BIOSAFETY MANUAL

SOUTH DAKOTA SCHOOL OF MINES & TECHNOLOGY

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The guidelines developed by the National Institutes of Health (NIH), Centers for Disease Control and Prevention (CDC), Occupational Safety and Health Administration (OSHA) are key components of this biosafety manual.

1.0 SDSM&T Laboratory Biosafety Policy

The School of Mines office of Environmental Health & Safety (EHS) has developed this manual to serve as a supplement to the campus Lab Safety Program, specifically addressing biological safety matters. The manual is part of School of Mines' biosafety program, which was established to accomplish the following goals:

- Protect personnel from exposure to infectious agents
- Prevent environmental contamination
- Provide an environment for high quality research while maintaining a safe work place
- Comply with applicable federal, state and local requirements

The biosafety manual provides university-wide safety guidelines, policies and procedures for the use and manipulation of biohazards. Planning for and implementation of biological safety must be part of every laboratory activity in which biohazard materials are used. In general, the handling and manipulation of biological agents and toxins requires the use of various precautionary measures depending on the material(s) involved. This manual will provide assistance in the evaluation, containment and control of biohazards. However, it is imperative that all parties involved or working with these materials seek additional advice and training when necessary.

Currently the School of Mines campus conducts research that requires Biosafety Level 1 and 2 (BSL 1 and BSL 2) containment only. Therefore, only the descriptions and assignments for BSL 1 and 2 are detailed herein.

2.0 Roles and Responsibility

2.1 Principal Investigator

The Principal Investigator has primary responsibility for assuring the safety of the laboratory personnel under his/her direction. This responsibility includes:

- Registration of potentially hazardous agents with the EHS Office;
- Assessment of the risks associated with the agents used and selection of appropriate safeguards;
- Preparation of a written safety plan;
- Training and supervision of staff and students in safe practices;
- Reporting of accidents and exposures of laboratory personnel to the EHS Office.

2.2 Environmental Health and Safety Office

The EHS Office is responsible for the biological safety program within the university and for implementation of policies established by the university. The duties include the following:

- Providing general surveillance over activities involving biohazardous agents, including routine inspections of all areas in which biohazardous agents are used;
- Determining compliance with university policies and safety guidelines;

- Monitoring and reviewing the performance and maintenance of containment systems designed for occupational or environmental protection;
- Providing consulting services on aspects of biological safety to personnel at all levels of responsibility;
- Providing guidance and assistance concerning the packaging and shipping of biohazardous agents leaving the institution;
- Maintaining records of agents used on campus, their risk classification, location and Principal Investigator;
    - This is accomplished using the SD Mines Biosafety Protocol form.
- Serving as liaison with NIH, CDC, and other research organizations on matters pertaining to biological safety;

3.0 General Lab Practices

The following information applies to all laboratories housing biological materials. Information specific to BSL 1 and BSL 2 will follow.

3.1 Routes of Infection

An infection occurs when disease-causing microorganisms enter the human body in sufficient numbers and by a particular route and overcome the body's defense system. The following routes of infection have been reported for laboratory-acquired infections:

1. Through the mouth

- Eating, drinking and smoking in the laboratory
- Mouth pipetting
- Transfer of microorganisms to mouth by contaminated fingers or articles

2. Through the skin

- Accidental inoculation with a hypodermic needle, other sharp instrument or glass
- Cuts, scratches

3. Through the eye

- Splashes of infectious material into the eye
- Transfer of microorganisms to eyes by contaminated fingers

4. Through the lungs

- Inhalation of airborne microorganisms

3.2 Access

When procedures are in progress, the lab door should be shut and when no one is present in the lab the doors should be locked. Anyone requesting access to the laboratory, as with any laboratory, should be questioned as

to their purpose.

3.3 Personal Protective Equipment (PPE)

Personal protective equipment is used to protect personnel from contact with hazardous materials and infectious agents. Appropriate clothing may also protect the materials from contamination. Personal protective devices and safety equipment, as well as training in the proper use of those devices and equipment, must be provided to all personnel under the appropriate circumstances. The personnel have the responsibility of properly using the equipment.

3.3.1 Eye and Face Protection

Safety glasses must be worn in the lab whenever procedures are underway involving a probability of splash, work with low hazard chemicals, or an impact hazard.

Whenever possible, lab operations should be performed in containment devices such as a biological safety cabinet or fume hood, or behind a bench-top shield in order to minimize the potential for skin or mucous membrane contact with a hazardous splash. If procedures do not permit containment of the hazard with a containment device, then appropriate PPE must be worn as outlined:

- Splash goggles are the only form of eye protection approved for splash hazards. If a chemical (including bleach) or biological splash hazard exists, splash goggles must be worn.
- Full face protection (i.e., face shield) must be used for procedures that have anticipated splashes or sprays of infectious or other hazardous materials to the face or if there is a high potential for aerosol generation. Face shields are not a replacement for eye protection.

3.3.2 Gloves

Gloves must be selected based on the hazards involved and the activity to be conducted. Gloves must be worn when working with biohazards, toxic substances, hazardous chemicals and other physically hazardous agents. Temperature resistant gloves must be worn when handling hot material or dry ice. Delicate work requiring a high degree of precision dictates the use of thin walled gloves.

When latex gloves have been chosen, alternatives should be made available. Gloves should be changed as soon as possible after they have become contaminated; when their integrity has been compromised or when necessary. Hands should be properly washed with soap and water after removing gloves. Disposable gloves must not be washed or reused.

Gloves should be removed and hands washed when work with potentially infectious materials is complete or when leaving the laboratory. If you are transporting potentially infectious materials (i.e., cultures, waste, etc.) to another part of the building use the one glove rule: use one gloved hand for handling the materials and use the other ungloved hand for touching common surfaces such as door knobs and elevator buttons.

