Section 6.3. Basic Laboratory Safety

Objective: To provide a safe and healthy environment for staff, volunteers and all personnel involved in PREDICT activities. This Guide is to provide basic information to ensure a safe laboratory environment and to comply with environmental standards. The recommendations in this Guide are consistent with the requirements of the U.S. Occupational Safety and Health (OSHA) Act of 1970, Executive Order 12196.

This document was made possible by the generous support of the American people through the United States Agency for International Development (USAID) Emerging Pandemic Threats PREDICT program. It was drafted to support activities conducted under PREDICT and is intended for an audience of qualified professionals trained in standard, associated best practices. This guide is not intended for use by untrained individuals.

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Table of Contents: Basic Laboratory Operations

Section 6.3.1. Learning Objectives
Section 6.3.2. Principles
Section 6.3.3. General Guidance for Laboratory Safety
  Ensure Safe Working Conditions
  Hazard Identification and Assessment
  Safe Laboratory and Operating Procedures
  Review of Material Safety Data Sheets
  Needle Stick Injury Prevention
Section 6.3.4. Biohazards of Zoonotic Pathogens
  Species-Specific Biosafety Considerations
  Biosafety Levels and Practices
Section 6.3.5. Medical Monitoring
Section 6.3.6. Medical Waste Management
  Safe Sharps Disposal
  Sharps Disposal Containers
  Medical Waste Disposal
Section 6.3.7. Special Chemical Storage and Handling
  Inventory Control
  Labeling
  Safe Handling and Transfer
  Secondary Containment & Spill Control
  Cabinet & Shelf Storage – General Precautions
  Refrigerators and Freezers – Flammable Storage
  Storage Chemicals by Class
Section 6.3.8. Training in Laboratory Safety and Procedures
Section 6.3.9. Basic Standards and Guide Checklist
Section 6.3.10. List of Equipment
Section 6.3.11. References
Section 6.3.1. Learning Objectives

If you understand the material in this Guide, you should be able to:

- Work safely in a basic laboratory environment.
- Recognize laboratory hazards and take the appropriate measures to reduce those hazards.
- Obtain a Material Data Safety Sheet (MSDS) for a hazardous material and explain the kinds of information in an MSDS.
- Explain important precautions to avoid needlestick injuries.
- Explain how to avoid exposure to pathogens in the laboratory.
- Describe the safety measures for a BSL 2 laboratory.
- Explain why medical monitoring of laboratory personnel is important.
- Describe the proper disposal of sharps and medical waste.
- Describe safety procedures for handling chemicals in the laboratory.

Confirm you understand the material of this Guide:
When you are familiar with the information in this Guide, take the PREDICT quiz on Basic Laboratory Safety (Section 8.4.16.).

This training module is mandatory for all laboratory and field staff.
Once you have passed the quiz please supply proof of training and completion to your supervisor.

Section 6.3.2. Principles

Guiding principles for PREDICT laboratory operations:
1. Prevent loss of life, personal injury or illness, property loss or damage, or environmental harm.
2. Comply with the PREDICT Environmental Compliance Protocol and local and national safety and health requirements.
3. Comply with applicable local building safety codes.
4. Ensure all PREDICT personnel understand relevant safe and healthy work practices.
5. Identify and assess hazards in the laboratory environment.
6. Establish overall safety and health guidelines that ensure employee safety and health at all times during PREDICT activities.
7. Periodically review and evaluate PREDICT plans, facilities, equipment, and activities to ensure that safety and health objectives are achieved.

Section 6.3.3. General Guidance for Laboratory Safety

This Laboratory Safety Guide describes safe work practices, personal protective equipment, and other control measures necessary for the safe use of chemicals and other hazardous materials
and procedures in the basic laboratory environment. PREDICT personnel involved in laboratory activities must review and follow this Guide. Staff, interns, visiting scientists, and volunteers are to receive this Guide prior to conducting laboratory activities for the PREDICT Program. This Guide will be updated as needed to improve safety procedures.

**Ensure Safe Working Conditions**
- Inspect your personal protective equipment (PPE), such as goggles and gloves, to ensure that each component fits well and works properly. Examine your gloves for cracks. Nitrile and latex gloves are disposable and a new pair should be used for each task.
- If you are working with PPE kits, ensure that the kit is complete (a list with the contents of the PPE kit should be available).
- Dispose of broken glass and biohazard materials in designated sharps and hazardous waste containers in the laboratory.
- Help provide a safe work environment by keeping the workspace neat and uncluttered.
- Sinks and eye wash stations should be kept clear.
- Wash your hands and forearms after you have removed and disposed of your PPE.

**Hazard Identification and Assessment**
Personnel should be able to recognize the possible hazards and inherent risks associated with laboratory procedures and equipment.

<table>
<thead>
<tr>
<th>Table 1: Hazards associated with laboratory procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procedure</td>
</tr>
<tr>
<td>Using autoclave or hotplate</td>
</tr>
<tr>
<td>Handling animal and human samples including</td>
</tr>
<tr>
<td>tissues, swabs</td>
</tr>
<tr>
<td>Reagent preparation</td>
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<tr>
<td>Disposal of needles and slides</td>
</tr>
<tr>
<td>Dry ice, liquid nitrogen or ultra-low freezers</td>
</tr>
<tr>
<td>Media preparation</td>
</tr>
<tr>
<td>Formaldehyde</td>
</tr>
<tr>
<td>TRIzol Reagent (or Tri reagent; phenol solution)</td>
</tr>
</tbody>
</table>
Safe Laboratory and Operating Procedures

Personnel must understand and follow the safe operating procedures of laboratory equipment and PPE to minimize health and safety risks. The use of the PPE for specific laboratory tasks, listed in Table 2, is mandatory and all PREDICT personnel must follow the special precautions listed for handling highly hazardous materials.

