NEW MEXICO STATE UNIVERSITY

BIOLOGICAL SAFETY MANUAL

NMSU INSTITUTIONAL BIOSAFETY COMMITTEE

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BIOLOGICAL SAFETY MANUAL

Materials contained in the Biological Safety Manual were prepared as a cooperative effort between NMSU Environmental Health, Safety, and Risk Management (EHS&RM) and the Office of Research Integrity & Compliance. Use of biohazardous materials at NMSU is regulated by federal, state and local requirements. The Vice President for Research and the Graduate School (VPRGS) has assigned the responsibility for ensuring compliance to the Institutional Biosafety Committee (IBC). The IBC guides the work of the Biosafety Officer (BSO) in maintaining the Biosafety Program to eliminate or minimize risks to the health of investigators, the community, and animals and plants in the environment.

All NMSU principal investigators who use biohazardous materials must have an approved IBC application. This manual is intended to provide information on the IBC application, administrative biosafety, regulations, and selected biosafety activities to faculty, staff, and students working with biohazardous materials.

The IBC and the BSO are committed to supporting the teaching and research mission at NMSU by working with faculty, staff, and students to ensure continued growth in biological, molecular microbiological, biomedical and agricultural research. Please forward comments and suggestions that may enhance future editions of this manual.

This manual has been reviewed by members of the IBC, and will be revised periodically to update regulations, guidelines, policies, and the names of university offices or titles. Substantive changes require approval from the IBC and VPRGS. Appendices contain operational procedures which may be updated as needed; these updates shall not require the IBC approval unless a change substantively affects a provision or policy of the Biosafety Program.

RECORD OF REVIEW
Nov 2005; Sept 2012; Nov 2013; Apr 2015; Dec 2015; Jan 2019; Aug 2019

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NMSU IBC Biological Safety Manual

Maintenance History

- Nov 2005 Issued
- Sept 2012 Reviewed; updated NMSU offices; reprinted and distributed to PIs
- Nov 2013 Reviewed; no revisions
- Apr 2015 (v5.1) Reviewed, revised for organization, content, format; IBC ratified 8/18/2015
- Dec 2015 (v5.2) Revised: updated IBC forms
- Jan 2019 (v5.3) Reviewed; updated NMSU titles, Risk Management, shipping, FSAT list
- Aug 2019 (v5.4) Updated webpage links for NMSU and external biosafety resources

NEW MEXICO STATE UNIVERSITY

EMERGENCY PREPAREDNESS

ASSISTANCE TELEPHONE NUMBERS

- Biological Safety Manager ..... (575) 646-4463
- Aggie Health & Wellness Center ..... (575) 646-1512
- Environmental Health Safety & Risk Management..... (575) 646-3327
- Facilities & Services Work Order Desk .....(575) 646-7114
- NMSU Fire Department (Non-emergency) ..... (575) 646-2519
- NMSU Police Department (Non-emergency) ..... (575) 646-3311
- NMSU Security Escort Service ..... (575) 646-1111
- Poison Control (West Texas Region) 1-800-222-1222
- Radiation Safety Officer ..... (575) 646-1023
- Research Integrity & Compliance ..... (575) 646-7177

EMERGENCY TELEPHONE NUMBERS

- Fire 911
- Police 911
- Ambulance 911

What is an emergency? An emergency exists any time there is a fire, someone needs immediate medical attention, a crime is in progress, or if a chemical, biohazard or radiological spill threatens safety and health. **If you are not sure which office to call, contact the NMSU Police.**

In case of any emergency, laboratory personnel should remain calm and do only what is necessary to protect life, without jeopardizing their own safety.

1. Summon help immediately by calling 911.
2. Render assistance to persons involved. Do not move an injured person unless he or she is in danger of further harm.
3. Warn personnel in adjacent areas of any potential hazards to their safety.
4. In case of splash contact/exposure to chemical or biological hazards, flood the exposed area for 15 minutes with running water and immediately remove any contaminated clothing. Rinse contaminated skin or eyes with plenty of water for 15 minutes. Seek medical attention as soon as possible, and report all exposures to your supervisor.

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I. INTRODUCTION

The purpose of this manual is to provide information on fundamental elements of biological safety pertaining to teaching and research at New Mexico State University campuses and research centers. The information is sourced from the US Government regulations, NMSU policy and a variety of publications available in the public domain. Teaching and research activities conducted at New Mexico State University and NMSU-affiliated sites may involve the use of biohazardous agents and other regulated materials or a potential for exposure to biohazardous agents. This manual provides guidance on research materials, facilities, work practices, and applicability to NMSU research projects to establish and maintain a compliant research program. Of course no single document or manual can account for every eventuality encountered in a dynamic teaching and research environment. Accordingly, this manual will be reviewed and if necessary, revised to communicate new regulations and reflect changes to established guidelines and University policy.

New Mexico State University is committed to the highest standards of integrity in all areas of research and academic activities. The university, through the Office of the Vice President for Research and the Graduate School, has established the Institutional Biosafety Committee (IBC) which oversees the use of biohazardous agents and/or recombinant nucleic acid molecules by university faculty and staff, or at university facilities. The IBC strives to develop awareness toward protecting the health of researchers, the community and the environment through an effective biological safety program that emphasizes risk assessment and biological containment. While the Principal Investigator remains responsible for overall compliance with regulations and policy for activities conducted at their direction, the IBC and Environmental Health, Safety and Risk Management (EHS&RM) are responsible for facilitating University safety by implementing programs that serve the faculty, students, employees and clients of New Mexico State University. This manual is a part of that effort.

II. SCOPE AND APPLICABILITY

General

The policy and regulatory content of this manual applies to activities in undergraduate and graduate teaching, and in research venues that use biohazardous agents, and includes internships and work-study programs, in clinics, teaching and research laboratories, greenhouses, and field studies of genetically modified plants and other bioengineered materials. The NMSU colleges of Arts and Sciences; Agriculture, Consumer and Environmental Sciences; Engineering; Health and Social Services; and the Dona Ana Community College's Health and Public Services Program conduct or sponsor research and teaching activities likely to involve biohazardous agents. The means for declaring the above-cited activities is the Institutional Biosafety Committee (IBC) Application. The form can be accessed online from the Research Integrity & Compliance webpage at http://compliance.nmsu.edu/IBC. Projects involving humans, animals, radioactive materials, or radiation-generating equipment require additional approval, by the Institutional Review Board (IRB), Institutional Animal Care and Use Committee (IACUC), and Radiation Safety Committee, respectively.

Teaching

This manual applies to teaching activities at NMSU and affiliated locations. It is incumbent on supervisors, instructors, and laboratory directors to ensure compliance of their activities with all applicable regulations and NMSU policy. Teaching activities involving the use of or having a potential for exposure to biohazardous agents must be communicated to the Institutional Biosafety Committee through submission of a completed and signed IBC application or comparable document that describes relevant experimental plans and safety precautions. Activities shall not be initiated until the application is approved.

Research

This manual applies to clinical, laboratory, greenhouse and field work. Persons conducting laboratory research using whole (live) animals, viable organisms, environmental biological samples, animal or human organs, tissues, cell lines or internal body fluids, biological toxins, recombinant organisms or synthetic nucleic acid molecules must submit a completed and signed IBC application. Research shall not be initiated until the application is approved.

Greenhouse and field research using genetically modified plants, plant pests, or pesticide-containing genes, and recombinant or synthetic nucleic acid molecules must submit a completed and signed IBC Application. Research shall not be initiated until the application is approved.

III. DEFINITIONS

Biohazardous Agents are defined as:

1) Any microorganism (including but not limited to bacteria, viruses, fungi, rickettsiae, or protozoa), or infectious substance, including prions, or naturally occurring, bioengineered, or synthesized component of any such microorganism or infectious substance that is capable of causing:
   a) Death, disease or other biological malfunction in a human, an animal, a plant or another living organism;
   b) Deterioration of food, water, equipment, supplies, or materials of any kind; or
   c) A deleterious alteration of the environment.
2) Any toxic material or product of plants, animals, microorganisms (including but not limited to bacteria, viruses, fungi, rickettsiae, or protozoa), or infectious substances, or a recombinant or synthesized molecule (whatever the origin and method of production), which includes any poisonous substance or biological product that:
   a) May be engineered as a result of biotechnology;
   b) Is produced by a living organism; or
   c) Is an isomer or biological product, homologue, or derivative of such a substance.
3) Infectious or pathogenic biological agent in humans, animals or plants defined by:
   a) CDC as biosafety level (BSL) 2-4 (*BMBL*, current edition) or
   b) NIH as risk group (RG) 2-4 agent (*NIH Guidelines*, current revision).
4) A regulated biological agent or toxin as identified by
   a) Title 42 Code of Federal Regulations (CFR) Part 73 (The Transfer, Use, and Possession of Select Biological Agents and Toxins);
   b) Title 7 CFR Part 331 and Title 9 CFR Part 121 list of High Consequence Livestock Pathogens and Toxins that pose a severe threat to "animal health or animal products" or to "plant health or plant products"

   *Note: Appendix I contains a combined list of select agents and toxins regulated by U.S. Department of Health & Human Services and U.S Department of Agriculture.
5) Recombinant and synthetic nucleic acids, defined by the *NIH Guidelines* as:
   a) molecules that i) are constructed by joining nucleic acid molecules and ii) that can replicate in a living cell, i.e., recombinant nucleic acids;
   b) nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids, or
   c) molecules that result from the replication of those described in (a) or (b) above.