3.4 Housekeeping

Good housekeeping in laboratories is essential to reduce risks and protect the integrity of biological experiments. Routine housekeeping must be relied upon to provide work areas free of significant sources of contamination. Housekeeping procedures should be based on the highest degree of risk to which personnel and experimental integrity is appropriate.

Laboratory personnel are responsible for cleaning laboratory benches, equipment and areas that require specialized technical knowledge. To facilitate decontamination, the laboratory should be kept neat and free of clutter - surfaces should be clean and free of infrequently used chemicals, glassware and equipment. Access to sinks, eyewash stations, emergency showers and exits, and fire extinguishers must not be blocked.

4.0 Laboratory Biosafety Level Criteria

4.1 Biosafety Levels 1-4 Definitions

4.1.1 Biosafety Level 1

Suitable for work involving well-characterized agents not known to cause disease in healthy adult humans and of minimal potential hazard to laboratory personnel and the environment.
Examples: *Bacillus subtilis*, *Naegleria gruberi* Infectious canine hepatitis virus,

4.1.2 Biosafety Level 2

Suitable for work involving agents of moderate potential hazard to personnel and the environment
Examples: Measles virus, Salmonellae, Toxoplasma species, Hepatitis B virus, primary human cells, viral vectors

4.1.3 Biosafety Level 3

Suitable for work with infectious agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route.
Examples: Mycobacterium tuberculosis, St. Louis encephalitis virus, Coxiella burnetii

4.1.4 Biosafety Level 4

Suitable for work with dangerous and exotic agents that pose a high individual risk of aerosol transmitted laboratory infections and life threatening disease.
Examples: Ebola Zaire virus, Rift Valley Fever virus

4.2 Biosafety Level 1 (BSL 1)

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is neither required nor generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

4.2.1 BSL 1 Laboratory Design and Facilities

The facilities required in a biosafety level 1 laboratory include the following:

- Doors
  Doors are required for access control. They should be kept locked when no one is present in the laboratory.

- Sink
  A sink must be available and supplied for hand washing (i.e., stocked with soap and paper towels).

- Easily cleanable
  Lab must be designed in a way that allows it to be cleaned easily. Carpets and rugs are not allowed. Spaces between benches, cabinets and equipment must be accessible for cleaning.

- Furniture
  Furniture in the lab must be appropriate for the anticipated use. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis and other chemicals. Chairs used in conjunction with lab work must be covered with a non-porous material that can be easily cleaned and disinfected.

- Windows
  If the lab has windows that can be opened to the outdoors, they must be fitted with screens.

4.2.2 BSL 1 Standard Practices

- Access to the laboratory is limited or restricted at the discretion of the Principal Investigator when experiments are in progress.
- Work surfaces are decontaminated at least once a day and after work with infectious materials is finished, and after any spill of viable material is cleaned with disinfectants that are effective against the agents of concern.
- All contaminated liquid or solid wastes are decontaminated before disposal.
- Mechanical pipetting devices are used; mouth pipetting is prohibited.
- Policies for the safe handling of sharps are instituted. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably autoclaved.
- Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only. No preparation, storage or consumption of food or drink is permitted in the lab.

- Persons wash their hands:
    - after handling materials involving organisms containing recombinant DNA molecules and animals
    - before exiting the laboratory
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- A biohazard sign must be posted at the entrance to the laboratory whenever infectious agents are present. The sign must include the name of the agent(s) in use and the name and the phone number of the investigator. Please see Appendix A for an example of an appropriate hazard warning sign.

4.2.3 BSL 1 Special Practices

- Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable, hard-walled, leak-proof container that is closed before being removed from the laboratory.

4.2.4 BSL 1 Special Equipment

Special containment equipment is generally not required for manipulations of agents assigned to BSL 1.

- Gloves should be worn if the skin on the hands is broken or if a rash is present.
- Protective eyewear should be worn for conduct of procedures in which splashes of microorganisms or other hazardous materials is anticipated.

4.3 Biosafety Level 2 (BSL 2)

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 BSCs or other physical containment equipment.

The following standard and special practices, safety equipment, and facility requirements apply to BSL-2:

4.3.1 BSL 2 Laboratory Design and Facilities

- Eye wash stations
  An eyewash station must be readily available.

- Decontamination
  A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

- Doors
  Self-closing doors are required for access control. They should be kept locked when no one is present

in the laboratory.

- Sink
  A sink must be available and supplied for hand washing (i.e., stocked with soap and paper towels). The sink may be manually operated, hands-free, or automatically operated. It should be located near the exit door.

- Easily cleanable
  The lab must be designed in a way that allows it to be cleaned easily. Carpets and rugs are not allowed. Spaces between benches, cabinets and equipment must be accessible for cleaning.

- Furniture
  Furniture in the lab must be appropriate for the anticipated use. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis and other chemicals. Chairs used in conjunction with lab work must be covered with a non-porous material that can be easily cleaned and disinfected.

- Biosafety cabinets
  BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.

  HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

- Vacuum lines
  Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps are required.

- Ventilation Systems
  There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.

- Windows
  If the lab has windows that can be opened to the outdoors, they must be fitted with screens.

4.3.2 BSL 2 Standard Microbiological Practices

- Access to the laboratory is limited or restricted at the discretion of the Principal Investigator.
- Work surfaces are decontaminated at least once a day and after work with infectious materials is finished, and after any spill of viable material is cleaned with disinfectants that are effective against the agents of concern.