Table 2: PPE required for laboratory tasks

<table>
<thead>
<tr>
<th>Lab Task</th>
<th>Health or Safety Hazards</th>
<th>Required PPE</th>
<th>Precautions for Highly Hazardous Materials*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Handling all samples from animals and humans (body fluids, tissues, swabs)</td>
<td>Zoonotic disease potential</td>
<td>Lab coat, closed shoes, disposable nitrile gloves, eye protection and respirator (N95 minimum)</td>
<td>Use of Biosafety Cabinet Class II and eye protection for samples known to be highly infectious or use PPE kits.</td>
</tr>
<tr>
<td>Handling acids or chemicals that are irritants (i.e. formaldehyde)</td>
<td>Respiratory irritation, acid or alkaline burns</td>
<td>Lab coat, laboratory gloves, face mask, closed toe shoes.</td>
<td>Chemical fume hood</td>
</tr>
<tr>
<td>Operation of autoclaves</td>
<td>Burns</td>
<td>Appropriate gloves, eye protection, closed toe shoes.</td>
<td>Care in opening the door to avoid burns from escaping steam.</td>
</tr>
<tr>
<td>Dry ice, liquid nitrogen</td>
<td>Burns, asphyxiation risk</td>
<td>Appropriate gloves, eye protection, closed toe shoes, and use in well-ventilated room.</td>
<td>Dispose of any unused dry ice or liquid nitrogen in ventilated fume hood.</td>
</tr>
<tr>
<td>Centrifuges</td>
<td>Aerosolized fluids, zoonotic disease</td>
<td>Lab coat, facemask, appropriate gloves when handling samples or cleaning centrifuge.</td>
<td>Ensure proper balancing of centrifuge and contents. Do not open until rotor has stopped. Use closed-top swinger rotors to spin biological materials.</td>
</tr>
<tr>
<td>Hot plate</td>
<td>Possible burns</td>
<td>Appropriate gloves, closed toe shoes.</td>
<td>Do not leave unattended for extended periods.</td>
</tr>
<tr>
<td>Use of bleach to disinfect</td>
<td>Possible burns, respiratory irritation</td>
<td>Lab coat, gloves, closed toe shoes, and eye protection.</td>
<td>Use of chemical fume hood recommended when preparing bleach.</td>
</tr>
<tr>
<td>Disposing of needles, glass slides</td>
<td>Cuts, zoonotic disease</td>
<td>Gloves, sharps container, closed toe shoes.</td>
<td>Follow sharps safety procedures in this guide.</td>
</tr>
<tr>
<td>TRIzol Reagent (or Tri reagent; phenol solution)</td>
<td>Contact burns, systemic poisoning</td>
<td>In lab: Gloves, lab coat, close toe-shoes, eye goggles</td>
<td>Aliquot TRIzol for sampling in the field in a ducted biosafety cabinet or fume hood; Perform RNA extraction of samples collected into TRIzol in a biosafety cabinet.</td>
</tr>
</tbody>
</table>
Definitions

*Highly hazardous materials* are chemicals, toxics and reactives that have the potential to cause immediate and permanent harm at feasible exposure levels. Chemicals that are highly toxic, are known to cause cancer or birth defects, have very low "permissible exposure limits," are highly reactive, or that react vigorously with common materials (such as water or air) should all be considered "highly hazardous materials." Chemicals that are under pressure, that can build up pressure, that can auto-ignite at possible temperatures, that burn vigorously and energetically, or that when burning cannot be extinguished with conventional methods, should be considered highly hazardous.


**Personal Protective Equipment (PPE)** is specialized clothing or equipment worn by an employee for protection against infectious and other hazardous materials. The warranted components of PPE vary according to the tasks being performed by personnel. A basic PPE kit may include: gloves, gowns or other protective clothing (e.g., plastic apron), shoe and head covers, mask or respirator, and face or eye protection (e.g., goggles).

**Review of Material Safety Data Sheets**

PREDICT personnel must verify that a Material Safety Data Sheet (MSDS) for each product to be used during PREDICT activities is readily available, complete and updated (less than three years old).

Coordinators must ensure that personnel have read and understand the MSDS BEFORE using a chemical product.

Personnel must be familiar with the name of the chemical and understand the hazards, safe handling and storage, and specific emergency procedures BEFORE using a chemical product.

Copies of MSDSs for all chemicals used in the laboratory should be kept together in a binder and placed in an accessible location known to all laboratory personnel.

**What is a Material Safety Data Sheet?**

A MSDS is prepared by the supplier or manufacturer of the material and contains information on the potential hazards (health, fire, reactivity and environmental) and safe use of the chemical product. It is an essential information resource for all health and safety programs. The MSDS also contains information on the safe use, storage, handling and emergency procedures for all hazardous materials. The MSDS contains much more information about the material than found on the product label including what to do if accidents occur, and how to recognize and treat overexposure to the chemical product.
What information is on the MSDS?
The information of greatest concern to workers is featured at the beginning of the data sheet, including information on chemical composition and first aid measures. More technical information that addresses topics regarding the physical and chemical properties of the material and toxicological data appears later in the document. The 16-section MSDS is now recognized internationally. Each MSDS must include:

1. Identification (name, manufacturer and supplier names, address and emergency phone numbers)
2. Hazard(s) identification
3. Composition/information on ingredients
4. First-aid measures
5. Fire-fighting measures
6. Accidental release measures
7. Handling and storage
8. Exposure controls/personal protection
9. Physical and chemical properties
10. Stability and reactivity
11. Toxicological information
12. Ecological information
13. Disposal considerations
14. Transport information
15. Regulatory information
16. Other information
Different jurisdictions have different content requirements for Material Safety Data Sheets. Despite the internationally recognized standard, a MSDS prepared in accordance with the United States OSHA Hazard Communication Standard is not necessarily acceptable in other countries. Check with local health authorities to ensure that your MSDSs are in compliance with local regulations.
Where to obtain MSDSs for chemical products?
A MSDS can be requested from the manufacturer or supplier of the product; in addition several MSDS databases exist online including:

SIRI MSDS index: http://siri.org/msds/index.php
MSDS online: http://www.msdsonline.com or http://www.msdsonfile.com
MSDS Hazard Communication Library: http://www.setonresourcecenter.com/MSDSs/comply1.htm

Needlestick Injury Prevention
Needlestick injuries are of concern in basic laboratory settings because they can result in the inoculation of personnel with infected materials. Additionally, skin breaks from needlesticks can act as portal of entry for environmental pathogens.