Containment means the physical control of pathogens, infections agents, and recombinant DNA within a laboratory and includes specific work practices and security measures that control access to materials within the laboratory.

Infectious Agent means any organism, protein, or nucleic acid molecule that is capable of invading body tissues, replicating itself and causing disease.

Infectious waste, as defined by New Mexico Administrative Code Title 20 Chapter 9 Part 2, (April 2015, http://www.nmcpr.state.nm.us/) means "a solid waste that carries a probable risk of transmitting disease to humans or animals, and includes the following which shall be considered infectious waste:

a) cultures and stocks of infectious agents and associated biologicals, including: cultures from medical and pathological laboratories; cultures and stocks of infectious agents from research and industrial laboratories; waste from the production of biologicals; discarded live and attenuated vaccines except for residue in emptied containers; and culture dishes, assemblies and devices used to conduct diagnostic tests or to transfer, inoculate, and mix cultures;

b) human pathological wastes, including tissues, organs, and body parts that are removed during surgery, autopsy, other medical procedures, or laboratory procedures, but not including hair or nails;

c) human and body fluid waste, including: (i) liquid waste human blood; (ii) blood products; (iii) items with human blood (caking, flaking, saturated or dripping); (iv) items with human blood, including serum, plasma, and other blood components, which were used or intended for use in patient care, specimen testing, or the development of biological products or pharmaceuticals ; (v) intravenous bags that have been used for blood transfusions; (vi) items, including dialysate, that have been in contact with the blood of patients undergoing hemodialysis at hospitals or independent treatment centers; (vii) items contaminated by body fluids from persons at trauma scenes, during surgery, autopsy, other medical procedures, or laboratory procedures; (viii) specimens of blood products, and their containers; and (ix) other potentially infectious materials as defined by the U.S. Department of Labor Occupational Safety and Health Administration at 29 CFR 1910.1030(b), including the following body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids;

d) contaminated animal carcasses, body parts, blood, blood products, secretions, excretions, and bedding of animals that were known to have been exposed to zoonotic infectious agents or non-zoonotic human pathogens, including during research (including research in veterinary schools and hospitals), production of biologicals, or testing of pharmaceuticals.

e) biological wastes and waste contaminated with bloody excretions, exudates, or secretions from: (i) humans who are isolated to protect others from rare diseases such as viral hemorrhagic fevers (Ebola, Lassa, Marburg) or other emerging infectious diseases whose biological wastes and waste contaminated with bloody excretions, exudates, or secretions are deemed infectious waste as described by advisory agencies such as the Centers for Disease Control (CDC); (ii) isolated animals known or suspected to be infected with rare diseases such as bovine spongiform encephalopathy (BSE) or other emerging infectious diseases identified by an advisory agency;

f) discarded sharps, used or unused (unless in original packaging), generated at a facility, that have, or are likely to have, come in contact with infectious agents while involved in human or animal patient care, treatment, or research, including hypodermic needles, syringes (with the attached needle), Pasteur pipettes, scalpel blades, blood vials, needles with attached

tubing, culture dishes, suture needles, slides, cover slips, and other broken and unbroken glass or plastic ware, unless properly treated or otherwise specifically exempted;

g) infectious waste does not include:

(i) wastes generated in a household (except for infectious wastes generated by home health care professionals);

(ii) human corpses, remains, and anatomical parts that are intended for interment or incineration as specified in Paragraphs (4) and (5) of Subsection E of 20.9.8.13 NMAC, or are donated and used for scientific or medical education, research, or treatment;

(iii) etiological agents being transported for purposes other than waste processing or disposal pursuant to the requirements of the United States Department of Transportation (49 CFR 171.1-190) and the New Mexico Department of Transportation and other applicable shipping requirements;

(iv) reusable or recyclable containers or other non-disposable materials, if they are cleaned and disinfected by a method approved by the secretary pursuant to NMSA 1978 74-9-3 P, or if there has been no direct contact between the surface of the container and materials identified as "infectious waste;"

(v) soiled diapers that do not contain materials identified as infectious waste;

(vi) body excretions such as feces and secretions such as nasal discharges, saliva, sputum, sweat, tears, urine, and vomitus unless visibly contaminated with blood or waste from a person or animal as described in Subparagraph (e) of Paragraph (5) of Subsection I of 20.9.2.7 NMAC; or

(vii) used or unused syringes that have not come into contact with human blood or other bodily fluids or infectious agents and do not have a needle attached."

Laboratory Biosafety Levels (BSL-1, BSL-2, BSL-3, BSL-4) refer to a set of laboratory work practices, facility requirements, equipment and training that are used to mitigate hazards when working with biological agents. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community. Biosafety levels are defined in the CDC/NIH publication, Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition. The current edition of the BMBL can be accessed online from the CDC website: www.cdc.gov .

Laminar Air Flow means unidirectional airflow at a constant velocity.

Pathogen means an organism, e.g., bacteria, virus, prion, fungus, or parasite that can cause disease in humans, animals, or plants.

IV. ROLES AND RESPONSIBILITIES

The University President has ultimate responsibility for establishing and maintaining health and safety programs and establishing a system for assessing safety performance for the university.

University Administration including all Vice-Presidents, Deans and Department Heads are responsible for:

1) Ensuring that facilities and equipment provided meet requirements for a safe work environment, or modifying those activities to come into compliance with applicable rules, regulations and standards.
2) Ensuring individuals under their management are in compliance with University, State and Federal environmental, health and safety policies, practices and programs.
3) Ensuring areas under their management are in compliance with University, State, and Federal environmental health, safety policies and programs.
4) Establishing priorities and committing resources for correction of environmental health and safety deficiencies.
5) Establishing procedures for disseminating safety-related policies and information;
6) Establishing procedures to implement policies.
7) Assessing safety performance to evaluate their areas of responsibility and reporting findings back to central administration.
8) Immediately notifying NMSU Environmental Health, Safety and Risk Management (EHS&RM) when they become aware of a violation of any University, State, or Federal environmental health or occupational safety rule or regulation. This includes any contact with a State or Federal regulatory agency regarding such a violation.

Supervisors, faculty, principal investigators, first-line supervisors, and all other persons in authority are responsible for:

1) Providing safe and healthy environments for those areas and personnel for whom they have supervisory or administrative responsibility, incorporating safety and health issues as an integral part of all activities at NMSU.
2) Being continuously cognizant of the safety and health needs of all co-workers and employees for whom they are responsible.
3) Initiating and enforcing preventive measures to control hazards.
4) Communicating with administrators and safety staff to ensure that necessary support such as engineering and administrative controls, personal protective equipment, occupational medical examinations and local exhaust ventilation are in place and adequate for operations.
5) Ensuring employees are trained prior to beginning new tasks.
6) Reporting injuries and illnesses to the Worker's Compensation Coordinator (Aggie Health & Wellness Center), and to the Biosafety Officer for incidents involving IBC-related activities.
7) Reviewing incident and injury reports for their area(s) and implementing corrective actions.
8) Serving as a focal point for employee safety and health concerns.

9) Immediately notifying Environmental Health, Safety and Risk Management when they become aware of a violation of any university, State or Federal environmental health or occupational safety rule or regulation. This includes any contact with a State or Federal regulatory agency regarding such a violation.

All New Mexico State University faculty, staff, and students are responsible for:

1) Participating in mandated training programs provided by Environmental Health, Safety & Risk Management, their supervisors, and other instructors.
2) Properly using university-supplied materials and equipment.
3) Using good judgment in carrying out work assignments and following established procedures.
4) Promptly reporting unsafe conditions, health hazards, injuries and illnesses to the cognizant supervisor or program director.
5) Giving due consideration to personal safety and the safety of others
6) Strictly adhering to Federal, State, and university safety requirements and guidelines.
7) Knowing that disregard or chronic negligence of established policies and procedures can result in disciplinary action.

The Biosafety Officer is responsible for:

1) Conducting annual inspection of facilities identified on IBC applications to ensure that laboratory standards are rigorously followed.
2) Maintaining a biosafety library of reference publications and training materials.
3) Providing biosafety training.
4) Reporting to the Institutional Biosafety Committee and the university any significant problems and violations of the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* and other standards of safety for the use of biohazardous research materials.
5) Reporting any significant research-related accidents or illnesses to the NIH Office of Biotechnology Activities and the university.
6) Reviewing emergency plans developed for handling accidental spills and personnel contamination and investigating laboratory accidents involving recombinant nucleic acid molecules or biohazardous materials.
7) Providing advice on laboratory security.
8) Providing technical advice to Principal Investigators, staff, the IBC and IACUC on biosafety guidelines, standards, and practices.
9) Serve as a member of the IBC and the point of contact with the NIH and other organizations on matters pertaining to biological safety.
10) Being aware of and reviewing testing programs designed to demonstrate the integrity of containment equipment and facility safeguards.
11) Supervising emergency laboratory decontamination measures.
12) Maintaining a database of IBC applications submitted for review and approval.
13) Facilitating shipping of biological materials to ensure safety and compliance.

