International
BIOLOGICAL THREAT REDUCTION

SAND2007-3855C

BSL-3 General Procedures

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Sandia National Laboratories

Rationale

- The general facility procedures are scoped to provide overall biosafety and biosecurity practices and procedures for working in any of the CSIR BSL-3 laboratories
- Specific standard operating procedures (SOP's) are required and will include biosafety and biosecurity practices unique to each BSL-3 laboratory and the work conducted within that laboratory space
  - Areas requiring specific SOPs will be identified

Priority Procedures

- Entry Procedures
- Proper PPE Procedures
  - Respiratory Protection
    - Fit Testing
    - Medical Evaluation and clearance
- Decontamination Procedures
- Incident Response Procedures
- Medical Surveillance

Risk Assessment

- **SOPs should be based upon a biosafety and biosecurity risk assessment designed to assess the unique risk:**
  - **Procedures**
  - **Agent Based Hazards**
    - **Safety**
    - **Security**
  - **Other Hazards**
    - **Chemical**
    - **Radiological**
  - **Environment**

SOP

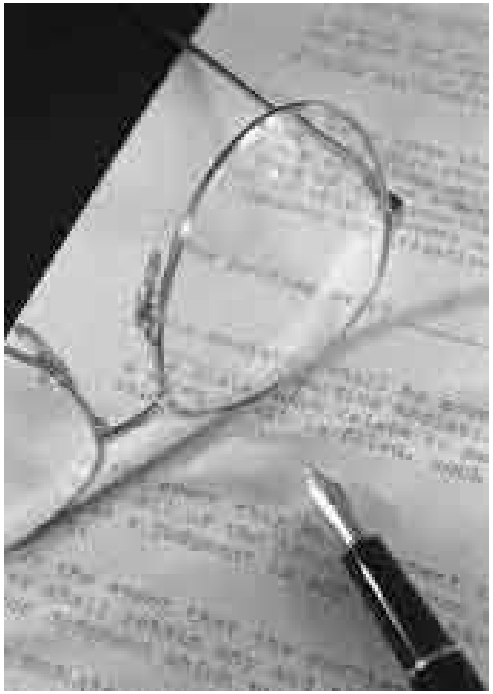
- **Standard Operating Procedures (SOPs)**
  - **Written instructions that document a routine or repetitive activity**
  - **Detail of processes necessary to perform a job safely and properly**
  - **Describe both technical and administrative practices and operations**
  - **Specific to an organization or laboratory**

SOP Purpose

- Ensure all relevant individuals understand the work
- Document how activities shall be performed
  - Facilitate consistency which supports data quality
  - Ensure compliance with regulations
  - Help maintain quality control
- Types
  - Repetitive technical activities
    - Clinical specimen receipt and processing
    - Proper use of biosafety cabinet
    - Diagnostic test procedures
  - Administrative procedures
    - The process for proper documentation of training
    - Laboratory Access Authorization

SOP Sections

- Title Page
- Table of Contents
- Required Controls
  - Limited Access
  - Containment
- Required Training
  - Personal Protective Equipment
  - Equipment
- Body
  - Brief description of the procedure
  - Risks
  - Definitions
  - Procedures
- Emergency Response Measures
- References
  - Other relevant SOPs
  - Relevant Guidelines and Regulations
- Contact Information
  - Principle Investigator
  - Responsible Official
  - Biosafety Officer
  - Engineering and Maintenance



Body of the SOP

- Format
  - Easy to read and concise
  - Sufficient detail to enable persons with basic knowledge to successfully reproduce procedure without supervision
- Title and brief description of the procedure or experiment
- Special health and safety precautions
- Associated Risks
  - Burns (Autoclaves; UV lights)
  - Rotor failure
- Definitions
- Experience Requirements
  - Formal training
  - Supervision
  - Documentation and evaluation of training
- Procedures
  - Step-by-step description of the procedure
  - Steps written in the order they occur

Biosafety/Biosecurity Manual Outline

1. Entry procedure
2. Working within the laboratory
   - Including PPE selection and Use
3. Exit procedure
4. Autoclaving/Decontamination
5. Movement of equipment in and out
6. Animal Procedures
7. Transport of infectious material
8. Communication and data transmission
9. Lab Status when not in use
10. Requirements of incident response plans
11. Medical Surveillance