Most needlestick injuries occur during the following activities
  • Recapping, bending, or breaking needles.
  • Inserting a needle into a test tube or specimen container and missing the target.
  • Carrying unprotected sharps.
  • Leaving sharps in unexpected places, such as clothing.
  • Handling or disposing of waste that contains used sharps.

Parts of a Syringe and Needle

Procedures to Prevent Needlestick Injuries
  • Follow proper techniques when using needles and syringes.
  • Be familiar with the different types and components of syringes and needles.
  • When uncapping a syringe needle, pull the cap straight off to remove it and expose the needle.
  • Never leave an uncapped needle lying around. A used syringe with the attached needle should be placed in a sharp disposal container immediately after use (a sharps disposal
container is designed for safe containment of medical articles that may cause punctures or cuts to those handling them – see below).

- **Removal of the syringe needle** may be necessary for transfer of the sample to another container, or for disposal of only the needle in the sharp container. When removal of the needle is necessary:
  - Make sure not to remove the cap--twist the entire needle to take it off the syringe along with the cap. Alternatively, the needle may be removed from the syringe by use of forceps.
  - Uncapped needles should never be removed from the syringe by hand.
  - **Syringes and needles** used on humans should never be recapped. However, when working with animals and in the field, it may be necessary to carefully recap a needle to avoid accidental sticks if a sharps container is not immediately available.

**If you recap a needle, use the **ONE HAND METHOD**

1. Lay the cap on a table or on a flat surface.
2. Hold the syringe by the end.
3. Tilt the end of the syringe up, so that the needle inside the cap is point down onto the surface.
4. Insert the needle on the syringe into the cap.
5. “Fish” up the cap with the needle.
6. Use the same hand to recap the needle.
7. Apply enough pressure to set the cap onto the needle.

If a needlestick occurs, it must be reported to your local PREDICT Supervisor and a medical professional immediately.

**Section 6.3.4. Biohazards of Zoonotic Pathogens**

Investigators working with domestic and wild animals and humans or with animal and human samples are at risk of disease due to exposure to zoonotic pathogens (pathogens transmitted between animals to humans). The zoonotic disease risk varies depending on the animal species being handled, but is generally caused by direct contact (e.g., contaminated/dirty hands), through open cuts, contact with blood and other body fluids, or inhalation of contaminated materials.
When performing tasks with risk of exposure to zoonotic pathogens (such as handling live or dead animals or samples from humans, collecting, testing, or packaging samples), PREDICT personnel should always wear the appropriate PPE as warranted by the assessed risk. It is the responsibility of the supervising veterinarian or medical specialist to determine the required PPE components for specific activities, based on an established PREDICT protocol or based on a risk assessment. (See Section 4. Biosafety and PPE Use for more information about determining the appropriate PPE.)

In the event that any personnel believe they have been exposed to material from a person or animal, they should immediately report the exposure to their supervisor, and if warranted seek the appropriate medical attention and follow-up.

**Species-Specific Biosafety Precautions**

The PREDICT Program will conduct surveillance and sampling among several groups of species. This section discusses special biosafety considerations for some of the key groups of species (bats, rodents, and non-human primates) likely to be handled as part of PREDICT activities.

Rodents, bats, non-human primates and other wild species may harbor pathogens that are transmittable to, and highly pathogenic in, humans. When handling these rodents, bats or non-human primates, careful consideration needs to be given to conscientious use of PPE, good personal hygiene (i.e., hand washing), safety training, and application of good animal handling and sampling techniques to minimize exposure to infection or injury.

In the event of an injury while handling animals that pose risk of zoonotic pathogen exposure, appropriate first aid must be applied. The risk of infection can be significantly reduced with immediate and thorough scrubbing of the wound with soap or antiseptic.

**Vaccination to prevent rabies infection:** Personnel who are handling animals that are known reservoirs for rabies (e.g., bats and dogs) should be immunized against rabies virus according to World Health Organization and CDC recommendations.

Investigators should familiarize themselves with known biohazards specific to species under study and with the procedures for the isolation and control of zoonotic pathogens. Specific considerations with regard to working with rodents, bats and non-human primates are discussed below:

**Rodents**

Wild rodents have the potential to carry a variety of zoonotic bacteria and viruses that can be passed on to those handling them. Because of the serious consequences of becoming infected, personnel must always follow good personal hygiene and animal handling procedures and use the provided PPE to protect against exposure.
Special Precautions:
- Wear the minimum PPE for handling rodents as specified in the PREDICT PPE Use Guide, this includes an N95 mask, eye-protection, gloves and coveralls, or clean dedicated clothing.
- Personnel who are handling animals should be immunized against rabies virus according to the World Health Organization and CDC recommendations.

**Bats**
Exposure to wild bat roosts (in caves or trees), handling of bats in the field or handling bat excreta (urine or feces) presents a potential for exposure to zoonotic pathogens. Rabies, Nipah virus, Ebola virus, and the fungal disease histoplasmosis are examples of zoonotic pathogens carried by some bat species. Bat bites, scratches and wound and mucous membrane exposure to bat saliva are the ways in which rabies can be transmitted. Spores of histoplasmosis can be present in soil and debris enriched with bird and bat droppings. When this dry soil is disturbed, spores can become airborne and cause infection by inhalation.

Special Precautions:
- When working around bats in enclosed spaces, such as in a cave, wear at a minimum an N95 respirator, goggles, gloves and Tyvek coveralls (or dedicated long-sleeved clothing).
- Personnel who are handling animals such as bats should be immunized against rabies virus and be aware of appropriate post exposure prophylaxis in the case of bites according to World Health Organization and CDC recommendations.

