

# Laboratory Biological Safety Manual And Standard Operating Procedure Guidance Document

## **What information is needed in a laboratory standard operating procedure?**

Well developed Standard Operating Procedures (SOPs), or Standard Laboratory Practices, are essential tools for any laboratory that manipulates biological research materials. SOP's serve as a resource to train new lab staff, supplement recurrent training curriculum, and as a valuable reference in the event of an emergency. The following components should be considered when establishing *minimum* best practices in a research laboratory. However, the value of an SOP only holds merit if it is implemented by all laboratory workers and enforced by the Principle Investigator.

### **Purpose**

What is the overall purpose of the SOP?

As an example:

*This SOP has been developed to outline the hazards involved with research using lentiviral vectors and how to safely manipulate these materials to avoid any lab acquired infection (LAI).*

### **Principal Investigator Responsibilities**

The Principal Investigator (PI) has the primary responsibility for ensuring that their laboratory is safe through establishment of the initial risk assessment, administrative controls, and by ensuring that all work is conducted with appropriate engineering controls. PI's must adhere to all applicable guidelines and regulations. The PI is responsible for the safe use of biological agents in their laboratory.

### **Laboratory Staff/Student Responsibilities**

The laboratory staff and students are responsible for knowing the potential hazards contained within their respective work areas, in particular the biological material and appropriate procedures and practices to be used in the laboratory. Laboratory employees must follow approved laboratory procedures and safety guidelines at all times. For information regarding minors working in laboratory areas, please contact EHS.

### **General Emergency Contact Information**

The first page of the SOP should include the following information so that it is quickly and easily accessible:

- Emergency Contact Information for:
  - Principle Investigator -
  - Lab Supervisor(s) -
  - Environmental Health & Safety (EHS) – 301-846-1451
  - Occupational Health Services (OHS) - 301-846-1096
  - Protective Services – 301-846-1091 (to be used after regular business hours)
- Locations of:
  - Fire Alarms
  - Fire extinguishers
  - Eyewashes (Note: eyewashes should be flushed monthly)

- Emergency Showers (Note: showers should receive maintenance every 6 months)
- Scrub Kits (Chlorhexidine and Betadine) - available from EHS

### **Principles of Biosafety**

According to the Center for Disease Control publication, Biosafety in Microbiological & Biomedical Laboratories (BMBL) 5<sup>th</sup> edition, a fundamental objective of any biosafety program is the effective management of potentially harmful biological agents through the use of administrative and engineering controls such as containment. Administrative controls are management tools that provide staff with a set of guidelines describing how to safely operate with biological hazards in an environment designed for containment of the hazard. The term "containment" is used in describing safe methods, facilities, and equipment for managing infectious materials in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents. The use of vaccines may provide an increased level of personal protection. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements.

### **Biological Risk Assessment**

(refer to [http://www.cdc.gov/od/ohs/biosfty/bmbl5/BMBL\\_5th\\_Edition.pdf](http://www.cdc.gov/od/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf))

Risk assessment is a process used to identify the hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a laboratory acquired infection (LAI), and the probable consequences of such an infection. The information identified by risk assessment will provide a guide for the selection of appropriate biosafety levels and microbiological practices, safety equipment, and facility safeguards that can prevent LAIs. The risk assessment will determine the biological safety containment level (BSL) at which the work can be safely conducted.

A risk assessment will identify the following:

- Pathogenicity of material: disease incidence and severity
- Route of transmission: parenteral, airborne or ingestion
- Agent stability-how easily the material can be decontaminated
- Infectious Dose
- Concentration of working quantities and infectious organisms per ml of stock solution
- Origin of material
- Availability of prophylaxis (vaccination)
- Medical surveillance programs and exposure management (post-exposure prophylaxis)
- Staff skill level and training

Additional risk assessment questions:

- Are procedures being used that may produce an aerosol?
- Are procedures being used that may use needles or sharps?
- Are cultures or cell concentrates purified?
- Are larger volumes being used (>10Liters)
- Has the research material been altered, if so, how, and how does that affect the hazards associated with the material?

- Is the material attenuated (to reduce the virulence/infectious nature of the material)?
- How could an exposure event occur?

### **Biological Safety Containment Levels**

(refer to Section IV of [http://www.cdc.gov/od/ohs/biosfty/bmbl5/BMBL\\_5th\\_Edition.pdf](http://www.cdc.gov/od/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf))

While the majority of the NCI-F biological research laboratories are considered Biosafety Level 2 (BSL2), BSL2-enhanced or BSL3, a general understanding of the different biosafety levels and their relevant best practices is recommended.

### **BSL-1, BSL-2, and BSL-3**

**Biosafety Level 1** is suitable for work involving well-characterized agents not known to consistently cause disease in immuno-competent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

**Biosafety Level 2** builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets (BSCs) or other physical containment equipment.