1. Entry Procedures

1. Secure Entry
2. Verify Airflow
3. Enter Laboratory Space

1.1 Secure Entry

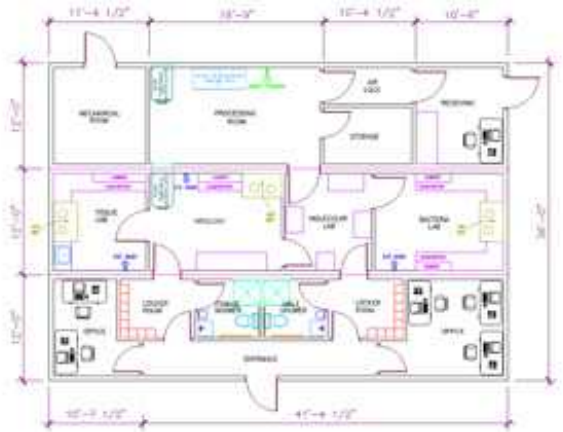
- Authorized and controlled access
- SOP to identify those authorized
- SOP to control access
  - Procedural
  - Physical

1.2 Verify Airflow

- No entry into laboratory until airflow checked
- SOP to identify proper flow rate
- SOP to identify logging mechanism
- SOP to determine response plan if airflow not in compliance

1.3 Entry into Laboratory Space

- Passage from outer area into anteroom and from anteroom into containment area
- Facility layout specific
- Location for Donning PPE



2. Working within the Laboratory

1. General procedures
2. Use of personal protective equipment
3. Use of BSC
4. Proper use of centrifuges and microfuge
5. Microfuge
6. Minimization of aerosol production
7. Sharps handling and disposal
8. Vacuum protection
9. Decontamination/disinfection (lab cleaning)
    1. Lab
    2. Equipment
10. Waste collection and disposal

2.1 General Procedures for working in a lab

- The laboratory itself is the first layer of containment
- The use of good laboratory practices and appropriate microbiological techniques
  - Being aware
  - Proper planning
  - Understanding the proper care and use of equipment
  - Avoiding distractions as much as possible
- The laboratories must have SOPs for the general procedures within each laboratory which are based upon the risk assessment

2.2 Use of PPE

- Dependant upon all hazardous material to be worked with
  - Biological
  - Chemical
  - Radiological
- Should be used in conjunction with engineered controls
  - Radiation shielding and chemical fume hoods may be required

1. Gowns
2. Gloves
3. Eye and mucosal membrane protection
4. Respiratory Protection program

2.2.1 Gowns

- Laboratory coats or gowns should be worn when there is a potential for splashing or spraying
- Gowns should be closed in the front and provide the appropriate level of fluid-resistance
- Gowns should have tight fitting wrists or elasticized sleeves when working in a Biosafety Cabinet (BSC)
  - Water resistance gauntlets can also be used to provide additional arm protection.
- Hoods, caps and shoe covers provide splash and spill protection
- Closed toe shoes are suggested

2.2.2 Gloves

- Gloves should be worn when performing all laboratory procedures
- Vinyl or latex gloves are effective against skin exposure to infectious materials
  - Not intended to protect against needle or sharp penetration
    - Multiple layers of vinyl or latex gloves
    - Heavyweight rubber utility gloves
  - Gloves should be changes when visibly contaminated, torn or defective and upon completion of work
- The outside of the gloves is considered contaminated
- Gloves should be removed using aseptic techniques to protect the skin from contamination

2.2.3 Eye and mucosal membrane protection

- A face mask or goggles must be used
  - When working with blood borne pathogens
  - Or when an infectious material may become aerosolized and work can not be conducted within a BSC
- A full face mask provides additional protection from the unintentional touching of mouth, eyes and nose with a contaminated gloved hand