**Non-Human Primates**
Non-human primates may be infected with a number of potentially serious zoonoses. For example, all macaque monkeys and their fluids should be considered to be infected with Herpes Simian B virus. Marmosets, although they do not carry the herpes B virus, can carry other disease agents that affect humans such as lymphocytic choriomeningitis virus and Trypanosoma cruzii, the cause of Chagas’ disease. It is critical that work with non-human primates be done while wearing the appropriate personal protective equipment and with the well-established safe protocols and procedures.

Special Precautions:
- Personnel must follow strict hygiene procedures. Frequent and thorough hand washing, although too often overlooked by the staff, is critical to physically remove bacterial contamination and prevent ingestion exposure.
- PREDICT personnel must wear the minimum PPE for handling non-human primates as specified in the PREDICT PPE Use Guide. This includes an N95 mask, eye-protection, gloves and coveralls or clean dedicated clothing.
Biosafety Levels and Practices

General
All laboratories handling biological agents must post signage indicating that the site is a potential biological hazard area, and identifying all agents in use. Supervisors shall ensure that employees are informed of biological hazards and that suitable biosafety controls are in place. Country Coordinators and lab and field supervisors managing surveillance and other field and laboratory activities should ensure that appropriate biosafety practices are implemented by personnel. Biological safety cabinets are to be certified annually.

It is important to know the biosafety level of the disease that you are working with before beginning work, so that the correct precautions can be taken.

Note: All samples collected for the PREDICT project are to be handled in a Class II Biosafety Laboratory.

Basics of Biosafety Level 1
Biosafety Level 1 (BSL1) practices represent a basic level of containment that relies on standard microbiological practices and basic safety equipment and lab design for laboratories that work with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans. However, many agents not ordinarily associated with disease processes in humans are opportunistic pathogens and may cause infection in the young, the aged, and immuno-deficient or immunosuppressed individuals.

BSL-1 Standard Microbiological Practices
1. Access to work areas is limited at the discretion of the supervisor.
2. Hands must be washed after handling biological materials, removing gloves, or before leaving the laboratory.
3. No eating or drinking is allowed in the laboratory.
4. Only mechanical devices are used for pipetting.
5. Safety devices such as self-protected injection syringe or non-sharps should be used as an alternative to sharps. Sharps used should be handled and disposed of properly.
6. Activities that are likely to create splashes, sprays, or aerosols should be minimized.
7. Work surfaces should be decontaminated with 10% bleach (70% ethanol for metal surfaces) at least daily (before and after work with infectious samples) and after any spills.
8. Waste materials should be disposed of properly.
9. Secondary containment should be used when transporting bio-hazardous materials outside of the laboratory. Avoid public areas during transport.
BSL-1 Safety Equipment (Primary Barriers)
1. BUTTONED lab coats should be worn to protect street clothes.
2. Barrier (preferably non-latex) gloves should be worn, particularly if hands have broken skin or a rash.
3. Appropriate eye/face protection (safety goggles as a minimum) should be worn if splashes or sprays are anticipated, or if wearing contact lenses during lab work.

BSL-1 Laboratory Facilities (Secondary Barriers)
1. The lab should have a sink for hand washing.
2. The lab should have an eye wash station.
3. The lab should have a door for access control.
4. The lab fixtures and floors should be easily cleaned and disinfected (no carpets or rugs); bench tops are to be impervious to water and resistant to both moderate heat and the chemicals used to decontaminate the work surface and equipment.

Note: BSL-1 is NOT APPROPRIATE for PREDICT samples.

Basics of Biosafety Level 2
Biosafety Level 2 is more restrictive than BSL-1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. All PREDICT samples are to be handled in a Biosafety level 2 laboratory. It differs in that (a) laboratory personnel have specific training in handling pathogenic agents and are directed by trained technologists, (b) access to the laboratory is limited when work is being conducted, (c) extreme precautions are taken with contaminated sharp items, and (d) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment. All PREDICT samples are to be handled in Class II biological safety cabinets, in Biosafety level 2 laboratory.

BSL-2 Standard Microbiological Practices
1. Personnel must wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
2. Eating, chewing gum, drinking, smoking, handling contact lenses, and applying cosmetics should not be permitted in the laboratory. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food should be stored outside the work area in cabinets or refrigerators designated for this purpose only.
3. Only mechanical pipetting devices are used for pipetting.
4. Policies for safe handling of sharps (when non-sharps are not available) should be instituted.
5. All procedures should be performed carefully to minimize the creation of splashes or aerosols.
6. Work surfaces should be decontaminated with 10% bleach (70% ethanol for metal surfaces) at least once a day (before and after working with infectious samples) and after any spill of viable material.
7. All cultures, stocks, and other regulated wastes are disposed of in the biohazard trash by placing them in a durable, leak-proof container, closed for transport from the laboratory, and transferred to the designated receptacle for disposal. Materials to be decontaminated at off-site locations from the laboratory should be packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.

BSL-2 Special Practices

1. Access to the laboratory is limited or restricted by the laboratory supervisor when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may be unusually hazardous are not allowed in the laboratory. Persons who are immuno-compromised, immunosuppressed, pregnant or at higher risk of acquiring infections, should not be permitted in the laboratory.

2. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet specific entry requirements (e.g., immunization) enter the laboratory.

3. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

4. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
   • Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a disposal area.
   • Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass should be decontaminated with 10% bleach before disposal, according to any local, state, or federal regulations.

5. Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.

6. Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant (such as 10% bleach) on a routine basis, before and after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility. Bleach (10%) can be used on all non-steel surfaces; however, 70% ethanol or other recommended disinfectant should be used when those chemicals are not available.
7. Spills and accidents that result in overt exposures to infectious materials should be reported immediately to the laboratory director. Medical evaluation, surveillance, and treatment should be provided as appropriate and written records should be maintained.

BSL-2 Safety Equipment (Primary Barriers)

1. Properly maintained biological safety cabinets, Class II, and other appropriate personal protective equipment or physical containment devices should be used.

Procedures with a potential for creating infectious aerosols or splashes are a hazard. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals, and harvesting infected tissues from animals, eggs or cell cultures.