- All contaminated liquid or solid wastes are decontaminated before disposal.
- Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
- Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport: Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, hard-walled, leak proof container and secured for transport.
- Mechanical pipetting devices are used; mouth pipetting is prohibited.
- Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
    - Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
    - Used disposable needles and syringes 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 processing area for decontamination, preferably by autoclaving.
    - Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
- Persons should wash their hands:
    - before exiting the laboratory
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- A biohazard sign must be posted at the entrance to the laboratory whenever infectious agents are present. The sign must include the name of the agent(s) in use and the name and the phone number of the investigator. Please see Appendix A for an example of an appropriate hazard warning sign.
- Potentially infectious agent hazard information should be posted in accordance with the institutional policy.
- An effective integrated pest management program is required.
- The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

4.3.3 BSL 2 Special Practices

- All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements depending on the space.
- Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
- When appropriate, a baseline serum sample should be stored.
- A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
- The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
- Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
- Laboratory equipment should be routinely decontaminated, as well as after spills, splashes, or other potential contamination.
- Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
- Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
- Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
- Animals and plants not associated with the work being performed must not be permitted in the laboratory.
- All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment device.

4.3.4 BSL 2 Special Equipment (Primary Barriers and Personal Protective Equipment)

- Properly maintained BSCs (preferably Class II), other appropriate personal protective equipment, or other physical containment devices must be used whenever:
    - Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
    - High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
- Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Dispose of protective clothing appropriately, or each department is responsible to determine an alternative method for safely laundering.. It is recommended that laboratory clothing not be taken home.
- Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with

other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.

- Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
    - Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
    - Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
    - Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
- Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

5.0 Recommended Work Practices

5.1 Autoclaves

This program establishes the following requirements:

- All potentially infectious materials and other biohazardous waste generated through research and teaching activities must be effectively decontaminated prior to disposal.
- Decontamination of materials must be verified using standard and approved procedures.

5.1.1 Autoclave Responsibilities

5.1.1.1 Responsible Technicians

This person is identified by the department who maintains the autoclaves for that space.

- Provide autoclave-specific training directly and schedule program management training, as needed.
- Develop and maintain standard operating procedures for proper autoclave use and management.
- Ensure that all documentation is current and accurate.
- Monitor autoclave use.
- Serve as the contact person and primary resource for answering questions and resolving problems related to the autoclave.
- Ensure all verification testing is performed and maintain associated testing supplies.
- Ensure that user log sheets, cycle charts, safety warnings, operational notices, and contact information for responsible parties are posted near the autoclave.

5.1.1.2 Autoclave Users

- Participate in required training.
- Follow safe and appropriate autoclave use procedures.
- Complete autoclave use log.
- Promptly report problems or misuse to the Responsible Technician.
- When in doubt, ask questions.

5.1.2 Procedure

5.1.2.1 Autoclaving Precautions

Autoclaving, or steam sterilization, is the most dependable procedure for the destruction of all forms of microbial life. Proper temperature and exposure time are critical factors in ensuring the reliability of this method. These critical factors are dependent upon steam penetration to every part of the waste load. Therefore, the autoclave user must be mindful to prevent the entrapment of air. If all the air is not allowed to escape from the waste during the cycle, steam will not replace the air. Saturated steam is employed under pressure (at least 15 pounds per square inch) to achieve a chamber temperature of at least 121 °C (250 °F) for a minimum of 30 minutes or as your procedure requires. This time is measured after the temperature of the steam saturated material being sterilized reaches 121 °C.

The hazards associated with autoclaves include extreme heat and high pressure, as well as large, heavy doors and loading carriage. When operating an autoclave the following safety procedures must be followed:

1. Become familiar with the autoclave's owner's manual. Though the principle is the same for each, manufacturer recommendations for use can vary widely.
2. Firmly lock autoclave doors and gaskets in place before you run the autoclave to prevent a sudden release of high-pressure steam. Some autoclaves do not have safety interlocks that prevent the autoclave from running if the door isn't closed properly. If your autoclave does not have safety interlocks, you will need to take additional precautions to ensure that the doors are closed.
3. If you have an older autoclave that has little or no heat shielding around the outside, attach signs warning of "Hot Surfaces, Keep Away" on or next to the autoclave to remind people of the hazard. Do not stack or store combustible materials (cardboard, plastic, volatile or flammable liquids, compressed gas cylinders) next to an autoclave.
4. Do not autoclave toxic, volatile or radioactive material. If you have biohazard waste that contains any of these materials, please contact EHS for guidance.
5. When a cycle is complete, wait until you are sure the pressure gauge registers 0 PSI before opening the door of the autoclave.
6. Wait at least 30 seconds after opening the door before reaching or looking into the autoclave.
7. Open the door slowly, keeping head, face, and hands away from the opening.
8. Allow contents to cool before removing them from the autoclave.
9. Remove solutions from the autoclave slowly and gently; some solutions can boil over when moved or when exposed to room temperature. Thick, heat-resistant gloves, safety goggles or face-shield and a rubber apron must be worn when removing hot liquids from the autoclave. Liquids should stand for over 1 hour before being handled without heat-resistant gloves.
10. Clean up any spills immediately.
11. Report any malfunctions or accidents to your supervisor immediately.

Procedures for School of Mines' autoclaves:

- Strong oxidizing material (chemicals) must not be autoclaved with organic material due to an increased risk of explosion.
- All biohazardous waste must be placed in orange biohazard bags with a heat sensitive "Autoclaved" indicator.
- Prior to autoclaving, a biohazard bag containing waste must be kept closed to prevent airborne contamination and nuisance odors. However, when autoclaving, the bag must be open to allow the steam to penetrate. Upon removal of the bag from the autoclave, it should be closed and disposed of in an opaque (black) waste bag
- It is recommended to add water to each bag before autoclaving
- Autoclave biohazardous materials using the recommended parameters posted on the autoclave

5.1.2.2 Cycle Optimization

Factors which must be considered for determining cycle types and times include:

- Shape/size/volume of containers and materials,
- Thermoconductive properties of containers and contents,
- Density of solid materials and viscosity of liquids,
- Position of load within the chamber, and
- Load configuration.