V. ADMINISTRATIVE BIOSAFETY

Administrative biosafety pertains to procedural and documentary record keeping on policy and regulatory compliance for each laboratory. The primary administrative biosafety documents at NMSU are the IBC application, the Activity Modification Report, EHS&RM training records, and award terms and conditions, if applicable, in Research Administration Services. The IBC application is addressed in Section VI of this manual.

Other documents that may have a Biosafety component are the application forms for the Institutional Animal Care and Use Committee (IACUC) for activities using live animals, the Radiation Safety Committee for use of ionizing radiation, and the Institutional Review Board (IRB) for research with human subjects. These applications are forwarded to the IBC for concurrent review when proposals include the use of recombinant nucleic acids and/or infectious agents.

Additionally, there are government and vendor-generated documents related to regulatory and policy compliance. For example, purchasing research materials from a commercial vendor necessarily creates a paper trail of the transaction. Documents related to the acquisition, transfer and use of some research materials are legally significant to the Principal Investigator and the university. In some instances there are statutory requirements for archiving these documents. Generally, records for obtaining and transfer of research materials should be kept for three years or more after the completion of the research or the researcher is no longer in possession of the material, financial records must be kept for seven years, and records for employee health should be kept for 30 years after separation of employment.

Research Administration Services (RAS), the office of Research Integrity & Compliance (RIC), the Principal Investigator (PI), and EHS&RM must work together to ensure the university maintains accurate records of research and documentation of regulatory compliance. Faculty and staff are not authorized to sign any legally binding document of terms and conditions on behalf of the university. The Vice President for Research and the Graduate School is responsible for signing all agreements related to research activities.

Grants and Contracts Proposal Award Review

All research grants under consideration for funding or having been awarded funding are internally reviewed for use of animals, hazardous chemicals, radiation, infectious materials and recombinant DNA. The PI is responsible for obtaining review and approval from each respective NMSU oversight committee and compliance with NMSU safety policies. For example, the hazardous chemical inventory must be updated at least annually, and each research facility must be operated and maintained to receive a satisfactory safety rating from EHS&RM and applicable regulatory entities.

The Vice President for Research and the Graduate School has established the following internal review to ensure compliance with the regulations and NMSU safety policy.

The Vice President for Research and the Graduate School forwards the PI name, grant title, and nature of materials intended to be used in the proposal (as indicated by markings in boxes in item 14 on the Proposal Award Review form) to Research Integrity & Compliance. The Compliance Coordinator reviews records to ensure that necessary committee approvals (IACUC, IBC, IRB and/or RSC) have been obtained and are in good standing. The Coordinator notifies the PI if a necessary approval is lacking and must be obtained, and notifies the Vice President for Research and the Graduate School on the status of compliance as incomplete, complete, or pending.

Principal Investigators (PIs) must submit an IBC application for any research involving infectious agents or recombinant DNA. All applications are administratively reviewed. Potential results of the administrative review are 1) approval without further action or 2) the application will be reviewed and voted upon by the IBC. The PI will be informed of the result.

Permits

Depending on the nature of the organisms or biological material and the type of experiment (laboratory, greenhouse, field trial or clinical site), a permit may be required. The trial sponsor or the NMSU Principal Investigator may submit the permit application for a specific research grant. In all cases it is the researcher's responsibility to learn of federal and state permitting requirements for their respective projects, and coordinate with NMSU Research Administration Services Office, EHS&RM and other appropriate NMSU administrative offices. Examples of permit issuing agencies are the NM Department of Agriculture (NMDA); NM Department of Game and Fish; the USDA Animal and Plant Health Inspection Service (APHIS), which includes Veterinary Services (VS), Plant Protection and Quarantine (PPQ), and Biotechnology Regulatory Services (BRS); the U.S. Department of Health and Human Services, and the U.S. Environmental Protection Agency.

There are restrictions on some items intended for import and export (including naturally occurring and genetically modified organisms, animals, animal tissues, and some regulated technologies). Issuance of a permit to possess, transfer, or use a particular research material is predicated on the applicant's agreement to fulfill specific terms and conditions defined in the permit. Contact Research Integrity & Compliance for assistance with these regulatory requirements.

- The importation of animals, crops, foodstuffs, or biological samples may require an import permit from the USDA, CDC and/or FDA.
- Materials and technologies listed on the Commerce Control List (CCR) of the US Department of Commerce, Bureau of Industry & Security (BIS), are prohibited from export and certain transfers, unless and until an export control authorization is received.
- These materials and technologies are deemed by the U. S. Government to have a "dual use" beyond bona fide research and may pose a threat to the public health or national security.
- Organisms that are known human pathogens or listed as a Select Agent or Toxin by HHS, or as a High-Consequence Livestock Pathogen or Toxin by the USDA, cannot be possessed, purchased, or transferred unless the University is registered with either or both federal agencies (depending on the agent or toxin**).** The application for registration to transfer, possess, or use Biological Select Agents or Toxins is available on the internet at http://www.selectagents.gov. In addition to the application, registration requirements

include a U.S. Department of Justice Security Risk Assessment (conducted by the FBI) of each person accessing Select Agents including the Responsible Official, and a facility inspection by the CDC or APHIS or both. This is a lengthy (8 – 12 months minimum) and cumbersome process since some of the plans, program descriptions and other information requested in the application do not exist and must be generated from "scratch". If the need arises, the Vice President for Research and the Graduate School will submit the Select Agent permit application for NMSU on behalf of a Principal Investigator interested in conducting research using Select Agents or Toxins. Research involving permissible amounts of National Select Agent Registry toxins must be approved by the NMSU IBC prior to receipt of the material.

- There may be permit requirements for organisms and toxins that are not on the Select Agents and Toxins list, but that are exotic to New Mexico or otherwise pose a threat to plants, animals, or the environment. The NM Department of Agriculture (NMDA) and USDA APHIS should be contacted for details. These agencies regulate the import of agricultural materials and organisms into the United States and movement between states.

A copy of the permit must accompany all IBC applications involving permitted materials. The Principal Investigator is responsible for discovery of import restrictions and permit requirements. In the event that a permit requirement is unknown or uncertain, contact the appropriate agency directly, or contact the Research Integrity & Compliance for assistance.

Purchase Orders

Many vendors have expanded the pre-conditions for the purchase of laboratory equipment, reagents and supplies routinely used in research. These conditions address regulatory and industry requirements (from US Department of Commerce, USDA, HHS, US Postal Service, commercial shipping companies, the airline industry and others,) as well as intellectual property and product development rights related to use of the item or material in question.

For example, consider The American Type Culture Collection (ATCC). The ATCC is a biological supply house that sells cell lines, bacterial and viral stocks, and nucleic acid molecules. The ATCC requires each purchaser to enter into a Material Transfer Agreement (MTA) prior to fulfilling a purchase order. The MTA defines the specific terms and conditions for subsequent product use. Briefly, ATCC products are restricted to research use in the laboratory of the purchaser. Purchasers are prohibited from subsequent distribution to colleagues (at NMSU or other sites) without the expressed written consent of the ATCC. The purchaser agrees to destroy the material at the conclusion of their work.

Another document tendered by the ATCC is the "Customer Acceptance of Responsibility" (CAR) form. Acceptance of the terms and conditions of the CAR apply to the purchase of certain bacteria and viruses vended by the ATCC that are included on the U.S. Department of Commerce's "commerce control list" of materials that may pose a risk to the public health, or have a potential for "dual use" and is therefore prohibited from export. A new CAR form is required for each purchase of these materials. **In summary, any document that mentions legal liability should be vetted through the Office of the Vice President for Research and the Graduate School for acceptance by NMSU.**

Required Training

The following are descriptions of training sessions provided by EHS&RM to faculty and staff as required by OSHA regulation or NMSU policy or both. Administrative review of IBC application submissions includes a review of training records for all persons listed on the IBC application, including the PI. Attendance at appropriate training sessions is a condition of IBC approval. Personnel must complete the required training before they start working in the laboratory.

Hazard Communication training is mandated under OSHA and NMSU policy for all employees of the university who work with or near chemicals. This is a one-time requirement for each employee and student as long as the work environment remains the same. Repeat the HazCom training when significant changes occur in the job duties, materials, locations, or procedures.

Hazardous Waste Disposal training is required for faculty, staff, students who are responsible for the chemical waste and are involved in disposing of chemical waste. A minimum of one staff member from each lab must attend. Completion of Hazard Communication training is the prerequisite to registering for Hazardous Waste Disposal training.

Laboratory Standard training is required for faculty, staff, graduate assistants and students that work in a laboratory where hazardous or toxic chemicals are present. The initial EHS&RM class provides information on compliance with the regulations. In addition, annual refresher training is required for review of the Chemical Hygiene Plan and other relevant laboratory safety procedures. Completion of the Hazard Communication training class is a prerequisite to registering for the Laboratory Standard training or online Laboratory Safety courses.

Biosafety Awareness training is required for faculty, staff and students identified on an IBC application for approval to work at BSL-2. Documentation of IBC approval will not be released until all persons have completed the Laboratory Biosafety Awareness training.