2.2.4 Respiratory Protection

- **Respiratory protection is used to provide protection from particles entry into the mouth, nose and lungs**
- **N95/N100 Respirator**
  - **Disposable**
  - **Classified by:**
    - **Filter efficiency – 95% (N95), 99% (N99), 99.97% (N100)**
    - **Series – N (not oil resistant), R (oil resistant), P (oil proof)**
- **Powered Air Purifying Respirator (PAPR)**
  - **Tight-fitting and loose-fitting models**
  - **Disposable hood**
  - **Breathing tube**
  - **Motor/blower unit**
  - **Cartridges**
  - **Nickel Cadmium (NiCad) battery pack**
  - **Used when**
    - **Persons with facial hair or facial anomalies that interfere with the seal cannot wear an N95 respirator**
    - **High-risk aerosol generating procedures present**
- **Surgical masks are not respirators**
  - **Provide droplet protection, not aerosol protection**
  - **Provide patient protection**
  - **Keeps hands out of mouth**

2.2.4 Respiratory Protection (con't)

- **Medical evaluation**
  - Determine individual's fitness to use a respirator
  - Physician or other healthcare provider
    - Medical evaluation questionnaire
    - Physical exam at physician's discretion
- **Fit test**
  - Accepted/approved qualitative or quantitative protocol
  - When
    - Prior to initial use
    - Annually
    - Whenever different respirator is worn
    - Whenever a problem reported
    - Whenever a change (e.g. facial change, weight loss) is reported
- **Training**
  - Criteria for respirator selection
  - Limitations of respirator types
  - Proper method for donning
  - Checking face piece for seal and proper operation
  - Respirator maintenance

Types of Cabinets

	Personnel	Product	Environment
Chemical Fume Hoods	X		
Laminar Flow Clean Benches		X	
Class I Biological Safety Cabinet	X		X
Class II Biological Safety Cabinet	X	X	X
Class III Biological Safety Cabinet	X	X	X
Isolators	X	X	X

2.3 Use of BSC

1. Start Up
2. Decontamination
3. Proper Use
4. Work Techniques
   - Waste Collection
   - Spill Clean up
5. Final purging and Wipe down
6. Shut down
7. Maintenance and Routine Cleaning

2.3.1 Start up

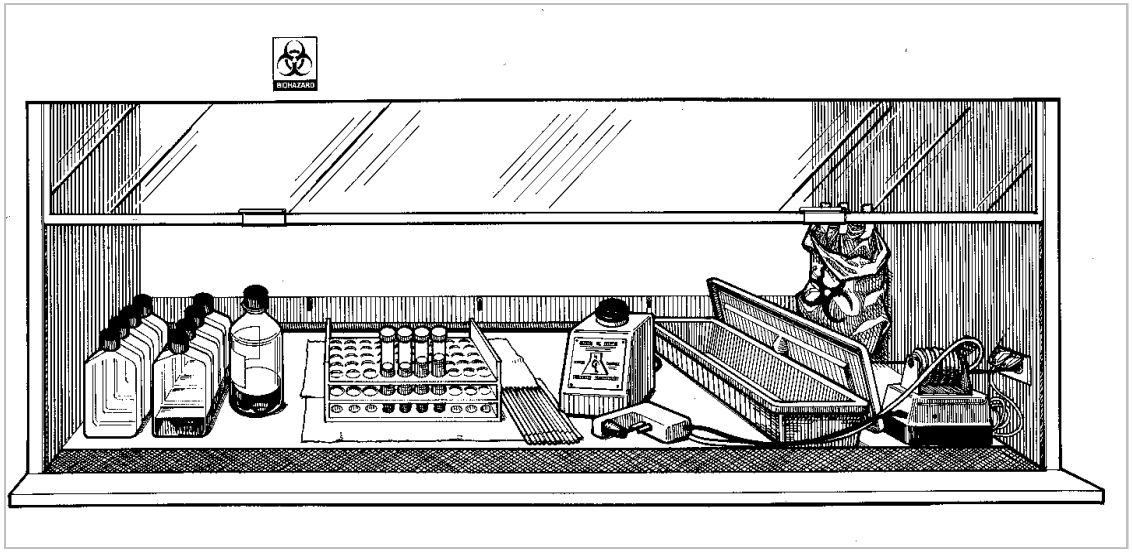
1. For class II B2 cabinets, the cabinets are controlled by the HVAC system and are therefore on at all times
2. For laboratories containing other types of BSC, before work starts the BSC should be powered for at least 1 minute before starting use
3. Sash alarms should not be powered off at any time
4. Gauges should be checked to insure cabinet is operating within the certified parameters