For most biohazardous waste, the autoclave must reach and maintain the following conditions at the most difficult location of the load to heat (e.g., inside of a package, autoclave bag): 121 °C and 15 psi for at least 30 minutes. However, longer lengths of time or greater temperatures or pressures may be needed for some materials.

Because the **entire** waste load must be exposed to the appropriate conditions, denser loads will require longer run times for effective steam/heat penetration. Cycle parameters for such loads must be developed, primarily by adjusting cycle times, so that successful sterilization is achieved.

Waste decontamination loads should always be run separately in the autoclave from loads of goods to be sterilized for use.

For every waste load that is decontaminated, regardless of cycle type, processing time, load composition or load configuration, a Class 5 Chemical Indicator (CI) must be used to determine sterilization effectiveness. This measure is necessary to ensure that waste, after autoclaving, can be handled and disposed of safely, and thus protects the health of research personnel as well as the general public.

5.1.2.2 Load Configuration

Always configure a waste load in a manner that avoids stacking, crowding, and touching the sides of the autoclave chamber. Load the waste to allow for air flow around waste containers.

Once a commonly-used load configuration has been proven to decontaminate waste successfully, it is recommended that it be documented and posted for reference by other autoclave users to save time, resources, and utilities.

5.1.2.3 Load Composition and Preparation

Primary containers must not be filled beyond 75% of holding capacity and must be made of materials that will remain structurally intact during autoclaving. All primary containers must allow steam penetration through loosened, vented openings or permeable materials. Secondary containers must be used which are deep enough to contain spills (e.g., stainless steel pans).

SPECIAL CONSIDERATIONS FOR LIQUIDS:

- Bottles or flasks of liquid waste, as well as liquids to be sterilized for further use, must ALWAYS be run using a Liquids Cycle that includes a slow exhaust to avoid boil-over.
- Do not decontaminate solid waste and liquid waste (in bottles or flasks) together in the same load.
- Minute volumes of liquid waste (e.g., in Petri dishes, conical tubes, microtiter plates, or vials), when bagged, can be mixed with solid waste (e.g., disposable gloves, paper towels) and autoclaved successfully using a Solids/Gravity cycle or Pre-vacuum cycle.
- Do not autoclave liquid waste containing hazardous chemicals or oxidizers.

5.1.3 Performance Verification/Monitoring

Each load processed in an autoclave must meet the operating parameters set up for that particular cycle, determined by the use of a Class 5 CI. Any load which fails to be exposed to the operating parameters must be processed again.

Performance verification per load must occur as follows:

- An externally placed, Class1 CI (i.e., autoclave indicator tape) must be used with each container, bag, or item processed in order to distinguish it from unprocessed materials.
- For every load, the user must verify and document operating parameters using an internally placed Class 5 CI. Chemical Indicators must be 1) within a commercially available challenge test pack, or 2) placed in a central position within the load. Products are available which aid in the placement and retrieval of CIs from within bags of waste.

Self-fashioned test packs which simulate commercial products can be tested and used with prior approval.

5.1.4 Monthly Performance Testing

Operating parameters (i.e., time, temperature, and pressure) for each programmed cycle (i.e., cycle type, time, and temperature) used for decontamination must be verified monthly by BI (biologic indicator) testing.

To perform this verification, BI vials must be 1) within a commercially available challenge test pack, or 2) placed in a central position within an actual or mock load of waste, to offer sufficient challenge for steam penetration.

5.1.4.0 Performance Failures

Any of the following must be taken as an indication that sterility was not achieved:

- Printout of process parameters shows sterilization conditions not met,
- Autoclave malfunctions, aborted cycles, or alarms during a cycle,
- Failure of monthly Biological Indicator Sterility Test, or
- Failure of CIs to show adequate processing occurred.

5.1.4.1 Failure Responses

Autoclave malfunction (e.g., printout, alarm, aborted cycle, power failure)

- The Responsible Technician must be notified immediately and the autoclave taken out of service for waste processing until the problem is rectified.
- The Responsible Technician will determine if service personnel are required to restore function and will arrange for repairs, if needed.

Failure of a Biological Indicator Test

- The Responsible Technician must be immediately notified of any BI test failure and the autoclave taken out of service for waste processing until the cause of the failure is found and rectified.
- Positive test results from BI can result from a variety of causes, such as inadequate steam quality, insufficient exposure time or temperature, poor loading practices, or product failure or operator failure.
- All possible causes should be investigated by the Responsible Technician.
- Proper autoclave function must be verified using repeat BI testing by the Responsible Technician.
- Once verified, the autoclave may be returned to service for waste processing.

Failure of Chemical Indicators (Class 1 or 5)

- The Responsible Technician must be immediately notified of any CI failure and the autoclave taken out of service for waste processing until the cause of the failure is found and rectified.
- CI failures can result from a variety of causes, such as inadequate steam quality, insufficient exposure time or temperature, poor loading practices, or product failure or operator failure.
- All possible causes should be investigated by the Responsible Technician.
- Proper autoclave function must be verified using repeat CI and/or BI testing by the Responsible Technician.
- Once verified, the autoclave may be returned to service for waste processing.

5.1.5 Documentation

Autoclave Use Log

- Must be maintained separately for each autoclave.
- Must be located in close proximity to the autoclave.
- All runs must be documented.