**Biosafety Level 3** is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

**Spills in the Laboratory** - Call EHS when a significant spill occurs and if assistance is needed for clean-up.

Steps to cleaning-up a biological spill:

- Post the area where the spill occurred to avoid the potential for cross contamination and unnecessary exposure to others in or near the work area.
- Since a spill has the potential to generate an aerosol, let the aerosol settle (~30min).
- Put on a clean lab coat and gloves and obtain or prepare fresh disinfectant solution for cleaning-up the spill (freshly prepared 10 % bleach solution or EPA-Approved Disinfectant). (Refer to EHS Safetygrams ISM-161 <http://home.ncifcrf.gov/ehs/uploadedFiles/IsM-161.pdf> and ISM-208 [http://home.ncifcrf.gov/ehs/uploadedFiles/ISM-208%20\(2\).pdf](http://home.ncifcrf.gov/ehs/uploadedFiles/ISM-208%20(2).pdf) )

- Lay paper towels or other absorbent material on top of the spill zone
- Starting from the outermost edge of the spill and working in toward the center of the spill, pour disinfectant onto the absorbent material and allow sufficient contact time to render any contaminants inactivated. Contact time is typically 10 to 30 minutes and should be noted on the bottle of disinfectant.
- Properly dispose of waste (including your gloves) in appropriate waste containers for final disposal.

### **Decontamination and Virus Inactivation**

(Refer to EHS Safetygrams ISM-145 <http://home.ncifcrf.gov/ehs/uploadedFiles/ISM-145.pdf> and ISM-208 [http://home.ncifcrf.gov/ehs/uploadedFiles/ISM-208%20\(2\).pdf](http://home.ncifcrf.gov/ehs/uploadedFiles/ISM-208%20(2).pdf) )

Laboratories should have specific SOP's for working with and effectively decontaminating and/or inactivating the following materials:

- HIV and other lentiviruses
- Adenovirus
- Viral vectors (Adeno-, Retro-, Lenti-, etc.)
- All human materials
- Prions
- Recombinant DNA/RNA
- Genetically modified animals
- Biological toxins
- All other BSL-2 and BSL-3 infectious materials
- Any other biological hazards

### **Training**

Laboratory staff should have both instructional and hands-on training for all biological hazards present in the laboratory. Technicians should demonstrate proficiency in techniques before being permitted to perform laboratory procedures independently. EHS provides health and safety training courses, not limited to the following:

- Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard 29 CFR 1910.1030
- Adenovirus and adenoviral vectors
- Lentivirus and lentiviral vectors
- Diphtheria toxin
- Toxoplasma gondii
- Listeria monocytogenes

Laboratory-specific training should be provided by the PI, lab manager or senior scientist who has several years experience working with the biological materials and can direct staff in safe handling of the materials so as to avoid any accidental exposures. All training sessions should be documented, to include the training session topic, information covered, instructor, date, and attendees. Depending on the significance of the hazards involved, curriculum training and proficiency testing may be warranted.

### **Medical Surveillance**

Depending on the biological materials manipulated in the laboratory, vaccinations and/or other medical surveillance programs may be warranted for employees. Please contact EHS for assistance with medical surveillance eligibility and enrollment.

### **Personal Protective Equipment (PPE)**

(Refer to EHS Safetygram ISM-139 <http://home.ncifcrf.gov/ehs/uploadedFiles/ISM-139.pdf> )

Aside from standard PPE that is required in a BSL2 laboratory, what other PPE is required to safely manipulate the agent? Does this PPE change if you are manipulating animals and if so, state this in your SOP. The following is a list of common PPE found in the laboratory:

- Gloves
- Safety Glasses/Goggles
- Gowns/Aprons
- Laboratory Coat
- Tyvek Suits
- Respiratory protection (fit test and medical clearance required)
- Surgical Mask
- Shoe Covers
- Bonnets
- Face Shields

### **Biohazard Warning Signs and Posting**

Each laboratory must clearly display a sign that provides safety information to visitors and service personnel. Contact EHS for more information.

**Biocontainment and Biological Safety Cabinets (BSC's)** (refer to Appendix A of [http://www.cdc.gov/od/ohs/biosfty/bmbl5/BMBL\\_5th\\_Edition.pdf](http://www.cdc.gov/od/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf) )

Appendix A presents information on the design, selection, function and use of BSCs, which are the primary means of containment developed for working safely with infectious microorganisms.

Please also reference the BSC purchase checklist for approved BSC models for purchase (<http://web.ncifcrf.gov/campus/als/downloads/ChecklistBiologicalSafety.xls>).

- Determine if augmentation of laboratory practices is necessary above and beyond facility biocontainment designation (i.e.- Biosafety Level 2 laboratory with Biosafety Level 3 practices and procedures, respirator usage, additional PPE, etc).
- Properly maintained Class I and II BSCs, when used in conjunction with good microbiological techniques, provide an effective containment system for safe manipulation of moderate and high-risk microorganisms (Biosafety Level 2 and 3 agents).
- Reference EHS Safetygrams ISM-144, ISM-203, and ISM-206 or contact EHS for additional training on BSC's and other engineering control equipment.

### **Biological Waste Disposal**

(Refer to EHS Safetygram ISM-174 <http://home.ncifcrf.gov/ehs/uploadedFiles/ISM-174.pdf> )

How will the biological material be disposed of?