2.3.2 Decontamination

- The BSC should be wiped down with a proper disinfectant
- The SOP will identify the proper disinfectant and contact time
  - E.g. A bleach solution with a following ethanol wipe down

2.3.3 Proper Use of BSC

- Place all needed equipment into the BSC
- Do not over crowd or block airflow
- Place the equipment so is it possible to work from a clean side to a dirty side
- Wait for the airflow to resume, approximately 2 to 3 minutes, before starting work



2.3.4 Work Techniques

- The laboratory SOP must define the proper work technique for working within the BSC
  - Must be specific to the work to be conducted
  - Must include the required PPE, address the use of flames in BSC, blocking airflow, arm movement, sash level, etc
- Waste Collection
  - Any waste should be disposed of within the BSC to avoid contamination outside the BSC
  - SOP must define proper waste handling
- Spill Clean up
  - Work should be halted as soon as possible after a spill occurs
  - Clean the spill as soon as safely possible
  - Broken glass or other sharps should be disposed of in sharps disposal with forceps or tongs
  - The laboratory SOP must detail the procedure for spill response
  - The SOP should include response to spills within the BSC grill and spills on persons working in the BSC

2.3.5 Final purging and Wipe down

- All biohazardous waste should be placed into a proper container and removed from the BSC
- The BSC should be wiped down with the proper disinfectant
  - Chemical Disinfectant
  - UV light*

* The US CDC, NIH and NSF agree that UV lamps are neither recommended nor required in BSC

2.3.6 Shut Down

- For hard ducted BSC, turn off only the lights
- Other BSCs may be powered off after use

2.3.7 Routine maintenance and routine cleaning

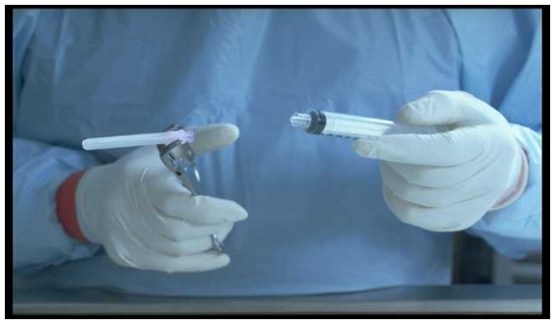
- BSC require routine maintenance and cleaning
- The laboratory SOP must include the process for routine cleaning and decontamination for maintenance

2.4 Proper use of centrifuges and microfuge

- **The laboratory SOP must define the proper use of centrifuges**
  - **Utilize aerosol containment devices**
  - **Follow preventive maintenance procedures**

- **The laboratory SOP must define the proper use of microfuges**
  - **Should be used in a aerosol-proof containment device**

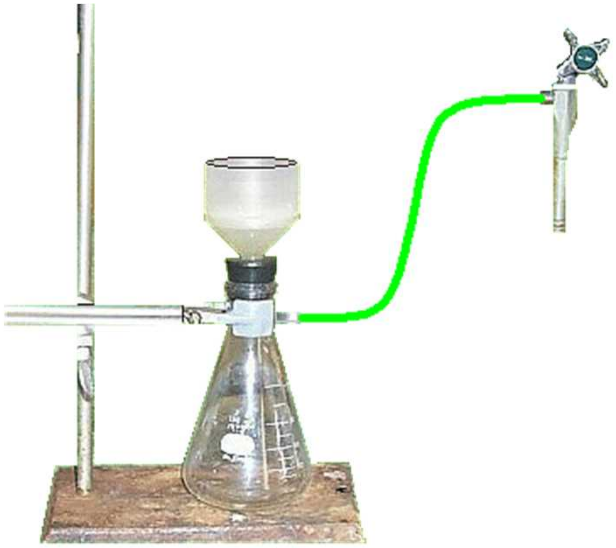
2.5 Sharps Handling



- Utilize safe sharp devices
- Minimize sharps and glass as much as possible
- Keep hands away from needles and use mechanical methods for needle removal
- Never bend, recap or manipulate sharps by hand
- Dispose of entire unit into sharps container and collect reusable sharps in labeled, leak-proof container
- Do not overfill containers
- The laboratory SOP must detail the proper methods for sharp handling and disposal