Service Log

- Must be maintained separately for each autoclave.
- Responsible technician must document maintenance, repair, and calibration services in this log.

Biological Indicator Test Results Log

- Must be maintained separately for each autoclave.
- New autoclave and monthly test results must be recorded in this log.

5.2 Sharps

Generally, the use of sharps should be restricted to procedures for which there is no alternative. Plastic alternatives should be substituted for glassware whenever possible to prevent the unnecessary potential for sharps related exposure incidents. If it has been determined that the use of sharps is unavoidable, the following practices should be adhered to:

- All personnel should be trained in safe sharps handling procedures.
- Use disposable sharps devices (i.e., scalpels, probes, needles) if at all possible.
- Procedures should be organized in a manner that limits personnel exposure to the sharp device. For example:
    - Do not expose/unsheathe sharp devices until the procedure actually requires the use of these items.
    - Do not leave exposed sharp items unattended.
    - If feasible, place an approved sharps container within arm's reach of the point of use for the sharp item to allow for immediate disposal (for reusable sharps, use a hard-walled container that encloses the sharp end of the device).
- Do not bend or break sharps.
- Do not recap sharps if possible. If recapping is required, use a one-handed scoop technique. Note: The need for recapping can be eliminated through the use of safer sharps devices.
- Do not handle sharps with two hands.
- Dispose of waste sharps in a properly labeled approved sharps container.
- Permanently close and dispose of sharps containers when they are ¾ full or within 90 days of the date of first use, whichever comes first. Do NOT overfill or shake containers because these actions can result in accidental sharps exposure.
- Reusable sharps should be placed in a hard walled container for storage until processing for reuse.
- Broken glassware should be handled with a mechanical device, such as tongs, forceps, or a broom and dustpan rather than directly by hand.

5.3 Decontamination

Decontamination is defined as the reduction of microorganisms to an acceptable level. Methods applied to reach this goal can vary and most often include disinfection or sterilization. Generally speaking, disinfection is used to make the acceptable level of microorganisms present is defined as being below the level necessary to cause disease. This means that viable microorganisms are still present. In contrast, sterilization is defined as the complete killing of all organisms present. Depending on the circumstances and tasks, decontamination of a surface (e.g., lab bench) is accomplished with a disinfectant, while decontamination of biomedical waste is done by sterilization in an autoclave.

Many different terms are used for disinfection and sterilization. The following are commonly used in biosafety:

- *Antimicrobial* - An agent that kills microorganisms or suppresses their growth and multiplication.
- *Antiseptic* - A substance that inhibits the growth and development of microorganisms without necessarily killing them. Antiseptics are usually applied to body surfaces.
- *Biocide* - A general term for any agent that kills organisms.
- *Chemical germicide* - A chemical or a mixture of chemicals used to kill microorganisms.

- *Disinfectant* - A chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects.
- *Microbicide* - A chemical or mixture of chemicals that kills microorganisms. The term is often used in place of "biocide", "chemical germicide" or "antimicrobial."
- *Sporocide* - A chemical or mixture of chemicals used to kill microorganisms and spores.

When choosing a method of decontamination, it is important to consider the following aspects:

- Type of biohazardous agents, concentration and potential for exposure;
- Physical and chemical hazards to products, materials, environment and personnel.

5.3.1 Decontamination Processes

Physical and chemical means of decontamination fall into four main categories:

Heat

In order to kill microbial agents, heat can be applied in dry or wet form. The advantage of wet heat is a better heat transfer to and into the cell resulting in shorter exposure time and lower temperature. Steam sterilization uses pressurized steam at 121-132 °C (250-270 °F) for 30 or 40 minutes. This type of heat kills all microbial cells including spores, which are normally heat resistant. In order to accomplish the same effect with dry heat in an oven, the temperature needs to be increased to 160-170 °C (320-338 °F) for periods of 2 to 4 hours.

Decontamination of Biohazardous Waste by Autoclaving
Autoclaving is accepted as a safe and effective procedure for sterilization when performed in accordance with the policies described in §5.1 Autoclaves. To ensure that any biohazardous waste created by the School of Mines community is properly decontaminated, each autoclave will be tested on a monthly basis as described in §5.1.4 Monthly Performance Testing.

Liquid chemicals

The appropriate liquid disinfectant should be chosen after carefully assessing the biohazardous agent and the type of material to be decontaminated. Liquid disinfectants are preferably used for solid surfaces and equipment. They vary greatly in their efficiency, depending on the chemical constituents and the agents involved. Variables to remember when disinfecting:

- Nature of the surface being disinfected - Porous or smooth; the more porous and rough the surface, the longer a disinfectant will need to remain on it to be effective.
- Number of microorganisms present - Higher concentrations require a longer application time and/or higher concentration of disinfectant.
- Presence of organic material - The proteins in organic materials such as blood, bodily fluids, and tissue can prevent or slow the activity of certain disinfectants.
- Duration of exposure and temperature - Increased exposure time increases the effectiveness of disinfectants. Low temperatures may slow down the activity requiring more exposure time.

EPA regulation of disinfectants

The Environmental Protection Agency (EPA) regulates pesticides, including chemical disinfectants, under the Federal Insecticide, Fungicide, and Rodenticide Act. They are required to be registered with the EPA. It is important to follow the directions on the manufacturer's label, including those for concentration and contact time, when using disinfectants to ensure compliance with the EPA requirements.

There are many different liquid disinfectants available under a variety of trade names. In general, these can be categorized as halogens, acids or alkalines, heavy metal salts, quaternary ammonium compounds, aldehydes, ketones, alcohols, and amines. Unfortunately, the most effective disinfectants are often corrosive and toxic.