- Refer to the following EHS website for additional information on medical waste and autoclave waste disposal. (<http://home.ncifcrf.gov/ehs/ehs.asp?id=66>).
- All recombinant material is considered infectious and must be disposed of as biological waste.

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**Housekeeping**

Special practices include: decontaminating work surfaces after completing the work with the infectious materials, keeping non-research animals out of the laboratory, and reporting all spills and accidents.

**Facility renovations**

- Laboratories that are relocating or closing should contact EHS for appropriate laboratory clearances
- New laboratories scheduled for occupancy require an EHS pre-occupancy walk through prior to commencement of laboratory operations. Contact EHS to schedule.
- Either of the above laboratory visits should also be coordinated with any applicable Facilities Maintenance and Engineering (FME) activities.

**Facility decontaminations**

(Refer to EHS Safetygrams ISM-123 <http://home.ncifcrf.gov/ehs/uploadedFiles/IsM-123.pdf> and ISM-124 <http://home.ncifcrf.gov/ehs/uploadedFiles/IsM-124.pdf> )

- Contact EHS to coordinate building or laboratory area decontaminations with an outside contractor

**Autoclaving**

(Refer to EHS Safetygram ISM-125 <http://home.ncifcrf.gov/ehs/uploadedFiles/IsM-125.pdf> )

- EHS monitors autoclave effectiveness and performance on a recurring basis with B.Stearothermophilus indicators
- Contact EHS to participate in the autoclave monitoring program (301-846-1451)

**Needles and Sharps Precautions**

(Refer to EHS Safetygram ISM-146 <http://home.ncifcrf.gov/ehs/uploadedFiles/IsM-146.pdf> )

- Substitute plastic-ware when possible
- DO NOT bend, shear, break, recap or remove needle from syringe
- Use sharps containers (don't fill more than ¾ full), which must be located as close to work area as possible.
- Non-disposable sharps should be placed in a rigid container for transport to appropriate area for decontamination, such as by autoclaving
- Only needle-locking syringes or disposable syringe-needle units (needle is integral to the syringe) should be used
- Syringes that re-sheath the needle, needleless systems, and other safety devices may also be used
- Do not handle broken glass directly by hand. Use a mechanical device such as forceps or protective gloves to pick up and dispose of glass in a glass box or sharps container

### **Shipping Biological Material**

(Refer to *EHS Safetygram ISM-191* <http://home.ncifcrf.gov/ehs/uploadedFiles/ISM-191.pdf>)

- Shipping is regulated by Department of Transportation (DOT) and International Air Transport Association (IATA)
- A Request for Shipment form must be completed for all shipments available on the EHS website (<http://web.ncifcrf.gov/campus/safety/wizard/>).
- Contact EHS (301-846-1451) for questions regarding the hazardous classification of a shipment

### **Intra-Facility Transportation of Biological Materials**

(Refer to *EHS Safetygram ISM-158* <http://home.ncifcrf.gov/ehs/uploadedFiles/ISM-158.pdf>)

- Transportation of biological material from one location to another should be done with the sample in a primary container, with a secondary sealed and leak-proof container with a sufficient quantity of absorbent between the primary and secondary containers. The transport container should also be
- labeled to identify the contents in the event the parcel is misplaced or dropped and spills.

### **Security**

- Call Protective Services with all issues concerning security (301-846-1091)
- Buildings secured by card key access only
- Containment laboratory entrance and exit
  - Restricted access
  - Minimize unnecessary access by casual visitors, vendors, or other persons

### **Select Agents/Toxins**

- Research involving Select Agents/Toxins is regulated by CDC/USDA
- Department of Justice security risk assessment and regulatory authorization and inspections must be successfully completed prior to receipt of any select agent
- Contact EHS for assistance if you are considering work with select agents and toxins (301-846-1451)

### ***Additional Resources for Information***

Resources for information, consultation, and advice on biohazard control, decontamination procedures, and other aspects of laboratory and animal safety management include:

#### **AAALAC International**

Association for Assessment and Accreditation of Laboratory Animal Care International  
5283 Corporate Drive  
Suite 203  
Fredrick, MD 21703-2879  
Telephone: (301) 696-9626  
Fax: (301) 696-9627  
Website: <http://www.aaalac.org>

**American Biological Safety Association**

American Biological Safety Association  
1200 Allanson Road  
Mundelein, IL 60060-3808  
Telephone: (847) 949-1517  
Fax: (847) 566-4580  
Website: <http://www.absa.org/>

**CDC Etiologic Agent Import Permit Program**

Centers for Disease Control and Prevention  
Etiologic Agent Import Permit Program (Mailstop: F-46)  
Atlanta, Georgia 30333  
Telephone: (404) 718-2077  
Fax: (404) 718-2093  
Website: <http://www.cdc.gov/od/eaipp/>

**CDC Office of Health and Safety**

Centers for Disease Control and Prevention  
Office of Health and Safety (Mailstop: F-05)  
1600 Clifton Road  
Atlanta, Georgia 30333  
Telephone: (404) 639-7233  
Fax: (404) 639-2294  
Website: <http://www.cdc.gov/od/ohs/>

**Select Agents General Information**

Website: <http://www.selectagents.gov/>