2. Face protection (goggles, mask, face-shield or other splatter guards) should be used for anticipated splashes or sprays of infectious or other hazardous materials to the face, when the microorganisms must be manipulated outside of the biosafety cabinet.

3. Protective laboratory coats, gowns, smocks, or uniforms designated for lab use should be worn while in the laboratory. This protective clothing should be removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing should be disposed of either in the laboratory or laundered by the institution; it should never be taken home by personnel.

4. Gloves (nitrile or latex) should be worn when hands may contact infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate, if a spill or splatter occurs; the hand will be protected after the contaminated glove is removed. Gloves should be removed and disposed of when contaminated, removed when work with infectious materials is completed, and should not be worn outside the laboratory. Disposable gloves are not washed or reused.

BSL-2 Laboratory Facilities (Secondary Barriers)

1. Each laboratory should contain a sink for hand washing.

2. The laboratory is designed so that it can be easily cleaned and disinfected. Rugs in laboratories are not appropriate, and should not be used because proper decontamination following a spill is extremely difficult to achieve.

3. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

4. Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.

5. An eyewash facility is readily available.

6. The laboratory should be at negative pressure with respect to areas outside the lab. Hoods and biosafety cabinets should be positioned away from doors, supply vents and air conditioner airflow.
**Biosafety Level 3**

Biosafety Level 3 is applicable to working with indigenous or exotic agents, such as brucella and tuberculosis, that may cause serious or potentially lethal disease through the inhalation route of 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. All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices. A BSL-3 laboratory has special engineering and design features.

**Biosafety Level 4**

Biosafety Level 4 is required for work with dangerous and exotic agents, such as Ebola, that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or re-designate the level. Laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors must be competent in handling agents and procedures requiring BSL-4 containment. The laboratory supervisor in accordance with institutional policies controls access to the laboratory.

**Section 6.3.5. Medical Monitoring**

The major purpose of medical monitoring is the early detection of disease or conditions for which treatment can prevent further illness. Medical monitoring is conducted to evaluate exposure to human and zoonotic diseases and unanticipated adverse health effects of exposure. It can also be a valuable tool for hazard control to monitor if initially effective control or work practice has lost effectiveness, or by detecting previously unknown exposures.

Medical consultations should take place:

- Whenever an injury occurs, such as a needlestick, or splash with contaminated material.
- Whenever an employee develops symptoms of exposure to a hazardous chemical or biological agent to which the employee may have been exposed in the laboratory.
- Whenever a spill, leak, explosion, or other occurrence results in the likelihood of an overexposure to a hazardous chemical or biological agent.
- When an employee requests a medical consultation due to health concerns related to assigned tasks and/or change in personal medical history, such as pregnancy, special medications, diagnosed hypersensitivities or other illnesses.
- When exposure monitoring results trigger medical surveillance requirements or when other regulations mandate medical consultations, such as for the use of respiratory protection.
Section 6.3.6. Medical Waste Management

Safe Sharps Disposal
The term “sharps” refers to any object that can cut or puncture the skin including, but not limited to, needles (hypodermic and suture), scalpels, lancets, broken vials or glass, broken capillary tubes, slides and coverslips, and exposed ends of contaminated wires. The primary cause of occupational exposure to blood-borne pathogens in field and laboratory personnel is injury from needlesticks or other sharp objects. At least 20 pathogens are known to have been transmitted following percutaneous exposure to blood. Infections with each of these pathogens are potentially life threatening – and preventable.

How to prevent sharp injuries:
- Do not bend, break, or cut sharps. Shearing or breaking of needles is prohibited.
- Concentrate on what you are doing and do not get distracted.
- Dispose of all sharps in an approved puncture-resistant container as soon after use as possible.
- Ensure this container is placed in the area where sharps are used.
- Ideally, needle and syringe should be disposed as one unit if possible. If a needle must be removed follow the directions on the Removal of the syringe needle section above.
- Do not recap needles unless absolutely necessary. If recapped, never use two hands, instead use the one-hand “scoop” technique (see Removal of the syringe needle section above).
- Do not overfill sharps disposal container. Seal the container and replace when it is ¾ full.
- Do not empty sharps containers. Dispose of whole container as one unit.
- Wear utility gloves when disposing of medical waste including sharps containers.
- To prevent sharp injuries during transport of medical waste, use a puncture-proof container that remain closed.
**Sharps Disposal Containers**

Never discard needles and sharps in waste bags, as personnel might be injured when they handle the bags.

Sharp containers are available commercially or can be adapted from some containers that comply with minimal safety standards.

There are four major criteria for sharps disposal container safety performance, functionality, accessibility, visibility, and accommodation:

**Functionality**: Containers should remain in a good state during their entire usage. They should be leak-resistant on their sides and bottoms and puncture-resistant until final disposal. Individual containers should have adequate volume and safe access to the opening.

**Accessibility**: Containers should be accessible to all workers who use, maintain, or dispose of sharp devices. Containers should be placed in all areas where sharps are used and, if necessary, be portable within the workplace or for fieldwork. Portable containers must have a lid to prevent spills and injuries during transport or while working in the field.

**Visibility**: Containers should be plainly visible to the workers who use them. Workers should be able to see the warning labels and the degree to which the container is full.

**Accommodation**: Container designs should be convenient, environmentally sound, and easy to store.
Medical Waste Disposal
Biological waste includes human and animal tissues, fluids and animal carcasses. These are generated along with the sharps and other biologically contaminated equipment that typically need to be discarded in all laboratories (e.g. pipette tips, gloves).

Animal carcasses should be bagged, sealed, and stored in freezers located in the facility until pick up for incineration.

All other biologically contaminated material should be placed in a red bag-lined medical waste box. When the medical waste box is full, it is the responsibility of the field and laboratory personnel to seal the bag, seal the box, and apply a label that contains information about the generating lab.