Alcohols:
Ethyl or isopropyl alcohol at a concentration of 70% are good general-use disinfectants. However, they evaporate fast and therefore have limited exposure time. They are less active against non-lipid viruses and ineffective against bacterial spores.

Formalin:
Formalin is a 37% solution of formaldehyde in water. Dilution of formalin to 5% results in an effective disinfectant. Formaldehyde is a suspected human carcinogen and creates respiratory problems at low levels of concentration.

Glutaraldehyde:
This compound, although chemically related to formaldehyde, is more effective against all types of bacteria, fungi, and viruses. Vapors of glutaraldehydes are irritating to the eyes, nasal passages and upper respiratory tract. They should always be used in accordance with the instructions on the label and the appropriate personal protective equipment.

Phenol and Phenol Derivatives:
Phenol based disinfectants come in various concentrations ranging primarily from 5% to 10%. These derivatives, including phenol, have an odor which can be somewhat unpleasant. Phenol itself is toxic and appropriate personal protective equipment is necessary during application. The phenolic disinfectants are used frequently for disinfection of contaminated surfaces (e.g., walls, floors, bench tops). They effectively kill bacteria including Mycobacterium tuberculosis, fungi and lipid-containing viruses. They are not active against spores or non-lipid viruses.

Quaternary Ammonium Compounds (Quats):
Quats are cationic detergents with strong surface activity. They are acceptable for general-use disinfectants and are active against Gram-positive bacteria and lipid-containing viruses. They are less active against Gram-negative bacteria and are not active against non-lipid-containing viruses. Quats are easily inactivated by organic materials, anionic detergents or salts of metals found in water. If Quats are mixed with phenols, they are very effective disinfectants as well as cleaners. Quats are relatively nontoxic and can be used for decontamination of food equipment and for general cleaning.

Halogens (Chlorine and Iodine):
Chlorine-containing solutions have broad spectrum activity. Sodium hypochlorite is the most common base for chlorine disinfectants. Common household bleach (5% available chlorine) can be diluted 1/10 to 1/100 with water to yield a satisfactory disinfectant solution. Chlorine containing disinfectants are inactivated by excess organic materials. They are also strong oxidizers and very corrosive. Always use appropriate personal protective equipment when using these compounds. At high concentrations and extended contact time,

hypochlorite solutions are considered cold sterilants since they inactivate bacterial spores. Iodine has similar properties to chlorine. Iodophors (organically bound iodine) are recommended disinfectants. They are most often used as antiseptics and in surgical soaps and are relatively nontoxic to humans.

Vapors and Gases

A variety of vapors and gases possess germicidal properties. The most commonly used are formaldehyde and ethylene oxide. Applied in closed systems under controlled conditions (e.g., humidity) these gases achieve sterility.

Formaldehyde gas is primarily used in the decontamination of spaces or biological containment equipment like biological safety cabinets. Formaldehyde is a toxic substance and a suspected human carcinogen. Considerable caution must be exercised in handling, storing, and using formaldehyde. Ethylene oxide is used in gas sterilizers under controlled conditions. Ethylene oxide is also a human carcinogen and monitoring is necessary during its use.

Incineration

Incineration is useful for disposing of animal carcasses as well as anatomical and other laboratory waste, with or without prior decontamination.

6.0 Biohazardous Waste

At SDSM&T, the term **biohazardous waste** is used to describe different types of waste that might include infectious agents. Currently, the following categories are all considered to be biohazardous waste, as defined by *South Dakota Administrative Rule 74:35*.

Medical waste

Medical waste means any solid waste which is generated in the diagnosis, treatment, or immunization of human beings or animals, in research, or in the production or testing of biologicals. It does not include any hazardous waste, radioactive waste, or household waste.

Regulated Medical Waste

Regulated medical waste is solid waste generated in the diagnosis, treatment, or immunization of human beings or animals, in research pertaining to diseases of humans or animals, or in the production or testing of biologicals, as listed in this section:

1) Cultures and stocks: cultures and stocks of infectious agents and associated biologicals, including the following:

    a) Cultures from medical and pathological laboratories;

b) Cultures and stocks of infectious agents from research and industrial laboratories;
c) Wastes from the production of biologicals;
d) Discarded live and attenuated vaccines; and
e) Culture dishes and devices used to transfer, inoculate, and mix cultures;

2) Pathological waste: human pathological waste, including:

a) Tissues, organs, and body parts and body fluids that are removed during surgery, autopsy, or other medical procedures except those extracted teeth that are returned to the patient; and
b) Specimens of body fluids and their containers;

3) Human blood and blood products, as follows:

a) Liquid waste human blood;
b) Products of blood;
c) Items saturated or dripping with human blood;
d) Items that were saturated or dripping with human blood that are now caked with dried human blood;
e) Serum, plasma, and other blood components, and their containers, which were used or intended for use in either patient care, testing, and laboratory analysis or the development of pharmaceuticals; and
f) Intravenous blood and blood product bags;

4) Sharps: sharps that have been used in animal or human patient care or treatment or in medical, research, or industrial laboratories, including the following:

a) Hypodermic needles;
b) Syringes with the attached needle or containing body fluids;
c) Pasteur pipettes;
d) Scalpel blades;
e) Blood vials;
f) Needles with attached tubing;
g) Culture dishes, regardless of the presence of infectious agents; and
h) Other types of broken or unbroken glassware that were in contact with infectious agents, such as used slides and cover slips;

5) Animal waste: contaminated animal carcasses, body parts, and bedding of animals that were known to have been exposed to infectious agents during research (including research in veterinary hospitals), production of biologicals, or testing of pharmaceuticals;

6) Isolation waste: biological waste and discarded materials contaminated with blood, excretion, exudates, or secretions from humans who are isolated to protect others from certain highly communicable diseases as identified by the health care facility or isolated from animals known to be infected with highly communicable diseases;

7) Unused sharps: the following unused, discarded sharps:

a) Hypodermic needles;
b) Suture needles;
c) Syringes with the attached needle; and
d) Scalpel blades.