2.6 Vacuum protection

- Vacuum pumps must be protected from contamination using a filter
- The risk assessment will aid in the definition of the filter type and location
- The laboratory SOP must detail the proper use and type of filter used



2.7 Chemical Storage

- The laboratory SOP must process the storage, labeling and handling techniques for hazardous chemicals within the laboratory
- The storage and handling methods should be based upon the risk assessment
- Identify proper methods of disposal
  - Incineration
  - Permissible limits for sewer disposal

2.9 Spill Response

- The laboratory SOP should define the proper spill response based upon the material spilled and other specifics include sharps handling
- The SOP must be developed based upon a risk assessment
  - Should identify the PPE
  - Should identify the proper disinfectants
- Precautions for each disinfecting agent defined within the SOP should also be listed
- Spill may exceed lab ability for clean-up
  - Fumigation by biosafety office may be necessary

3 Exit Procedures

The laboratory SOP must detail the exit procedures for each laboratory.

1. Secure infectious materials
2. Waste disposal/decontamination
3. Disinfection of work surfaces
4. Doff, disposal/decontamination of PPE
5. Hand washing
6. Exit
7. Consider emergency evacuation procedures

3.1 Waste Disposal/Decontamination

- All waste should be placed into a biohazard disposal bag
  - This bag should be clearly labeled as containing biohazardous material and be water tight
  - Any sharps to be disposed of should be placed into a sharps container which is also properly labeled
    - A sharp container should be resistant to perforation by any sharps within the container and be resistant to spills
    - Sharps should be able to be placed inside the container without opening the lib.
- The container and all contents must be autoclaved before removal from containment
- If an autoclave is not available within the containment area, the biohazard container should be placed into a second outer bag
- The outer bag should be sealed and submerged into a decontamination dunk tank or decontaminated before removed from containment

3.2 Disinfection of work surfaces

- The surface area of the laboratory should be disinfected with the proper disinfecting agent
- Each disinfecting agent has different properties
  - Concentration
  - Age of effectiveness
  - Contact time
  - Impact to the biological agent
- Disinfecting agents may also corrode equipment and surfaces.
- The laboratory SOP will define the exact disinfecting agent, contact time and procedure
- The SOP should also define the hazards associated with the disinfecting agent and proper handling and storage techniques
- MSDS should be available for all disinfectants

3.3 Disposal/Decontamination of PPE

- PPE should be removed as directed in the SOP before exiting the lab or anteroom
- Disposable PPE should be placed into a biohazard container
- Reusable PPE should be decontaminated based upon manufactures recommendations and stored

3.4 Hand washing

- All personnel should wash their hands prior to leaving the anteroom or containment area
- If soap and water are not present, a chemical hand cleanser may also be used

3.5 Exit

- **Before exiting the laboratory area**
  - **Double check all equipment is in the proper state**
  - **All doors are closed**
  - **All biohazards are properly disposed of or stored**
- **Sign out where required**
- **The laboratory area should be secured as determined by the SOP**

4 Autoclaving

- An autoclave is a device which uses extreme heat in the form of steam and pressure to sterilize items
- Add water to a sealed container
- Items should be placed loosely within the autoclave
- The laboratory SOP must define the time and pressure required to fully sterilize items
  - The proper loading and unloading procedures
  - The SOP should also identify those which can not be autoclaved
  - The SOP must also define the verification methods for use within the laboratory

5 Movement of equipment in and out

- The laboratory should have been designed to allow for large equipment movement in and out
- Equipment must be decontaminated before removal from the laboratory
- The decontamination procedure must be identified with the equipment SOP
- The method of decontamination must consider:
  - The contaminants
  - The physics of the equipment
  - The reason for its removal from the laboratory