Section 6.3.7. Special Chemical Storage and Handling Practices

Laboratory chemical storage and handling hazards can be effectively managed if you:

- Maintain good inventory control and purchase/use the least amount possible.
- Label all stored and in-process chemicals clearly and completely.
- Adopt safe handling practices.
- Use secondary containment and practice your spill response plan.
- Segregate incompatible chemicals and store them in separate appropriate cabinets or cold-storage.
- Develop special controls for highly hazardous materials.

Inventory Control
- Purchase chemicals only in the quantities needed and in containers of the smallest practical size. Although the cost may be higher, significant savings will be gained by reduced hazardous waste disposal or clean-up costs.
• Inventory your chemical supplies at least annually and actively share or distribute excess stocks with other departments to minimize waste. Dispose of all unused and outdated chemicals through appropriate hazardous waste programs.

• Products that could also be purchased for home use, such as soap, oil, or cleaning sprays, should be part of your chemical inventory and have an MSDS on file if the product will be used in an occupational setting and could cause a health exposure in the workplace.

• Before laboratory personnel leave the laboratory, all leftover chemicals should be inventoried and distributed or disposed of.

**Labeling**

Personnel should ensure that labels on containers of hazardous chemicals are not removed or altered, particularly the manufacturer’s original label. Empty chemical containers must never be reused for another purpose, even if the labeling is changed as reactions with new liquid and residual chemical could be extremely dangerous. All bottles, containers, and other apparatus containing chemicals should be accurately and clearly labeled as to contents, hazards, and where practical, the appropriate precautions required when handling the chemical.

Avoid the use of grease pencils or other markers that will wear off.

There are three levels of complexity to labeling: original container, secondary transfer containers, and small container (vials, flask, beakers) for immediate, same-day use.

1. The manufacturer’s original labels must contain the following information:
   - Name of chemical or solution
   - Manufacturer name and emergency telephone number
   - Hazard warning (health effect or target organs)

   **When opening you must add:**
   - Date received and opened
   - Initials

2. For laboratory-prepared solutions and when chemicals are transferred to secondary containers not intended for immediate use, labels should include:
   - Name (no abbreviations) of the chemical and its concentration.
   - For prepared solutions or any secondary containers: initial and date prepared.
   - Hazard warning on the most serious health or safety hazard posed (consult MSDS). Stickers can be applied indicating "corrosive," "carcinogen," "water-reactive," "flammable," etc.
   - If special precautions are critical, expand the hazard warning to include the target organ and the required protection (e.g., "Corrosive, esp. to skin and eyes. Use gloves and goggles").
3. Containers for immediate (same-day) use should have:
   - Chemical name and its concentration
   - Date
   - Initials

**Safe Handling and Transfer**
Hand-carried chemicals should be placed in unbreakable secondary containers such as bottle carriers or acid-carrying buckets. Wheeled carts used to transport chemicals should have side guards and lipped surfaces capable of containing a break, and sturdy wheels that move easily over uneven surfaces.

Staff should wear protective aprons, gloves, goggles and closed-toed shoes when transporting chemicals.

Class I flammable liquids (any liquid having a flash point below 37.7°C should not be stored or transferred from one vessel to another in an exit access corridor, open plan building, or in an ancillary space unprotected from the exit access corridor.

Transfer of Class I liquids to smaller containers from bulk stock containers not exceeding 5 gallons in capacity should be performed in a laboratory hood, in an area provided with ventilation adequate to prevent accumulations of flammable vapor exceeding 25% of the lower flammable limit, or within an inside liquid storage area approved for dispensing.

Class I liquids should not be transferred between conductive containers of greater than 1.1 gallons, unless the containers are bonded and grounded (the process of providing an electrically conductive pathway - usually by clipping connecting wires - between a dispensing container and a receiving container [bonding], and the receiving container and an earth ground).

**Secondary Containment and Spill Control**
Liquid chemicals should be stored in corrosion-resistant trays or on spill pallets or other secondary containment to contain a break or leak.

Concentrated acids and bases should be stored in acid or caustic storage cabinets. If possible, keep corrosives stored in their original (e.g. Styrofoam cubes) shipment containers.

In the event of a chemical spill, try to turn off all reaction apparatus, especially heat sources, notify supervision immediately and follow the response steps in your facility.

**Cabinet and Shelf Storage – General Precautions**
Cabinets and other storage areas should be marked with the general class of chemical stored, and any other pertinent warnings.
Storage areas should have good general ventilation and be well lighted.

On shelves, containers should be staggered for easy access, with labels facing out. DO NOT ALPHABETIZE STORED CHEMICALS; SEPARATE BY COMPATIBILITY (see next section).

Heavy and large containers are to be placed on bottom shelves. Chemicals, especially liquids, should be stored below eye level. Larger containers should be stored on lower shelves. Exposure to heat or direct sunlight should be avoided. Avoid storing chemicals on the floor unless in approved shipping containers. Minimize open shelf or bench top storage, except for those chemicals currently being used, to prevent accidental spills and reduce the risk of fires.

Cabinets specifically for corrosives (either acids or bases) should have corrosion-resistant paint. Flammable storage cabinets should provide an airtight seal; vent holes should be kept covered and flame-arrestor kept in place.

Oxidizers MUST be stored in separate cabinets from flammables and combustibles. Oxidizers, explosives, and organic peroxides must be separated from combustibles and placed in a metal cabinet, or in an approved dry, cool, and well-ventilated location.

If acids and bases must be stored together in the same cabinet, place each in separate secondary containers (non-reactive trays) on opposite sides of the cabinet to minimize intermingling in case of a spill or drip (in other words, do not store all the acids on one shelf, and all the bases on the shelf below).

Initially assign each chemical to broad hazard classes, for example: flammable, corrosive (acids and bases), reactive oxidizer or reducer, special hazard (air/water reactive, peroxide forming chemical, store at reduced temperature or under an inert atmosphere, highly toxic).

Chemicals that possess more than one hazard (i.e., oxidizer and corrosive) are assigned to the class that represents the greater hazard for that laboratory.

Post incompatibility lists (from your MSDSs) for reference.