6.1 General Labeling, Packaging and Disposal Procedures

The responsibility for decontamination and proper disposal of biohazardous waste lies with the producing facility (e.g., laboratory and department).

All biohazardous waste needs to be packaged, contained and located in a way that protects and prevents the waste from release at any time at the producing facility prior to ultimate disposal. If storage is necessary, putrefaction and the release of infectious agents into the air must be prevented.

If not stated otherwise, biohazardous waste will be disposed of in biohazard bags. All waste disposed of in these bags is to be autoclaved in an approved autoclave until the waste is decontaminated. After successful autoclaving (decontamination), all biohazard bags need to be bagged in opaque (black) plastic non-biohazard bags that are leak proof. These opaque bags can be picked up by custodial services. Biohazardous waste that has been successfully decontaminated by autoclaving is no longer considered hazardous.

Since autoclaves are an integral part of School of Mines' biohazardous waste treatment procedure, proper operation and maintenance as described in §5.1 Autoclaves is very important. All users of autoclaves need to be trained in the proper operating procedures by either through the laboratory supervisor or the technician assigned to that autoclave. Maintenance and repair of autoclaves used for the decontamination of biohazardous waste are the responsibility of the individual departments. If the department chooses to not use autoclaves for their biohazardous waste treatment, alternative procedures (e.g., outside biomedical waste hauler) need to be established.

6.1.1 BSL 1-Specific Waste Procedures

Cultures, Stocks and Related Materials

Cultures and stocks of infectious agents and associated biologicals, shall be placed in biohazard bags and decontaminated by autoclaving. Double or triple bagging may be required to avoid rupture or puncture of the bags.

Bulk Liquid Waste, Blood and Blood Products

All liquid waste from humans or animals such as blood, blood products and certain body fluids, not known to contain infectious agents, can be disposed of directly by flushing down a sanitary sewer. However, due to coagulation, flushing of large quantities of blood is impractical. Contact the EHS Office for additional information on disposal of large volumes of blood. All other liquid biohazardous waste needs to be autoclaved prior to disposal or treated with a disinfectant.

Sharps

All sharps must be placed in a rigid, puncture resistant, closable and leak proof container, which is labeled with

the word **"Sharps"** and the biohazard symbol. Food containers (e.g., empty coffee cans) are **not permissible** as sharps containers. When a sharps container is first put into use it must be labeled with a completed sharps label. All sharps must be handled with extreme caution. The clipping, breaking, and recapping of needles is not recommended. Sharps containers should not be filled more than ¾ full.

Contaminated Solid Waste

Contaminated solid waste includes cloth, plastic and paper items that have been exposed to agents that are infectious or hazardous to humans, animals, or plants. These contaminated items shall be placed in biohazard bags and decontaminated by autoclaving. Double or triple bagging may be required to avoid rupture or puncture of the bags. **Contaminated Pasteur pipettes are considered sharps** and need to be disposed of in a sharps container.

Contaminated Animal Carcasses, Body Parts or Human Tissue

Small animal carcasses and unrecognizable body parts inoculated with infectious agents are disposed of by autoclaving. Incineration is currently not an available technique, so if a large animal carcass is used a disposal plan must be developed prior to experiment start date.

Unrecognizable human tissues can be autoclaved and disposed of as described under Contaminated Solid Waste. If the tissues have been chemically preserved, the tissue should be removed from the chemical prior to autoclaving. The chemical would then be disposed of as chemical hazardous waste.

7.0 Spill Cleanup Procedures

Since spills of biological materials will happen, it is important to be prepared prior to dealing with the problem. In BSL 1 labs, although it is preferable to have the contents of a spill kit in one location, as long as the materials are easily accessible to everyone in the lab, prior assembly might not be necessary.

The following procedures are provided as a guideline to biohazardous spill clean-up and will need to be modified for specific situations. As with any emergency situation, stay calm, call 911 if necessary, and proceed with common sense. Call the Environmental Health and Safety Office 605.394.6020 or Campus Safety 605-394-6100 if further assistance is required, especially if the spill outgrows the resources in the laboratory.

7.1 Spills with NO broken glass/sharps

1. Put on two layers of gloves. Put on splash goggles.
2. Prepare the disinfectant solution, following the manufacturer's recommendations for concentration.
3. Cover the spill area with absorbent material (i.e., Superfine or paper towels).
4. Using the broom and dustpan, remove absorbent powder and deposit it in the biohazard bag, or if using paper towels, place them in the biohazard bag for disposal.
5. Spray the contaminated area with disinfectant and wait the appropriate contact time. Remove disinfectant with paper towels and place the paper towels in the biohazard bag for disposal.
6. Repeat step 5 to allow for sufficient disinfection of contaminated surfaces.
7. Remove outer pair of gloves only and dispose of them in the biohazard bag.

8. Remove goggles with inner gloves still on, and clean the goggles with an antimicrobial towelette. Also wipe down contact surfaces of disinfectant container.
9. Remove inner gloves and dispose of them in biohazard bag.
10. Place the biohazard bag in a biohazardous waste container for treatment and disposal.
11. Wash your hands with anti-bacterial soap and water as soon as possible.