Bloodborne Pathogen (BBP) Exposure Control training is required for persons whose routine tasks and duties involve reasonably-anticipated exposure to blood, internal body fluids, unfixed cells or tissues from humans or non-human primates. BBP training is also required for laboratory work with human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) or other bloodborne pathogens. Refresher training is required annually for each employee or student whose work meets the above criteria.

Site-specific training is required and must be documented. To comply with the Hazard Communications standards and legal obligations, supervisors must ensure training on the specific workplace hazards before the employee begins procedures or operations involving new hazardous work conditions. Written records must include the name of the individual, the date of the training, a description of the training provided, and the means used to verify that the employee understood the training. These records should be maintained by the lab supervisor as part of the Laboratory Safety Plan.

Examples of specialized laboratory procedures that require documentation of site-specific training are:

- Autoclave operation and sterilization procedures (see Chapter VIII, "Autoclaving" and "Disposal Procedure for Laboratory Microbiological Wastes");
- Standard operating procedures (SOPs) that are developed by the laboratory supervisor to use hazardous equipment and perform specialized techniques.
- The Bloodborne Pathogen Exposure Control Plan or other comparable biological hazard exposure control plan specific to the materials in use; the exposure control plan should be presented to new lab members for signature during an initial lab orientation.
- Transport of infectious materials through public areas of a building, between buildings on campus, between locations in an official or private vehicle, or to be offered for shipping through a commercial service. Hazardous Materials shipping through a public contractor (FedEx, UPS, US Postal Service, etc.) must be performed by a certified shipper with specialized training (see Chapter VIII, "Shipping Research Materials"); the NMSU employee responsible for shipping must be trained to comply with 49 CFR 172, Subpart H, HM-181 and HM-126F before performing shipping duties. Retraining must occur every two years to satisfy international air transport regulations, and a copy of the training certificate should be forwarded to EHS&RM to be maintained in the employee's Training Central record.

VI. THE IBC APPLICATION

The Institutional Biosafety Committee (IBC) Application is used to document the "who, what, where, and how" for all teaching and research projects involving biohazardous agents and recombinant nucleic acids at NMSU and NMSU-affiliated locations (see Section III for the definition of biohazardous agents). From a Federal compliance perspective, IBC applications can be divided into two classes, those that are exempt from the *NIH Guidelines* and those that are non-exempt. In conformance with the *NIH Guidelines*, the Principal Investigator makes the initial determination to classify the experiments, and then the IBC reviews the application to confirm, or in some cases reevaluate, the classification. Exempt activities are usually administratively approved and non-exempt activities are reviewed and voted on by the IBC. As a matter of NMSU policy, the final determination of exempt or non-exempt status rests with the IBC.

The ability of the IBC to provide a timely review of applications depends in large part on the completeness of the information contained in the submission. The narrative sections required in the IBC application prompts the applicant to submit questions or solicit advice on matters related to procedural or facility biosafety for the proposed project to EHS&RM or to members of the IBC. All applications are administratively reviewed for completeness, regulatory and policy compliance prior to distribution to the IBC membership. Compliance is evaluated by checking the training records of the Principal Investigator (PI) and staff in the NMSU training database, and a survey of the laboratory facility. If necessary, the Biosafety Officer (BSO) will contact the PI for additional information or clarification of information included in the application.

Once the BSO evaluation is completed, the application is then forwarded for review by the IBC Chair. The application is either administratively approved or remanded to review and vote by the IBC. Approval granted administratively or by a vote of the IBC is valid for three years from the date of issue.

The PI on applications scheduled for IBC review will be notified via email of the scheduled IBC review date. Although not required, PIs are encouraged to be present at the IBC meeting while their application is being reviewed. Scheduling is coordinated through the BSO.

Major and minor changes in the research and teaching conducted under an IBC-approved application must be communicated to the IBC in a timely manner. The Activity Modification Report form is used for communicating both major and minor modifications to approved applications. The form is available from the Research Integrity & Compliance webpage and is included as Appendix B of this manual.

Section I: Administrative Information

The information requested in this section identifies the Principal Investigator, co-Principal Investigators (if any), the project title, the funding source and a proposed Biosafety Level for the project. The IBC may accept or revise the Biosafety level proposed by the applicant. The "Category of Application" distinguishes new applications from continuing approvals and grant-

specific applications from general teaching and research activities. The information provided may also be used to coordinate related internal administrative processes.

Section II: Institutional & Regulatory Approval / Registrations

The Institutional and Regulatory Approval / Registration section identifies projects subject to university oversight (other than the IBC) and Federal or State permit requirements. A brief description of each of the four sub-sections follows.

Use of Animals: The care and use of animals in teaching and research at NMSU is reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). The university maintains a U.S. Public Health Service-approved "assurance" with the NIH Office of Laboratory Animal Welfare as required under the Health Research Extension Act of 1985, Public Law 99-158, "Animals In Research" (November 20, 1985). No work with vertebrate animals can begin without IACUC approval.

Use of Radiation: Use of radiation generating devices and radioactive materials is reviewed and approved by the NMSU Radiation Safety Committee. The university maintains a Radiation License granted by the New Mexico Environment Department Radiation Control Bureau. No acquisition of or work with radioactive materials or x-ray generating equipment may begin without Radiation Safety Committee approval.

Use of Human Subjects: The use of human study subjects is reviewed and approved by the NMSU Institutional Review Board (IRB). The university maintains a Federal Wide Assurance as required under Title 45 of the Code of Federal Regulations Part 46, Protection of Human Subjects. No research involving human subjects, including the collection of data about or from human subjects using surveys, existing data, or specimens, can begin without IRB approval.

Federal Permits: Acquisition, possession, transfer (interstate, intrastate, import or export) and use of certain bacteria, viruses, rickettsia, parasites, biological or plant toxins, plants, plant pests, genetically modified organisms, and whole or parts of the genetic elements from these biological agents, require obtaining a permit. Permitting agencies include the US EPA, CDC, USDA Animal and Plant Health Inspection Service (APHIS), the NM Department of Agriculture (NMDA), and the New Mexico Department of Fish and Wildlife. It is incumbent on the Applicant to obtain the required permit(s) for materials to be used in the proposed research. There is no university-wide permit to acquire, possess, transfer (interstate, intrastate, import or export) or use permitted materials. Permit applications under review by the permitting entity at the time of application can be reported as such on the application; however IBC approval is contingent on IBC receipt and administrative review of the permit. Due to the expanding regulatory and enforcement climate, applicants are encouraged to contact the Biosafety Officer for assistance with discovery of permit requirements and if necessary, assistance with the application process.

Section III: Location of Activities

The information provided in this section is used to verify that the facility is appropriate to support the scope of work proposed in the application. Identify each laboratory, preparation room, shared equipment space or rooms, off-campus and satellite campus locations, animal facility or greenhouse used for the proposed research. Applications for field trials must identify the location and include a rough diagram shading or boxing in the area of the field to be used for the trial and marked or shaded in a manner that clearly indicates separation from other growing areas.

Section IV: Type of Biologicals and Biosafety Activity

There are three sections that ask for information relevant to the risk assessment of the proposed research or teaching project. For each agent or material identified (bacteria, virus, fungi, parasite, toxin, other agent or component), this section asks the applicant to comment on the following:

- Strain or type of bacteria, cell line, or virus, or other biological materials,
- If a biological safety cabinet will be used for experiments with the listed materials,
- If there is a protective vaccine against the disease caused by the agent, and if the Public Health Service Advisory Committee on Immunizations Practices recommends the vaccine,
- Special precautionary measures warranted with the proposed research.

Responses to application items about recombinant DNA and the Biosafety Level demonstrate that the PI is familiar with the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* and the CDC/NIH publication, *Biosafety in Microbiological and Biomedical Laboratories*. For recombinant materials, the applicant identifies (i) the source(s) of DNA; (ii) the nature of the inserted DNA sequences; (iii) the host(s) and vector(s) to be used; (iv) if an attempt will be made to obtain expression of a foreign gene, and if so, indicate the protein that will be produced; and (v) the containment conditions that will be implemented as specified in the *NIH Guidelines*.

Note: The *NIH Guidelines* describes a number of places where judgments are to be made. In all these cases, the Principal Investigator shall make the judgment on these matters as part of his/her responsibility to "make the initial determination of the required levels of physical and biological containment in accordance with the *NIH Guidelines*" (see *NIH Guidelines* Section IV-B-7-c-(1)). For cases falling under *NIH Guidelines* Sections III-A through III-E, *Experiments Covered by the NIH Guidelines*, this judgment is to be reviewed and approved by the Institutional Biosafety Committee as part of its responsibility to make an "independent assessment of the containment levels required by the *NIH Guidelines* for the proposed research".

Section V: Description of Activity

Section V requires the applicant to identify the procedures that will be used to conduct the activities. Part A asks for a description of the activity in terms easily understood by a non-

scientist. Useful information includes the research problem or question to be explored, brief description of methods, and the projected outcome or the intended use of the data to be obtained.

A sample lay summary for a teaching experiment might be "We will grow a well characterized, commercially obtained strain of *E. coli* that does not cause illness in healthy humans. We have obtained a group of genes of interest. We will express these genes in the E. coli to see if the gene works and the trait is expressed." Similar wording should be used for research projects.

