



Biosafety Level 2 Standard Practices and Containment

Office of Research Safety Affairs



Purpose and Objectives

Purpose

- To promote the safe conduct of research with biohazardous materials known to present a moderate threat to human health.
- Comply with training requirements established by the CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) and the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines).

Objectives

Upon completing this course you should be able to:

- Obtain hazard information and the exposure response procedure for the BSL2 agents that you handle.
- Identify containment requirements necessary for work at BSL2.
- Perform work practices required for work at BSL2.
- Describe the role of the Institutional Biosafety Committee (IBC).

Hazard Specific Checklist

- Guides supervisors through BSL2 agent specific training.
- Ensures communication of hazards, precautions and response activities.
- Provides researchers and opportunity to ask questions.
- Documents that training occurred.

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Handling BSL2 Agents – Hazard Specific Training Checklist

Biological materials with a moderate potential to hazard to personnel and the environment are typically handled under BSL2 precautions. The CDC BMBL and NIH Guidelines require that personnel handling agents at BSL2 be trained to work safely with the hazardous agents they handle. Given the potential hazardous agents and exposure risks that vary from project to project the Principle Investigator is typically considered the appropriate subject matter expert to provide their personnel with this training.

This checklist is intended to guide Principle Investigators through the necessary information that must be included when training their personnel. The actual training content can be communicated by whatever means is effective (verbally, written, video) provided it addresses each of the nine elements below while providing each researcher with an opportunity to have questions answered about the risks, precautions, and safe work practices for the activities that they will perform.

Identify the biological agents to be handled under BSL2 precautions.				
If applicable, identify IBC Protocols with which the agent is associated.	1.	2.	3.	4.
	5.	6.	7.	8.

Training Element <i>(Supervisor: Provide information for each item below.)</i>	PI Initials	Researcher Initials
1. Health hazards associated with exposure to BSL2 agent.		
2. Symptoms of exposure to BSL2 agent.		
3. Availability of vaccines or medical surveillance.		
4. Required PPE (e.g. gloves, respirator, face-shield, lab coat, etc.), when it must be worn, how PPE is to be maintained, and limitations of its use.		
5. Engineering controls (e.g. biological safety cabinet) to be used and when.		
6. How to respond in case of a possible exposure and who to report it to.		
7. Review of SOPs and, if applicable IBC protocols in their entirety.		
8. Decontamination methods for surfaces, liquid waste, and equipments and lab coats* <small>*Lab coats MUST be autoclaved prior to being taken to any laundry facility (in-house or vendor).</small>		
9. Waste handling procedures.		

By initialing and signing this form the PI and staff member certify that they have either provided or received the information requested above.

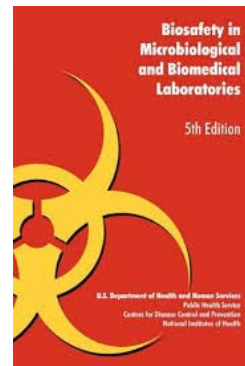
Principle Investigator: _____
Print Name Signature and Date

BMBL

International
Biohazard Symbol



CDC
Biosafety in Medical and
Biological Laboratories
(BMBL)



Pathogen Risk Groups

	Risk Group 1	Risk Group 2	Risk Group 3	Risk Group 4
Characteristics	Does not cause disease in healthy adults	Can cause infection of varying severity; Rarely lethal	Agents associated with moderate to severe disease outcome; Can be lethal	Capable of causing severe disease with lethal outcome
Availability of Treatment	Not applicable	Treatment usually available or host immune system is capable of controlling the infection	Treatment may not be available	Treatment is generally not available; Experimental treatment regimens possible
Routes of Transmission	Not applicable	Ingestion, through the skin and via facial mucous membranes	Same as Risk Group 2 plus inhalation	Same as Risk Group 3
Disease Severity to Individual	None in healthy adults	Low to moderate	Moderate to high; Higher mortality and morbidity	High; Highest mortality rates in this category
Community Risk	Low	Low	Low to moderate	High
Infectious Dose	Not applicable	Generally high (variable)	Lower doses capable of infection	Can be as low as 1 organism
Example Agents	Non-conjugative strains of <i>E. coli</i> , rodent cell lines, <i>Saccharomyces cerevisiae</i>	Parasites (<i>Plasmodium</i>, <i>Trypanosomes</i>, <i>Leishmania</i>) GI pathogens (<i>Salmonella</i>, <i>Shigella</i>) Bloodborne Pathogens (HBV, HCV, <i>Borrelia</i>)	<i>Mycobacterium tuberculosis</i> , West Nile Virus, Yellow Fever Virus, <i>Rickettsia rickettsii</i>	Ebola virus, Marburg virus, <i>Sabia virus</i> , Equine Morbillivirus
Gwladys Caspar's Quick Guide and Associated Safe Practices	Don't drink it Never eat, drink, or smoke in the laboratory	Don't touch it Wear gloves, decontaminate work surfaces, avoid touching your face, make sure wounds are covered, wear face protection, and work behind a shield	Don't breathe it Because of inhalation risk, perform all work inside of a biosafety cabinet; Wear respiratory protection if needed	Don't do it (in your state unless you have a federally approved BSL4 laboratory); Risk Group 4 agents require significant containment