Hazardous chemicals should be disposed of in clearly labeled containers, and as with storage, separated by class. For example, acids should not be disposed of with bases but should be separated. The same is true for corrosives and flammables.

**Refrigerators and Freezers – Flammable Storage**

All refrigerators or freezers should be distinctly marked as to whether they are suitable for the storage of flammable liquids.

Standard household-variety refrigerators should not be used to store flammable liquids.

Flammable liquids stored in refrigerated equipment should be in closed containers.
Storage of Chemicals by Class

**Flammables and Combustibles**
Flammables are chemicals that have a flash point less than 37°C (100°F). Combustible chemicals have flash points that are 37-93°C. If stored or used improperly, flammables and combustibles can be a fire hazard.

Examples of flammable liquids include benzene, alcohols, acetone, ethers, organic acids (i.e., glacial acetic acid).

The quantity of flammable/combustible hazardous chemicals within a laboratory unit or in a laboratory work area, that is stored in the open, shall be limited to the minimum necessary to perform required tasks.

Bulk supplies of alcohol (such as 95% EtOH in drums) should be stored in an approved flammable liquids storage room.

To the greatest degree possible, the storage of flammable liquids in a laboratory work area, outside of an approved flammable liquids cabinet, or storage room should be limited to what is needed for a single day’s use. Otherwise, flammable liquids should be stored within an approved flammable liquids cabinet when not in use.

**Corrosives: Acids**
Acids are corrosive and react violently with bases. There are two main groups of acids: organic acids, and inorganic (mineral) acids. Some inorganic (mineral) acids are oxidizers and will react with organics, increase burning rate of combustibles and contribute an oxygen source to a combustion reaction. Therefore, inorganic (mineral) acids should be stored separately from organic acids.

Examples of inorganic OXIDIZING acids: perchloric acid (particularly dangerous at elevated temperature), chromic acid, nitric acid, sulfuric acid (particularly dangerous at elevated temperature).

Examples of inorganic MINERAL acids: hydrochloric acid, hydrofluoric acid, phosphoric acid.

Examples of organic acids: acetic acid, formic acid, butyric acid, propionic acid, picric acid, acrylic acid.

Oxidizing inorganic acids should be segregated from organic acids, flammable and combustible materials. Most mineral acids can be stored together, except perchloric acid (see below):

Nitric acid shall be stored separate from other acids.

Segregate acids from bases and active metals such as potassium and magnesium.
Segregate acids from chemicals that could generate toxic gases upon contact, such as sodium cyanide.

Segregate acids from solvents such as toluene and xylene.

Organic acids (e.g., glacial acetic acid) are combustible and should be stored separately or with flammables rather than with inorganic acids. Several inorganic acids are oxidizers and are therefore incompatible with organics.

**Corrosives: Bases**
Bases are corrosive and react violently with acids.

Examples: ammonium hydroxide, sodium hydroxide, calcium hydroxide, organic amines.

Segregate bases from acids. Bases are also corrosive to skin and tissue. Pay meticulous attention to PPE when using bases.

**Reactive: Oxidizers**
Oxidizers react vigorously with reducing materials. The reaction can lead to fires or explosions. Oxidizers will increase the burning rate of combustible materials and contribute oxygen to a combustion reaction.

Examples: halogens, ammonium persulfate, hydrogen peroxide, sodium dichromate, potassium permanganate, perchloric acid; at elevated temperature, ammonium nitrate (and other nitrate salts).

Keep oxidizers away from flammables, combustibles (such as paper, wood) and other reducing agents.

**Reactive: Reducers**
Reducing materials react vigorously with oxidizers. The reaction can lead to fires or explosions.

*Examples: ammonia, carbon, metals, metal hydrides, phosphorus, silicon, sulfur.*

*Store reducing materials away from oxidizers.*

**Water-reactive Chemicals**
Water reactive materials react with water, water solutions, moisture, or humidity in the air to produce heat and/or flammable gases, which can ignite.

Examples: sodium (elemental), potassium (elemental), calcium carbide, phosphorous pentachloride.
Store water reactives away from any sources of water or moisture. Review manufacturer's recommendations for special storage conditions, such as under an inert atmosphere or, as in the case of elemental sodium, under mineral oil.

*Peroxide Forming Chemicals*

Potentially explosive peroxides are formed by a free-radical reaction of hydrocarbons with molecular oxygen. Distillation, evaporation or other concentration of the peroxide can cause an explosion in contaminated hydrocarbons.

Examples: diethyl ether, tetrahydrofuran, acetaldehyde, isopropyl ether.

Store peroxide-forming chemicals away from light and heat. Carefully label all containers with the date received and the date opened. Monitor container dates and avoid keeping peroxide-forming chemicals on hand for more than a year after receipt and 6 months after opening.

*Highly Hazardous Chemicals*

Highly hazardous chemicals are defined as chemical carcinogens, reproductive toxins, acutely toxic substances, and highly reactive materials (ex. Ethidium bromide used in molecular laboratories).

Designate a Restricted Work Area. Conduct all transfers and work with these substances in a "controlled area" (i.e., a restricted access hood, glove box, or portion of a lab designated for use of highly-toxic substances) for which all personnel with access are aware of the substances being used and the necessary precautions that must be taken. Only trained and authorized personnel should work in or have access to controlled areas.

Signs and labels. Assure that the controlled area is conspicuously marked with restricted access and warning signs, such as, "WARNING: Highly-Toxic Substance in Use: Authorized Personnel Only" or "WARNING: Cancer-Suspect Agent: Authorized Personnel Only." All containers of these substances must be appropriately labeled with identity and warning such as, "Warning: High Chronic Toxicity or Cancer Suspect Agent."

Storage. Store containers of these chemicals in a ventilated, limited access area in appropriately labeled, unbreakable, chemically resistant, secondary containers.

Establish Decontamination Procedures. The need for routine decontamination of designated work area, equipment, or personnel depends on the laboratory circumstances.