Part B requires a list of procedures used in the experiment. For example "We will use standard molecular biology techniques as described in "Molecular Cloning" by Maniatis et al, 2nd Edition, 1989. General procedures include bacterial cell culture, pipetting, centrifugation, nucleic acid purification and restriction, agarose gel electrophoresis. Support procedures include preparation of bacterial media (LB), buffer (PBS, Tris) and reagents, steam sterilization of pipette tips and other supplies, chemical decontamination of liquids, and autoclaving of contaminated solid waste. The (bacteria or virus) will be expanded in cell culture using the prepared media. No more than 2.0 L will be in culture at any given time. The cells will be harvested and lysed to recover cellular DNA or antigen by a series of filtration and centrifugation steps (identify steps). Aseptic procedures will be performed in a certified biological safety cabinet. Finally, we will prepare a solution of the recovered viral antigen and isolate DNA by agarose gel electrophoresis to discover if the component nucleic acids migrate across the gel according to our predicted model."

Part C item 1 contains a template version of routine substance disposal and decontamination procedures that are based on NMSU policy and applicable regulations. Part C item 2 asks the PI to specify additional waste handling, decontamination, and disposal operations beyond those described in item 1.

Part D asks the PI to indicate if a biological safety cabinet (BSC) or clean air bench (CAB) will be used and if an autoclave will be used for decontamination of solid laboratory waste. For each piece of equipment used the PI must state the equipment location (building and room), the manufacturer, model, serial number and date of the most recent certification (BSC, CAB) or autoclave challenge test using microbial spore vials or strips or chemical indicator strips.

Section VI: Personnel

Section VI asks for the names of personnel assigned to work on the proposed project and for a description of their training and education. Experience with specific laboratory techniques and equipment is requested for each person listed in this section, including the PI. Examples include gel electrophoresis, cell culture, centrifugation, type of PCR, media and buffer preparation. When appropriate, state that a new hire has "no experience" and will be working under supervision with the experimental techniques and equipment.

Under no circumstances will an inexperienced and untrained person be left unsupervised while performing experimental procedures and techniques. The PI maintains a record of all

training. The IBC requires that inexperienced personnel be trained according to the following protocol:

1) Inexperienced personnel will read and understand the written descriptions of experimental procedures.
2) Inexperienced personnel will observe as the PI or other person trained by the PI demonstrates the experimental procedures and techniques.
3) Inexperienced personnel then perform experimental procedures and techniques under direct supervision of the PI or other person trained by the PI until the inexperienced personnel demonstrates competency in the experimental procedures and techniques.

The Biosafety Officer will check training records for all personnel listed on the application, including the PI, and enter the EHS&RM training dates on the application form. The PI is responsible for ensuring that personnel complete the appropriate safety training for the work to be performed.

Section VII: Safety Plans

Each applicant must generate a Laboratory Safety Plan, and ensure that personnel are trained on the departmental Emergency Response Plan (alternately referred to as an Emergency Action Plan or Disaster Response Plan). Each laboratory Director or Principal Investigator must provide emergency contact information. Contact name, business phone and after hours phone contact information should be posted at the laboratory entrance.

The laboratory safety plan answers the following:

1) What types of hazards (biological, chemical, radiological) are present in the lab?
2) What are the safety training requirements for persons entering the lab?
    a) Hazard Communication and Lab Standard are required for all personnel;
    b) Specify other applicable training requirements such as Bloodborne Pathogens, Biosafety Awareness, Radiation Safety, and Respiratory Protection.
3) What personal protective equipment is required? Specify minimum equipment and task-specific requirements.
4) Description of spill response procedures for biological, chemical, and/or radiological incidents.
5) Who is to be notified in the event of an emergency? Provide contact information.
6) Describe laboratory security (i.e., when are doors locked, access by visitors).

The Emergency Response Plan instructs occupants what to do in the event of a natural or man-made disaster. Natural disasters include fire, flood, or high-wind event that may pose a threat to the building integrity or occupants. Man-made disasters include spills involving large quantities of hazardous materials or an explosion. The plan includes posting an emergency egress map that identifies paths to exit the building and designates a rendezvous location outside the building. Additional information is available on the webpage of NMSU Environmental Health, Safety, and Risk Management, Emergency Information. This plan may be authored at the college, academic department or the laboratory level.

Section VIII: Principal Investigator Statement

The "Principal Investigator Statement" lists expectations for the safe conduct of IBC-approved research and attests to the PI's commitment to complying with all applicable regulatory and NMSU policy requirements. Briefly, the statement informs the PI of the following requirements:

1) Conduct research and teaching activities in compliance with the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules,* current national standards in the Public Health Service publication, *Biosafety in Microbiological and Biomedical Laboratories,* and other applicable standards and regulations.
2) Ensure that laboratory workers receive training on emergency procedures, good laboratory work practices, the safe operation of laboratory equipment, and that they are familiar with the hazards and symptoms of exposure relevant to the biological materials used within the lab.
3) Provide staff with necessary personal protective equipment.
4) Report to the IBC through the Biosafety Officer of all instances of:
    a) Occupational injury or exposure to biohazardous agents or recombinant or synthetic nucleic acid molecules (through needle sticks, wounds, inhalation, ingestion, or splashes to the face).
    b) Events (known or likely) resulting in environmental release of biohazardous agents or recombinant or synthetic nucleic acid molecules.
    c) Instances of containment equipment breakdown and facility system failures.
5) Submit an Activity Modification Report for the following MINOR modifications of IBC-approved research:
    a) When new staff are added or removed.
    b) Laboratory renovation.
    c) Research relocation to a different laboratory.
    d) When the project is temporarily suspended or terminated.
    e) When research no longer involves live animals, animal cells or tissues, infectious or pathogenic organisms, or recombinant nucleic acid molecules.
6) Submit a new IBC Application for the following MAJOR modifications.
    a) Change in PI.
    b) The project expands to include live animals, animal cells or tissues, recombinant or synthetic nucleic acids, or biological agents that are infectious, pathogenic or toxic..
    c) Research needs to progress from BSL-1 to BSL-2 facility and work practices.
    d) Substantial changes in the IBC-approved procedures (new technology or novel recombinant genetic construct) or initial acquisition of new organisms or toxins. Submit description of changes to the IBC Chair through the biosafety officer for review on a case-by-case basis.
7) The signed Principal Investigator statement binds the signatory to the conditions that must be maintained to conduct the IBC-approved activities.

VII. BIOSAFETY LEVELS AND WORK PRACTICES

This section reviews the standard requirements for laboratory and field research conducted at NMSU that involves biological procedures, including molecular and microbiological techniques, in cells, tissues and organisms.

The term, "biosafety level" describes a combination of administrative controls, work practices, safety equipment, and facility design requirements that are used to manage the conditions under which harmful biological agents can be safely maintained and manipulated. Information here is referenced from the following publications:

- **Biosafety in Microbiological and Biomedical Laboratories ("BMBL"), 5th Edition**, 2009, DHHS, Public Health Service, Centers for Disease Control and Prevention, Atlanta, Georgia, and National Institutes of Health, Bethesda, Maryland. Available online at https://www.cdc.gov/labs/BMBL.html
- ***NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* ("*NIH Guidelines*"), November 2013 or latest revision,** available at https://osp.od.nih.gov/biotechnology/nih-guidelines/
- ***A Practical Guide to Containment: Plant Biosafety in Research Greenhouses***, 2008, Adair, D. and R. Irwin, ISB Virginia Tech; available at https://vtechworks.lib.vt.edu/handle/10919/78423

BSL-1 and BSL-2 describes standard practices, special work practices, safety equipment and facility requirements used for biomedical, microbiological and molecular biology research in laboratories, animal facilities, and greenhouses.

Research at NMSU typically is conducted in laboratories using Biosafety Level 1 (BSL-1) and BSL-2 containment. Research at BSL-3 must have a laboratory-specific biosafety manual and therefore is not included in this section.

Animal Biosafety Level (ABSL) criteria describes standard practices, special work practices, safety equipment and facility requirements for use of animals housed in indoor research facilities. Animals may be experimentally infected (ABSL-1 or ABSL-2), or may naturally harbor infectious agents that are harmful to the health of other animals, to humans or to both animals and humans. In general, the biosafety level recommended for working with infectious agents *in vivo* and *in vitro* are comparable. In addition to the BMBL recommendations, the animal facilities, operational practices, and quality of animal care must meet standards and regulations in accordance with the IACUC approval and guidance from the NMSU veterinarian and EHS&RM.

Plant containment levels refer to conditions that prevent the release of a plant pathogen, plant pest or plant-associated organism or agent outside of the experimental facility. In addition to worker protection, great emphasis is placed on minimizing the possibility of an unanticipated deleterious effect on local agricultural organisms and ecosystems. BL1-P and BL2-P describe the use of plant tissue culture rooms and growth chambers within laboratory facilities and in greenhouses and quarantine facilities. The greenhouse director and the IBC may require additional biological containment practices as regulations, guidance, and standards change, and

as necessary based on the risk assessment of an IBC application, especially if botanical reproductive structures are produced that have the potential to escape containment.

