator medical questionnaires, and respirator fit testing

Servicing EHS Department	
Building	Servicing EHS Department
NRI	UNC EHS
PHHI	NCRC EHS
CORE LAB	NCRC EHS


3.9 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 must be taken 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.

4.0 SPECIAL PRACTICES

4.1 Entry/exit requirements are established

All persons entering designated BSL-2 areas are advised of the potential hazards and they meet any and all specific entry/exit requirements when manipulations involving materials from the Biosafety Protocol. These requirements are reviewed with new staff and the Biosafety Protocol is reviewed annually.

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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.

4.2 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 Biosafety Protocol.

The DHMRI Occupational Health Program (SOP-0267) for animal handlers is a requirement for entry into the CLAS facility. The Biosafety Protocol submission should identify the need for additional medical surveillance, if necessary.

4.3 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 medical surveillance and immunization are required by the PI's risk assessment they will be listed on the Biosafety Protocol (FM-0088).

If the lab group is working with a known pathogen for which there is an effective vaccine, the vaccine must be made available to all workers.

Anyone in the lab working with BBP or OPIM (including treating waste) are required by the OSHA standard to have, or officially decline the Hepatitis B vaccination series.


4.4 Worker proficiency has been demonstrated

The PI 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 PI must ensure 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. 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 Biosafety Protocol is reviewed annually.

For more information about animal handling technical proficiency, please contact the CLAS Attending Veterinarian.

4.5 Proper containerization will be followed

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

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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.

4.6 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 with the Biosafety Protocol submission.

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 also listed on the Biosafety Protocol.

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

4.7 Spill kit & clean-up procedures are in place

Workers are properly trained and equipped to contain, decontaminate, and clean up spills involving BSL-2 material. The procedures to be followed if an accident contaminates personnel or the environment must be listed in the Biosafety Protocol submission.


Minimally, a spill clean-up kit must include:

- Nitrile gloves
- Housekeeping gloves (vinyl heavy duty)
- Kevlar gloves and sleeve protectors
- Shoe covers (2)
- 1 Gallon of bleach, 5.25% sodium hypochlorite-dated and replaced as required
- Plastic dustpan
- Large sponges (2)
- Autoclave bags (2)
- Spill control pads (2)
- Spill control booms (2)

If you are bringing biohazardous agents into the DHMRI CLAS you are expected to provide the appropriate spill clean-up materials for your project.

4.8 Exposure incidents will be reported properly

Any incident that may result in exposures to materials handled at BSL-2 (including recombinant DNA) will be immediately reported and evaluated by the BioSafety Officer and the IBC.

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4.9 Pets & house plants are excluded from BSL-2 area

Animals and plants not associated with the project are not permitted in the BSL-2 laboratory.

4.10 Aerosol generation precautions are taken

All procedures involving the manipulation of infectious materials that may generate an aerosol will be conducted within a BSC or other physical containment device.

REVISION HISTORY		
Superseded Revisions	DCO Number	Effective Date
N/A	15-072	20FEB2016
Current Revision:	R1.0	
Section Number	Description of Changes	Justification of Changes
All	Form used to capture safety information regarding BSL-2 project as part of SOP-0207	New DHMRI document