	Biosafety Level 1	Biosafety Level 2	Biosafety Level 3	Biosafety Level 4
Practices	Basic foundational work practices	Level 1 practices plus safe sharps work practices	Level 2 work practices, with all work performed inside primary containment devices.	Same as Level 3
Protective Clothing	Gloves and Lab coat recommended	Gloves and lab coat required; Face protection added if potential for splash or splatter	Same as Level 2 plus additional respiratory protection.	Supply airline respirator and fully encapsulating protective suit (Suit laboratories)
Containment Equipment	None required	Biosafety cabinet to contain aerosols based on risk assessment; Centrifuge safety cups and other primary containment may be required if aerosols are generated.	Primary containment equipment such as biosafety cabinet required.	Configuration of BSL4 is referred to as a Cabinet lab. All work performed in sealed glove boxes
Lab Design Features	General lab, easy to clean surfaces, sink, and door	Same as Level 1, with possible requirements of controlled airflow into the lab and a biosafety cabinet for aerosol containment	Same as Level 2 with additional requirement of controlled airflow into the lab, dedicated HVAC system, no recirculation of exhaust, airflow monitoring devices at entry, two-door separation from general traffic, and fan failure alarms.	Same as Level 3, with many more advanced features; Level 4 is a building within a building approach; All systems for lab separated from non Level 4 areas
Other			HEPA filters for exhaust air may be required.	Double HEPA filtered exhaust air; HEPA filtered supply air; Effluent decontamination system

BSL2 practices



BSL2 FACILITY REQUIREMENTS

BSL2 Facility Requirements

Pathways for Biohazardous Agents:


- Transported with researchers
- Plumbing
- Air currents



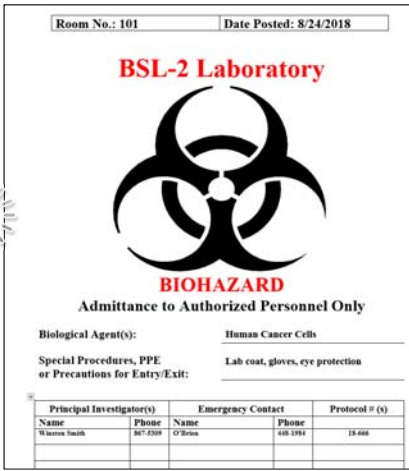
Restricted Access 


- Door should remain closed.
- Unauthorized personnel should not enter.
- Be aware of the risk of contamination within the room.



Warning Sign 

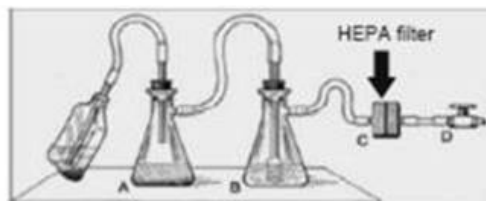
- Must be posted at entrances to BSL2 work areas.
- Identifies the following:
 - Hazardous agents
 - Responsible individuals
 - Necessary precautions for entry



Room No.: 101		Date Posted: 8/24/2018	
BSL-2 Laboratory			
			
BIOHAZARD			
Admittance to Authorized Personnel Only			
Biological Agent(s):		Human Cancer Cells	
Special Procedures, PPE or Precautions for Entry/Exit:		Lab coat, gloves, eye protection	
Principal Investigator(s)		Emergency Contact	
Name	Phone	Name	Phone
Warner Smith	907-2107	G Brown	606-2104
			18-466

Building Vacuum Line Filters

- Use filters to protect building vacuum system.
- 1.0 μm or smaller pore size filter.
- Available through General Stores in the GEB.




Vacuum Line HEPA Filter (.22um pore size)	Vacuum Line HEPA Filter (.3um pore size)
Fisher Cat. # slfg05010	Fisher Cat. # 09-744-79

No plants or animals







Equipment

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- Label equipment with biohazard symbol.
- Use equipment with safety cups or sealed rotors.
- In case of breakage or spill follow response procedure.

Safety Cups	Sealed Rotors
	
	

Biosafety cabinet for aerosols

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- BSL2 work that may generate aerosols must take place in a biosafety cabinet (BSC).
- Disinfect surfaces before and after use.
- Must be certified annually.






BSL2 STANDARD PRACTICES

Standard Practices

Review of Standard Microbiological Practices for BSL 1

- 
- Proper technique for donning and doffing gloves.
 - Wash hands after removing gloves.
 - No eating, drinking of food stored in lab.
 - No mouth pipetting.
 - ***Use sharps handling precautions.***
- Minimize splash and aerosol creation.
 - Decontaminate work surfaces at least once a day or after spills.
 - Decontaminate stocks and cultures before disposal.
 - Complete training and immunizations if applicable.