Biosafety Level 1 (BSL-1) for Agricultural, Molecular and Microbiological Experiments

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 not required, but may be used as determined by appropriate risk assessment. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with training in microbiology or a related science.

The following standard and special practices, safety equipment and facilities apply to agents assigned to Biosafety Level 1:

A. Standard Microbiological Practices for BSL-1

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens are in progress.
2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food or cosmetics for human use are not permitted in the work areas. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware, are implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.
6. All procedures are performed carefully to minimize the creation of splashes or aerosols.
7. Work surfaces are decontaminated at least once a day and after any spill of viable material.
8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leak-proof container that is closed prior to transporting from the laboratory. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. An effective integrated pest management program is required.
10. 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 childbearing 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.

B. Special Practices: None for work at BSL-1

C. Safety Equipment (Primary Barriers) for BSL-1

1. Special containment devices or equipment such as biological safety cabinets are not generally required for manipulations of agents assigned to Biosafety Level 1.
2. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination or soiling of personal clothing.
3. Gloves should be worn if the skin on the hands is broken or if a rash is present. Alternatives to powdered latex gloves should be available.
4. Protective eyewear should be worn for conduct of procedures in which splashes of microorganisms or other hazardous materials is anticipated.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratories should have doors for access control.
2. Each laboratory contains a sink for hand washing.
3. The laboratory is designed so that it can be easily cleaned. Carpets, rugs and cloth-covered furniture in laboratories are not appropriate.
4. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate work surfaces and equipment.
5. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning.
6. Laboratory windows that open to the exterior are fitted with screens.

Biosafety Level 2 (BSL-2) for Agricultural, Molecular and Microbiological Experiments

Biosafety Level 2 is similar to Biosafety Level 1 and is suitable for work involving agents of moderate potential hazard 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 with experience in the procedures; (2) access to the laboratory is restricted when work is being conducted; and (3) all procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

In addition to general laboratory training requirements, all personnel attend Laboratory Biosafety Awareness training.

The following standard and special practices, safety equipment, and facilities apply to agents assigned to Biosafety Level 2.

A. Standard Microbiological Practices for BSL-2

1. Access to the laboratory is restricted when experiments are in progress.
2. Persons wash their hands after removing gloves and just prior to leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in the work areas. Food and cosmetics for human use are stored outside the laboratory area in cabinets or refrigerators designated for this purpose only.
4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. Use of sharps is minimized, and spent sharps are disposed of in red, puncture-resistant containers manufactured for the purpose of sharps disposal.
6. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated by autoclaving or other means prior to contacting EHS&RM for pick up.
7. All procedures are performed carefully to minimize the creation of splashes or aerosols.
8. Work surfaces are decontaminated on completion of work and at end of day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern.
9. All cultures, stocks, and other regulated wastes are decontaminated before disposal by autoclaving or before being picked up by EHS&RM for disposal. Methods to demonstrate sterility must be used when disinfecting infectious waste by autoclaving prior to disposal. Materials to be decontaminated outside of the immediate laboratory are transported in a closed, leak-proof secondary container labeled with the biohazard symbol.
10. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the investigator's name and contact information and the name and contact information of a second person familiar with the laboratory as an emergency contact, any personal protective equipment that must be worn in the laboratory, the required immunizations, and the procedures required for entering and exiting the laboratory. Agent information (e.g., organism name) is posted according to departmental emergency procedures for safety and security.
11. An effective integrated pest management program is required.
12. The laboratory supervisor must insure 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 childbearing 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.

B. Special Practices for BSL-2

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.
2. The laboratory director establishes and the IBC approves procedures for personnel to receive appropriate immunizations or medical surveillance for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB testing).
3. The laboratory director should consider the need for collection and storage of serum samples from at-risk personnel, depending on the agents handled or the function of the facility. Circumstances and procedures must be developed in consultation with the Aggie Health & Wellness Center medical director.
4. This biosafety manual must be adopted as policy, and supplemented with laboratory-specific safety information prepared by the laboratory director. The biosafety manual must be available and accessible. Personnel are required to sign a laboratory-specific safety statement certifying that they have been advised of special hazards and agree to follow instructions on practices and procedures.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents. Personnel receive annual updates or additional training as necessary or as procedures change. The laboratory director maintains a record of training for all laboratory personnel, including laboratory-specific training as well as the safety classes required by Environmental Health, Safety & Risk Management. Personnel who have not completed the necessary training are not allowed to work in the laboratory.
6. Cultures, tissues, body fluid specimens, or potentially infectious materials are placed in a durable container with a cover that prevents leakage during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be decontaminated routinely, as well as after spills, splashes, or other potential contamination.
    a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious materials.
    b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory. A record of the decontamination must be prepared and kept for three years. Follow EHS&RM decommissioning procedures for disposal of large equipment such as refrigerators or incubators regardless of working or non-working condition.
8. Incidents that may result in exposure to infectious materials are immediately evaluated and treated according to the laboratory exposure control plan. All such incidents are reported to the laboratory director and Biosafety Officer. A physician provides medical evaluation, surveillance, and treatment, and appropriate records are maintained by Aggie Health & Wellness Center, the laboratory director and EHS&RM.

9. Animals and plants not associated with the work being performed are not permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials that may generate an aerosol are conducted in a certified biological safety cabinet or other physical containment device.

C. Safety Equipment (Primary Barriers) for BSL-2

1. Properly maintained and certified biological safety cabinets (BSC), preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:
    a. procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
    b. 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.
2. Protective laboratory coats, gowns, smock, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; personnel do not take laundry home
3. 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 is disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should wear eye protection in laboratories.
4. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. 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:
    a. Change gloves when contaminated, glove integrity is compromised or when otherwise necessary.
    b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
    c. Disposable gloves are not washed or reused. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

D. Laboratory Facilities (Secondary Barriers) for BSL-2

1. Laboratory doors should be self-closing and are locked when personnel are not present.
2. Each laboratory contains a sink for hand washing; hands-free operation is preferred.

3. The laboratory is designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
4. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Bench tops are impervious to water and are resistant to heat, organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment. Chairs and other furniture used in laboratory work are covered with a non-porous material that can be easily decontaminated with appropriate disinfectant.
5. Laboratory windows that open to the exterior must be fitted with screens.
6. Install biological safety cabinets so that fluctuations of the room supply and exhaust do not interfere with proper operations. Locate biological safety cabinets away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions to maintain the biological safety cabinets' air flow parameters for containment.
7. Vacuum lines are protected with liquid disinfectant traps.
8. An eyewash station is readily available within 50 feet of the work area.
9. There are no specific ventilation requirements. 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.
10. HEPA filtered exhaust air from a Class II BSC can be safely recirculated 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 directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.
11. A method for decontaminating all laboratory wastes is available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

Animal Biosafety Level 1 (ABSL-1) for Vertebrate Animals

Animal Biosafety Level 1 (ABSL-1) is suitable for work involving well-characterized agents that are not known to cause disease in healthy adult humans, and present minimal potential hazard to laboratory personnel and the environment. Personnel must have specific training in animal facility procedures and must be supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures.

A. Standard Practices for ABSL-1

1. The animal facility director establishes and enforces policies, procedures, and protocols for daily operations and emergency situations. Animal studies must be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee (IBC). Worker safety and health concerns are addressed as part of the animal protocol review. Animal experiments cannot begin until IACUC approval is given.
2. A safety manual specific to the animal facility is prepared, or this manual is adopted and supplemented as needed in consultation with the animal facility director, the veterinarian, and safety personnel. The safety manual must be available and accessible. Personnel are advised of potential hazards, and are required to read and follow instructions on practices and procedures.
3. The supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to prevent exposures, and hazard/ exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates and additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.
4. An appropriate medical surveillance program is in place, as determined by risk assessment.
    a. The need for an animal allergy prevention program should be considered.
    b. Animal research supervisors should ensure that Aggie Health & Wellness Center professionals are informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.
    c. Personal health status may impact an individual’s susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of childbearing age should be provided 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.
    d. Personnel using respirators must be enrolled in the EHS&RM respiratory protection program.
5. A sign incorporating safety information must be posted on the entrance to the animal room whenever infectious agents are present. The hazard warning sign identifies the infectious agent(s) in use, lists the name and telephone number of the responsible person(s), and indicates the special requirements for entering the animal room (e.g., the need for immunizations and respirators).

- Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.

6. Only those persons required for program or support purposes are authorized to enter the facility. All persons including facility personnel, service workers, and visitors are advised of the potential biohazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.
7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing. Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials, and when handling animals. Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.
   a. Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.
   b. Eye and face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.
8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption should only be done in designated areas. These activities are not permitted in animal or procedure rooms.
9. All procedures are carefully performed to minimize the creation of aerosols or splatters.
10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
11. Policies for the safe handling of sharps such as needles, scalpels, pipettes, and broken glassware are implemented. Sharps are disposed of in puncture proof containers suitable for autoclaving, or red biohazard containers labeled for pickup by EHS&RM personnel.
12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.
13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/ or animals are housed or are manipulated.
14. An effective integrated pest management program is required.
15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional or local requirements.
    - Decontaminate all potentially infectious materials before disposal using an effective method.