6 Animal Biosafety Procedures

1. ***Animal Care and Handling (1999 manual?) CEPSCA***
2. **Animal care worker**
3. **Cage cleaning / waste disposal**
4. **Animal Disposal**

6.1 Animal Care workers

- Animal care workers should be giving proper training
  - Handling
  - Risks
  - PPE
- Each laboratory must have a unique SOP for each species
  - A supplemental SOP for working with infected animals
- PPE for animal care workers should include:
  - Protection from allergens
  - Infectious agents
  - Injuries acquired from the animals
- Medical surveillance program

6.2 Cage cleaning/waste disposal

- Cage cleaning releases aerosols which may include infectious agents and are also a strong source of allergens
- Respiratory protection should be used while cleaning cages which have not been sterilized via autoclave
- Cage cleaning may also be preformed in a BSC
- The laboratory SOP must define how the cages are to be cleaned and sterilized
- The SOP must also define the method for waste disposal and transport

6.3Animal Disposal

- Autoclaving animals larger then mice not recommended
  - To sterilize a mouse with an autoclave 1.5 hours of time is required
- Decontamination of the animal storage container via autoclave or chemical decontamination prior to transport to an incinerator or chemical digester
- The laboratory SOP must define the methods for decontamination and disposal of animals

7 Transport of Infectious Materials

- CSIR must develop a policy for movement of material in and out of the containment space
- This should include:
  - The validation requirements if the material is to be used outside of a BSL-3 laboratory
  - The procedures for transporting material between BSL-3 laboratories

7.1 Transport Between BSL-3 lab space

- Use leak proof non-breakable primary container
- Place into similar secondary container
- Label as biohazard
- Decon outer container before removal from containment

7.2 Transportation of material outside

- **Prior to movement outside of BSL-3 laboratory space:**
  - **Infectious material must be inactivated**
  - **The inactivation methods must be verified**
- **The inactivation and validation methods must be defined within the SOP**

8 Communication and Data Transmission

- Communication and data as part of biosafety and biosecurity can include:
  - Information regarding the laboratory airflow
  - Power grid
  - Security system
  - Personnel procedures
  - The SOPs
- Most biosafety information should be available for all persons working within the laboratory area
- Biosecurity information should be protected to a level appropriate to the information

9 Lab Status when not in use

- CSIR needs to work with the laboratory engineers to determine the engineered control limitations
- The laboratory must remain negative, but a risk assessment along with the engineering limitation can be used to determine how negative
- SOPs should also identify equipment and other systems which can be shutdown when the laboratory space is not in use
- The SOP must also define the proper procedures for restoring the laboratory to operational levels prior to use
- CSIR must also define the proper methods for laboratory decontamination and scheduled maintenance cycles
  - The decontamination method should be developed based upon a risk assessment

10 Incident Response Plan

- Each facility must develop an incident response plan which includes the procedures for handling incidents within the BSL-3 laboratory space
- The response plan should be scoped to include:
  - Large level disasters
  - Infrastructure disruptions
  - Medical emergencies
  - Biosecurity incidents
- A specific SOP should be developed to define the response mechanism for each incident within the laboratory
- The SOPs should be developed based upon the risk assessment
- Plans should be practices and reviewed on a periodic basis
- Training should include all agencies involved in the response

11 Medical Surveillance

- Each facility should define the medical surveillance program for any employees with access to the laboratory and infectious material (including animal care takers)
- The medical surveillance program should be developed based upon the hazards and the facilities limitations
  - The surveillance program requirements should fall out of a risk assessment
- The program should identify:
  - The pre-screening requirements
  - The suggested/required immunizations
  - Monitoring procedures for symptoms
  - Post-exposure practice and procedures
  - Provide information to the workers as well as healthcare providers as to the potential exposures
    - Emergency care options

Summary

- CSIR define policies and general procedures
- Laboratories to develop and implement SOPs
- Priority Procedures
  - Entry Procedures
  - Proper PPE Procedures
    - Respiratory Protection
  - Decontamination Procedures
  - Incident Response Procedures
  - Medical Surveillance