BSL2 Standard Practices



Training and Supervision

- Complete training.
- Review Institutional Biosafety Committee (IBC) protocols for the work that you perform.
- Work must be supervised by competent scientists.



BSL2 Standard Practices



Routine Disinfection

- Decontaminate surfaces and equipment after completion of work.
- Equipment must be decontaminated before removal from the laboratory.
- Transport materials in durable, leakproof container.
- Disinfect liquids (30 min.) prior to drain disposal.
- Potentially contaminated materials discarded as infectious waste in red or orange bags. (**only solids**)
- **Do not use ethanol to disinfect surfaces!**



Sharps Handling

- Do not use bent, sheared, or broken needles.
- Do not recap needles.
- Used needles discarded in puncture resistant, FDA approved sharps container.
- No direct handling of broken glass.
- Secure sharps including razor blades, scalpels and similar materials when not in use.



BSL2 Standard Practices

Aerosol Generating Procedures

- Aerosol or splash procedures must be conducted in BSCs or physical containment.
- This includes:
 - Pipetting,
 - Centrifuging,
 - Sonicating
 - Mixing
 - Shaking
 - Inoculating animals intranasally
 - Loading or using syringes
 - Opening containers of infectious materials



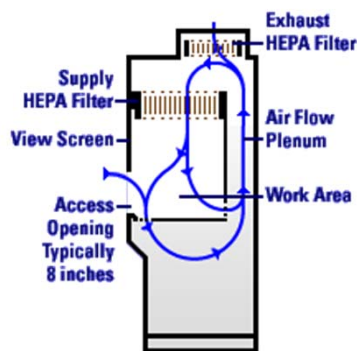
Using a Biosafety Cabinet



Biosafety Cabinet Performance

Effective performance depends on:

- Proper airflow.
- Integrity of High Efficiency Particulate Air (HEPA) filters.
- Work practices:



Using a Biosafety Cabinet



Proper Use

- Check sticker for certification (must be within last 12 months)
- Turn on blower and wait ≥ 5 minutes.
- Decontaminate the sides, sash, and work surface by wiping with a suitable decontaminant.



Yearly
Certification

Using a Biosafety Cabinet



Biosafety Cabinet Work Practices

- ***Do not block the front grill!***
- Work at least 4 inches from front grill.
- Enter BSC straight on using smooth hand and arm movements.
- Wear PPE while at the cabinet.
 - Gloves
 - Lab coat
 - Eye protection



As required by the CDC BMBL

BSL2

PERSONAL PROTECTIVE EQUIPMENT

PPE

Lab Coat

- Lab coats (or equivalent) must be worn.
- Lab coat must be closed (i.e. buttoned)
- Remove before leaving work area or dispose of appropriately
- Tucked sleeves into the top of gloves.



PPE

Gloves

- Gloves must be worn.
- Discard as infectious waste after use.
- Do not re-use gloves.
- Remove gloves before leaving the lab.



Eye Protection

- Eye protection always required when handling hazardous chemicals.
- Eye protection required when splash or spray may be reasonably anticipated.
- Face shield when handled large volumes (>4 liters)
- Decontaminate face and eye protection after use or discard as contaminated waste.



BIOHAZARDOUS WASTE DISPOSAL

Packaging Infectious Waste for Transport

Step 1:
Get Stericycle box and tape the bottom closed
(Make sure arrows are pointing upwards)



Step 2:
Place red liner bag into the box



Step 3:
Place closed biohazards waste bag or closed sharp container into liner inside the box



Step 4:
Once box is full close the box liner and tape the box closed



Step 5:
Place the shipping label on the appropriate area on the box



Step 6:
Place the box in the designated area for Stericycle to pickup



Note:

- All waste bags and sharps containers must have the PI name and room number written on them.
- Do not leave sharps containers on the floor.
- Do not obstruct the door, stack full boxes.



INCIDENT RESPONSE

Medical Surveillance

- Medical surveillance may be appropriate and available.
- Occupational Health is located in 910 Madison Ave.
- Immunizations may be available for some infectious agents.



Exposure Response

Be familiar with the following:

- Symptoms of exposure.
- First aid/exposure response practices.
- Incident reporting procedure.
- Where to obtain follow-up medical care.



Exposures



Incident Reporting

Exposure must be evaluated and treated immediately.



- Provide first aid or emergency treatment.
- All incidents must be reported lab supervisor.
- Call Corvel 24/7 nurse line at 1-866-245-8588.

BSL2 Spill Response



In the event a spill of a BSL2 material:

- Exit the spill area
- Close the door
- Post warning sign
- Wait 30 minutes for aerosols to settle
- Return wearing PPE
- Clean spill by:
 - Covering with paper towels or rags
 - Pouring 1-10% solution of household bleach.
 - Waiting 10 minutes for bleach to work.
 - Discard material as infectious waste.
 - Use tongs to handle broken glass and sharp materials.



<https://www.youtube.com/watch?v=aGrPG3zla2I>

(Click link to watch video)

Quiz



Questions?

Email labsafety@uthsc.edu or call
448-6114.