B. Special Practices for ABSL-1: None required.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment) for ABSL-1

1. A risk assessment is conducted to determine the appropriate type of personal protective equipment (PPE) that must be worn.
2. Special containment devices or equipment may not be required as determined by appropriate risk assessment.

3. Protective laboratory coats, gowns, or uniforms may be required to prevent contamination of personal clothing. Protective outer clothing is not worn outside areas where infectious materials and/or animals are housed or manipulated. Gowns and uniforms are not worn outside the facility.
4. Protective eyewear is worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations of airborne particulates. Persons having contact with non-human primates must assess the risk of mucous membrane exposure and wear protective equipment (e.g., masks, goggles, face shields, etc.) as appropriate for the task to be performed.
5. Gloves are worn to protect hands from exposure to hazardous materials. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to protective gloves should be available. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary. Gloves are removed before exiting the animal room. Gloves and PPE should be removed in a manner that prevents transfer of contamination. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.
6. All personnel must wash hands after handling animals, after removing protective gloves, and before leaving the areas where infectious materials and/or animals are housed or manipulated.

D. Facilities (Secondary Barriers) for ABSL-1

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking. Access to the animal facility is restricted. Doors to areas where infectious materials and/or animals are housed open inward, are self-closing, and are kept closed when experimental animals are present. Cubicle room inner doors may open outward or slide horizontally or vertically.
2. The animal facility must have a sink for hand washing. Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.
3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. It is recommended that penetrations in floors, walls and ceiling surfaces be sealed, including openings around ducts, doors and doorframes, to facilitate pest control and proper cleaning.
4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning. Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.
5. External windows are not recommended; if present windows must be resistant to breakage. Where possible, windows should be sealed. If the animal facility has windows that open, they are fitted with fly screens. The presence of windows may impact facility security and therefore should be assessed by security personnel.

6. Ventilation should be provided in accordance with criteria from *Guide for Care and Use of Laboratory Animals, Eighth Edition* (Institute for Laboratory Animal Research, National Research Council; Washington, DC: National Academies Press, 2011). No recirculation of exhaust air may occur. It is recommended that animal rooms have inward directional airflow. Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
8. If floor drains are provided, the traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
9. Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F. If manual cage washing is utilized, ensure that appropriate disinfectants are selected.
10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
11. Emergency eyewash and shower are readily available; location is determined by risk assessment.

Animal Biosafety Level 2 (ABSL-2) for Vertebrate Animals

Animal Biosafety Level 2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1. ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

ABSL-2 requires that: 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of pathogenic agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and 4) BSCs or other physical containment equipment is used when procedures involve the manipulation of infectious materials, or where aerosols or splashes may be created.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Implementation of employee occupational health programs should be considered.

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

A. Standard Practices for ABSL-2

1. The animal facility director establishes and enforces policies, procedures, and protocols for daily operations and emergency situations. Worker safety and health concerns are addressed as part of each animal protocol. Prior to beginning a study, animal protocols

must be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee (IBC). Experiments cannot begin until IACUC approval is given.

2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety officers. The PI may prepare an entirely unique biosafety manual or adopt this NMSU biosafety manual with the addition of supplemental information that advises personnel of special hazards.
    - Consideration should be given to specific biohazards unique to the animal species and protocol in use.
    - The safety manual must be available and accessible. The safety manual is required reading as part of instruction on work practices and procedures. A record is maintained listing the names of persons who read the manual and the date they read the manual.
3. The supervisor must ensure that animal care, laboratory, and support personnel receive appropriate training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.
4. An appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered.
    - Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.
    - Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of childbearing age should be provided 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.
    - Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.
5. A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/ or animals are housed or are manipulated when infectious agents are present. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or names of other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of all infectious agents is necessary when more than one agent is being used within an animal room.
    - Security-sensitive agent information and occupational health requirements should be posted in accordance with the departmental policy based on a biosecurity risk assessment with input from EHS&RM, IBC, and IACUC.
    - Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.

6. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or manipulated.
   - All persons including facility personnel, service workers, and visitors are advised of the potential hazards (physical, naturally occurring, or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.
7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
   - Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials and when handling animals.
   - Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.
   - Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.
   - Eye, face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.
8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in animal holding areas or procedure rooms. Food must be stored outside of the laboratory in cabinets or refrigerators designated and used for this purpose.
9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries.

    Precautions must always be taken with sharp items. These include:

    a. The use of needles and syringes or other sharp instruments in the animal facility is limited to situations where there is no alternative such as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
    b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used, disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.
    c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
    d. Broken glassware must not be handled directly; it should be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
    e. Use of equipment with sharp edges and corners should be avoided.

12. Equipment and work surfaces in the room are decontaminated at least daily with an effective disinfectant after work with the infectious agent, and after spills, splashes, or other contamination by infectious materials.
13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/ or animals are housed or manipulated.
14. An effective integrated pest management program is required. See the IPM section of this manual.
15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof containers for appropriate disposal in compliance with applicable institutional, local and state requirements.
    - Decontaminate all potentially infectious materials before disposal using an effective method.

B. Special Practices for ABSL-2

1. Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms. When appropriate, a base line serum sample should be stored
2. Procedures involving a high potential for generating aerosols should be conducted within a biosafety cabinet or other physical containment device. When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used.

   Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications) should be used whenever possible.
3. Decontamination by an appropriate method (e.g. autoclave, chemical disinfection, or other approved decontamination methods) is necessary for all potentially infectious materials and animal waste before movement outside the areas where infectious materials and/or animals are housed or are manipulated. This includes potentially infectious animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse.
   - A method for decontaminating routine husbandry equipment, sensitive electronic and medical equipment should be identified and implemented.
   - Materials to be decontaminated outside of the immediate areas where infectious materials and/or animals are housed or are manipulated must be placed in a durable, leak proof, covered container and secured for transport. The outer surface of the container is disinfected prior to moving materials. The transport container must have a universal biohazard label.
   - Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements. Autoclaving of content prior to incineration is recommended.
4. Equipment, cages, and racks should be handled in a manner that minimizes contamination of other areas.

- Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated.

5. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
6. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in facility-specific safety plans. All such incidents must be reported to the animal facility supervisor and the Biosafety Officer. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment) for ABSL-2

1. Properly maintained Biological Safety Cabinets (BSCs), personal protective equipment (e.g., gloves, lab coats, face shields, respirators, etc.) and/or other physical containment devices or equipment, are used whenever conducting procedures with a potential for creating aerosols, splashes, or other potential exposures to hazardous materials. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals.
   - When indicated by risk assessment, animals are housed in primary biosafety containment equipment appropriate for the animal species, such as solid wall and bottom cages covered with filter bonnets for rodents or other equivalent primary containment systems for larger animal cages.
2. A risk assessment should determine the appropriate type of personal protective equipment to be utilized.
   - Scrub suits and uniforms are removed before leaving the animal facility. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home.
   - Gowns, uniforms, laboratory coats and personal protective equipment are worn while in the areas where infectious materials and/or animals are housed or manipulated and removed prior to exiting. Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.
3. Eye and face protection (mask, goggles, face shield or other splatter guard) are used for manipulations or activities that may result in splashes or sprays from infectious or other hazardous materials and when the animal or 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 should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.

   Persons having contact with NHPs should assess risk of mucous membrane exposure and wear protective equipment (e.g., masks, goggles, face shields) appropriate for the task to be performed.
   - Respiratory protection is worn based upon risk assessment.
4. Gloves are worn to protect hands from exposure to hazardous materials. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.

- Gloves are changed when contaminated, glove integrity is compromised, or when otherwise necessary.
- Gloves must not be worn outside the animal rooms.
- Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials.
- Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.
- Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

D. Facilities (Secondary Barriers) for ABSL-2

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.
   - Doors to areas where infectious materials and/or animals are housed open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
2. A hand-washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility.
   - If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink must also be available for hand washing at the exit from each segregated area.
   - Sink traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) are water resistant.
   - Penetrations in floors, walls and ceiling surfaces are sealed, including openings around ducts, doors and doorframes, to facilitate pest control and proper cleaning.
   - Floors must be slip-resistant, impervious to liquids, and resistant to chemicals.
4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
   - Furniture should be minimized. Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.
5. External windows are not recommended; if present, windows must be sealed and resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.

6. Ventilation should be provided in accordance with criteria from *Guide for Care and Use of Laboratory Animals, Eighth Edition* (Institute for Laboratory Animal Research, National Research Council; Washington, DC: National Academies Press, 2011). The direction of airflow in the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms.
    - Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas, to facilitate cleaning and minimize the accumulation of debris or fomites.
8. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
9. Cages should be autoclaved or otherwise decontaminated prior to washing. The mechanical cage washer should have a final rinse temperature of at least 180°F. The cage wash area should be designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures during the cage/equipment cleaning process.
10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
11. If BSCs are present, they 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, 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 directly to the outside through an independent, hard connection.
    - Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be recertified at least once a year to ensure correct performance.
    - All BSCs should be used according to manufacturer's specifications to protect the worker and avoid creating a hazardous environment from volatile chemicals and gases.
12. If vacuum service (i.e., central or local) is provided, each service connection should be fitted with liquid disinfectant traps and an in-line HEPA filter placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement.
13. An autoclave should be present in the animal facility to facilitate decontamination of infectious materials and waste.
14. Emergency eyewash and shower are readily available; location is determined by risk assessment.