7.2 Spills involving broken glass/sharps

1. Retrieve a sharps container for disposal of glass/sharps.
2. Put on two layers of gloves. Put on splash goggles.
3. Prepare the disinfectant solution, following the manufacturer's recommendations for concentration.
4. Using tongs or forceps, place broken glass/sharps in sharps container.
5. Cover the spill area with absorbent powder.
6. Using a broom and dustpan, remove absorbent powder and deposit it in the biohazard bag.
7. Spray the contaminated area with disinfectant and wait the appropriate contact time. Remove disinfectant with paper towels and place the paper towels in the biohazard bag for disposal.
8. Repeat step 7 to allow for sufficient disinfection of contaminated surfaces.
9. Remove outer pair of gloves only and dispose of them in the biohazard bag.
10. Remove goggles with inner gloves still on, and clean the goggles with an antimicrobial towelette. Also wipe down contact surfaces of disinfectant container.
11. Remove inner gloves and dispose of them in biohazard bag.
12. Place the biohazard bag in a biohazardous waste container for treatment and disposal.
13. Wash your hands with anti-bacterial soap and water as soon as possible.

7.3 Biological Spill Kit Contents

The following is a list of items that should go into a basic biological spill kit. It should be enhanced to meet the needs of your unique situation.

- Disinfectant (e.g., bleach, to be freshly diluted in tap water to a 1:10 (10%) dilution at the time of spill cleanup)
- Absorbent material (e.g., paper towels, absorbent powder)
- Waste container (e.g., biohazard bags, sharps containers)
- Personal protective equipment (e.g., gloves, eye and face protection)
- Mechanical tools (e.g., tongs, dustpan and broom)
- Antimicrobial towelettes
- Spill cleanup procedures

Appendix A - Sample Biohazard Notification Sign

Printable sign follows on next page.

Room: _____ **DATE POSTED:** _____

BIOHAZARD NOTIFICATION

ADMITTANCE TO AUTHORIZED PERSONNEL ONLY

Custodial Staff Can :

☐ **Empty Trash** ☐ **Clean Floors**

Entry By: ☐ **Lab Staff** ☐ **Facility Management** ☐ **Emergency Personnel**

Biological Agents: _____ **Biosafety Level** ☐ **1** ☐ **2**

_____

Biological Agents: _____ **Biosafety Level** ☐ **1** ☐ **2**

_____

Special Lab Entry Requirements: _____

_____

Special Lab Exit Requirements: _____

_____

Contacts:

Principal Investigator _____ **Phone #** _____

Lab Contact _____ **Phone #** _____

SDSM&T EHS 605.394.6020

Appendix B - Sample Sharps Safety Sign

Printable sign follows on next page.

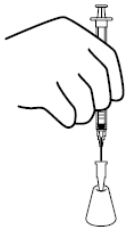
Sharps Safety

Needle Disposal

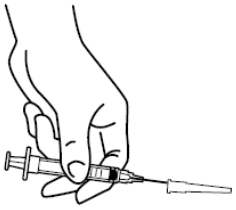
Always dispose of needles and other sharps in a rigid, puncture-resistant container immediately after use.

Avoid Recapping

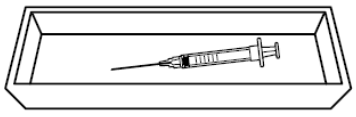
Do not recap needles for disposal whenever possible. If recapping is required for the procedure being done, you must use tongs, a recapping device or one-hand scoop method to recap the needle. Never recap needles using one hand to hold the cap and the other to hold the needle!



Recapping device



One-hand scoop



Rigid tray

Why is recapping needles dangerous?

It is extremely dangerous to hold a needle in one hand and attempt to cover it with a small cap held in the other hand because the following might happen:

- The needle could miss the cap and stab the hand holding it.
- The needle could pierce the cap and stab the hand holding it.
- The poorly fitting cap could slip off a recapped needle and stab the hand holding it.

How to protect yourself from needle-stick injuries:

- Avoid the use of needles if safe and effective alternatives are available.
- Select, evaluate and use devices with safety features that reduce the risk of needlestick injury.
- Avoid recapping needles.
- Plan for safe handling and disposal of needles before using them.
- Put uncapped needles in a rigid tray during procedures.
- Promptly dispose of used needles in appropriate sharps-disposal containers.
- Report all needle-stick and sharps-related injuries promptly to ensure that you receive appropriate follow-up care.
- Substitute plasticware for glass when possible.
- Follow safety guidelines for all sharps hazards (razor blades, scalpels, slides).
- Participate in training.

Appendix C – References

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3. National Institute of Health publication, *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*, http://oba.od.nih.gov/oba/rac/guidelines_02/NIH_Gdlnes_lnk_2002z.pdf
4. World Health Organization publication, *Laboratory Biosafety Manual*, 3rd Edition, http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf
5. AIHA Publication, *Biosafety in the Laboratory*
6. Lea & Febiger, *Disinfection, Sterilization, and Preservation*, 4th Edition
7. *South Dakota Administrative Rule 74:35*, http://legis.state.sd.us/rules/DisplayRule.aspx?Rule=74:35:01:09
8. University of Wisconsin – Milwaukee, *Biological Safety Program Policy Manual*, http://www.uwm.edu/Dept/EHSRM/BIO/biomanual.pdf
9. University of North Carolina, *Biological Safety Manual*, http://ehs.unc.edu/manuals/biological/docs/bsm_full.pdf
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11. University of Texas at Austin, *Autoclaving*, http://www.utexas.edu/safety/ehs/biosafety/autoclaving.html
12. Sandia National Laboratory, *Laboratory Biosafety and Biosecurity Workshop* - Cairo, Egypt, 3-5 April 2007