Medical surveillance. When using a highly toxic substance on a regular basis (e.g., 3 times per week), consult with your supervisor concerning medical surveillance or other health concerns you may have.

Cleanup and Waste Disposal. Use chemical decontamination whenever possible. Use a vacuum cleaner equipped with a High Efficiency Particulate Air (HEPA) filter, instead of dry sweeping.
when the toxic substance is a dry powder. A wet mop may also be used when the chemical is not water reactive or otherwise incompatible with water. Ensure that all vacuum filters, bag debris, mop heads or cleaning rags, as well as waste chemicals are transferred from the designated control according to a hazardous waste disposal container. Ensure that contingency plans, equipment, and materials are available to minimize exposures to personnel and property in the event of an accident. Do not ask/expect custodial staff to clean hazardous materials spills, unless they are already members of the facility’s trained response team.

**Hazardous Waste Disposal and Spill Control**

Each container of hazardous waste is to be labeled with the following legends:

“HAZARDOUS WASTE”
Contents (be specific as to chemical):
Accumulation start date:

If a reagent container label has been removed or becomes illegible, and the identity of the contents is unknown, the container must be disposed of as soon as possible by arrangement with the facility hazardous waste coordinator.

Prior to the departure of staff, chemicals for which that person was responsible should be inventoried and discarded or returned to storage.

**Pouring hazardous waste chemicals down the drain, adding them to regular trash, or evaporating them in a local exhaust hood could be illegal actions!**

**Section 6.3.8. Training in Basic Laboratory Procedures and Protocols**

Training and education in laboratory safety need to be an ongoing process, not just an annual presentation. The most effective way to reinforce good work practices is to involve all personnel from principal researchers to volunteers in regular, periodic reviews and updates of this Basic Laboratory Safety Guide. Documentation of all forms of training is to be maintained in the laboratory as well as reported to the facility safety coordinator.

**INITIAL BASIC LAB HAZARD AWARENESS TRAINING is mandatory for all staff** and must be provided to all employees doing field and laboratory work prior to actual lab and field work, and prior to assignments involving new potential exposures. Information provided during trainings should include:

*The location and availability of the Laboratory Safety Plan, chemical inventory, Material Safety Data Sheets (MSDSs), applicable regulatory exposure limits, and other reference material regarding the safe handling, storage, and disposal of hazardous chemicals (or hazardous collections) in the lab.*
Signs and symptoms associated with exposures to hazardous chemicals and biological agents used in the laboratory, as well as the health hazards themselves.

Methods that may be used to detect the presence or release of a hazardous chemical. This could include industrial hygiene monitoring, the use of continuous monitoring devices, visual appearance, or odors of chemicals.

Methods employees can take to protect themselves from hazards, including work practices, personal protective equipment and emergency procedures listed in the LSP. This should include a discussion of the proper use and limitations of engineering controls and safety devices, including chemical and biological hoods.

Emergency response plans established by each facility’s Emergency/Disaster Response Plan, any medical or first aid response specifically recommended, extinguishment of clothing fires (Stop, Drop, and Roll), and Chemical Spill Response Plans established by each facility.
Section 6.3.9. Basic Standards and Guide Checklists

- Coordinators should provide a “Useful Contacts” list with address and numbers of local medical and emergency response services.
- Personnel should know the locations of the emergency supplies (fire extinguishers, first aid kits, spill kits, safety showers and eye wash stations), phone numbers of supervisor and exits.
- Coordinators must verify that a Material Safety Data Sheet (MSDS) for each product to be used during PREDICT activities is readily available, complete and updated.
- Personnel should know where the MSDSs are located.
- Coordinators must ensure that personnel have read and understood the MSDS before using a chemical product.
- Coordinators must have MSDS data available for emergency responders.
- Individuals that have been exposed to any hazardous chemical or biological agent should immediately report the exposure to medical authorities and supervisor.
- A complete list with the contents of the PPE kit should be available to the personnel.
- Personnel should wear appropriate PPE (lab-coat, protective glasses, gloves, closed toed shoes) for laboratory procedures.
- Inspect your PPE to ensure that it is in proper working condition before use (goggles, gloves, etc.).
- If you are working with PPE kits, ensure that the kit is stocked and material has not expired.
- Personnel must use a chemical, fume or laminar flow hood when indicated.
- All needles, scalpel blades and any other sharp instruments should be used and disposed of in a manner that prevents accidental human injury.
- All stored and in-process chemicals should be labeled clearly and completely.
- Segregate incompatible chemicals and store in appropriate cabinets or special cold-storage.
- Develop special controls for highly hazardous materials.
- Purchase chemicals only in the quantities needed and in containers of the smallest practical size.
- Inventory your chemical supplies at least annually and actively share or distribute excess stocks with other departments.
- Dispose of all unused and outdated chemicals through appropriate hazardous waste programs and NOT down the drain or by adding them to regular trash.
- Sinks and eye wash stations should be kept clear and in proper working condition.
- Staff should wash their hands and forearms after they have removed and disposed their PPE or after removing gloves.
- Food and beverages are NOT allowed in any of the labs.
- Report any lab failure (equipment, facilities, etc.) to the supervisor.
- Staff should keep BUTTONED lab coats at all times when working in the laboratory.
- All human and animal tissues, fluids and excrement should be handled in a Class II Biosafety Cabinet so that the potential for human exposure is minimized.
- Specific Biosafety levels 1 and 2 practices should be followed by personnel as warranted.
- Personnel must be familiar with hazard controls and safe operating procedures.
Section 6.3.10. List of Equipment and Supplies

☐ Lab-coat
☐ Nitrile gloves ideal, latex if not available
☐ Face-mask
☐ Goggles
☐ Face-shield
☐ Closed toed shoes
☐ Disposable (Tyvek) suit
☐ Sharp-container
☐ Medical waste box
☐ Respirator
☐ PPE Kits or Supplies
☐ Eyewash station
☐ Liquid nitrogen gloves
Section 6.3.11. References


Occupational Safety and Health Administration, Publications.  
http://www.osha.gov/pls/publications/publication.html