Plant Biosafety Level 1 (BL1-P) Greenhouse Containment

The BL1-P designation provides for a moderate level of containment for experiments involving plant pests, plant-associated organisms, and transgenic plants in which there is no evidence that the agent would be able to survive and spread in the environment and, if accidentally released, would not pose an environmental risk. For example, an experiment designed to study transgenic potato plants containing cloned genes for insect resistance obtained from primitive potato cultivars would be classified as BL1-P.

BL1-P also applies to DNA-modified common microorganisms that cannot spread rapidly and are not known to have any negative effects on either natural or managed ecosystems, such as *Rhizobium* and *Agrobacterium*. A BL1-P designation would be assigned, for example, to an experiment that uses a transgenic strain of *Rhizobium* containing *Agrobacterium* genes known to affect root colonization, or plants using *Agrobacterium* DNA segments as part of the transformation process.

A. Standard Practices for BL1-P

1. Access to the greenhouse shall be limited or restricted, at the discretion of the greenhouse director, when experiments are in progress.
2. Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL1-P greenhouse practices and procedures. All procedures are performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism.
3. A record shall be kept of experiments currently in progress in the greenhouse facility.

B. Decontamination and Inactivation (BL1-P)

1. Experimental organisms shall be rendered biologically inactive by a validated chemical method or by autoclaving before disposal outside of the greenhouse facility.
2. A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens), by methods appropriate to the organisms and in accordance with applicable state and federal laws.
3. Arthropods and other motile macroorganisms must be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions must be taken to minimize escape from the greenhouse facility, such as adding of additional doors, UV/fan traps, and multi-panel plastic drapes across the doorway.

C. Concurrent Experiments Conducted in the Greenhouse (BSL-1P)

Experiments involving other organisms that require containment level lower than BL1-P may be conducted in the greenhouse concurrently with experiments that require BL1-P containment, provided that all work is conducted in accordance with BL1-P greenhouse practices.

D. Facilities (BL1-P)

1. The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.
2. The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area.
3. The greenhouse floor may be composed of gravel or other porous material. At a minimum, impervious (e.g., concrete) walkways are recommended.
4. Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to contain or exclude pollen, microorganisms, or small flying animals (e.g., arthropods and birds); however, screens are recommended.

Plant Biosafety Level 2 (BL2-P) Greenhouse Containment

BL2-P is assigned to experiments with plant pests, plant pathogens, transgenic plants, and plant-associated organisms, which, if released outside the greenhouse, could be viable in the surrounding environment but would have a predictably minimal biological impact or could be readily managed. BL2-P is required for transgenic plants that may exhibit a new weedy characteristic or that may be capable of interbreeding with weeds or related species growing in the vicinity. For example, greenhouse tests of transgenic sunflower containing wheat genes intended to confer resistance to the fungus *Sclerotinia* would be classified BL2-P because sunflower is capable both of hybridizing with wild relatives, and becoming established as a volunteer weed.

BL2-P containment is assigned to transgenic experiments that use the entire genome of an indigenous infectious agent or pathogen. This level of containment is also appropriate for transgenic plant-associated microorganisms that are either indigenous to the area and potentially harmful to the environment but manageable, or are exotic but have no potential for causing serious harm to managed or natural ecosystems. The BL2-P classification likewise applies to experiments using plant-associated transgenic insects or small animals as long as they pose no threat to managed or natural ecosystems.

A. Standard Practices (BL2-P)

1. Access to the greenhouse is limited or restricted, at the discretion of the greenhouse director, to individuals directly involved with the experiments when they are in progress.
2. A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

3. If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated on a sign posted on the greenhouse access doors.
4. If there is a risk to human health, a sign shall be posted incorporating the universal biohazard symbol.
5. Personnel shall be required to read and follow instructions on BL2-P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.
6. A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.
7. Materials containing experimental microorganisms, which are brought into or removed from the greenhouse facility in a viable or intact state, shall be transferred in a closed non-breakable container.
8. A record shall be kept of experiments currently in progress in the greenhouse facility.
9. A greenhouse practices manual shall be prepared or adopted. This manual shall: (i) advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms.
10. The Principal Investigator must report any greenhouse accident involving the inadvertent release or spill of microorganisms to the greenhouse director and to the Biosafety Officer who will then notify the Institutional Biosafety Committee, NIH/OBA and other appropriate authorities if applicable. Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail); 301-496-9838 (phone), 301-496-9839 (fax). Documentation of any such accident shall be prepared and maintained.

B. Decontamination and Inactivation (BL2-P)

1. Experimental organisms shall be rendered biologically inactive by appropriate methods (chemical denaturing or autoclaving) before disposal outside of the greenhouse facility.
2. Decontamination of run-off water is not necessarily required. However, if the floor of the greenhouse is composed of gravel or similar porous material, the flooring should be periodically treated with a compound known to denature, or render inactive, any organisms potentially entrapped by the gravel.

C. Control of Undesired Species and Motile Macroorganisms (BL2-P)

1. A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.
2. Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility. Overlapping plastic panels across the doorway, UV traps, suction fans or similarly acting devices can minimize the escape of flying insects, arthropods or nematodes.

3. Experiments involving other organisms that require containment level lower than BL2-P may be conducted in the greenhouse concurrently with experiments that require BL2-P containment provided that all work is conducted in accordance with BL2-P greenhouse practices.

D. Transfer of Materials (BL2-P)

Materials containing experimental microorganisms, which are brought into or removed from the greenhouse facility in a viable or intact state, shall be transferred in a closed non-breakable container.

E. Greenhouse Practices Manual (BL2-P)

A greenhouse practices manual shall be prepared or adopted. This manual shall: (i) advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms.

F. Facilities (BL2-P)

1. The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.
2. The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas and is considered part of the confinement area.
3. A greenhouse floor composed of an impervious material. Concrete is recommended, but gravel or other porous material under benches is acceptable unless propagules of experimental organisms are readily disseminated through soil. Soil beds are acceptable unless propagules of experimental organisms are readily disseminated through soil.
4. Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to exclude pollen or microorganisms; however, screens are required to exclude small flying animals (e.g., arthropods and birds).
5. An autoclave shall be available for the treatment of contaminated greenhouse materials.
6. If intake fans are used, measures shall be taken to minimize the ingress of arthropods. Louvers or fans shall be constructed such that they can only be opened when the fan is in operation.
7. BL2-P greenhouse containment requirements may be satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macroorganisms in a manner that satisfies the intent of the foregoing clauses.

Table 1. Summary of Recommended Biosafety Levels for Infectious Agents

Source: BMBL, 5th Ed., Section IV

BSL	Agents	Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults	Standard microbiological practices	• No primary barriers required. • PPE: lab coats, gloves, eye and/or face protection as needed	Laboratory bench and sink required
2	Associated with human disease • Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers: • Biosafety cabinet (BSC) or other physical containment devices are used for all manipulations of agents that cause splashes or aerosols of infectious materials • PPE: Lab coats, gloves, face and eye protection as needed.	BSL-1 plus: • Autoclave available
3	Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure	BSL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering	Primary barriers: • Biosafety cabinet (BSC) or other physical devices used for all open manipulations of agents • PPE: Protective lab clothing, gloves, face, eye and respiratory protection as needed.	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhausted air is not recirculated • Negative airflow into lab • Entry through airlock or anteroom • Hand washing sink near lab exit

Table 2. Summary of Recommended Animal Biosafety Levels for Activities with Experimentally or Naturally Infected Vertebrate Animals

Sources: BMBL, 5th edition, Section IV; also see, *Report of the Trans-Federal Task Force on Optimizing Biosafety and Biocontainment Oversight*, July 2009

Animal Biosafety Level	Agents	Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
ABSL-1	Not known to consistently cause diseases in healthy adult humans	Standard animal care and management practices, including appropriate medical surveillance programs	As required for normal care of each species. • PPE: lab coats and gloves; eye and/or face protection as needed	Standard animal facility: • No recirculation of exhaust air • Directional airflow recommended • Hand washing sink is available
ABSL-2	Associated with human disease • Hazard: percutaneous injury, ingestion, mucous membrane exposure	ABSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual • Decontamination of infectious wastes and animal cages prior to washing	ABSL-1 equipment plus: • Containment equipment appropriate for animal species PPE: Lab coat, • gloves, face, eye and respiratory protection as needed	ABSL-1 facility plus: • Autoclave available • Hand washing sink available • Mechanical cage • washer recommended • Negative airflow into animal and procedure rooms recommended
ABSL-3	Indigenous or exotic agents with potential for aerosol transmission • Disease may have serious or potentially lethal consequences	ABSL-2 practice plus: • Controlled access • Decontamination of clothing before laundering • Cages decontaminated before bedding is removed • Disinfectant foot bath as needed	ABSL-2 equipment plus: • Containment equipment for housing animals and cage dumping activities • Class I, II or III BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols • PPE: appropriate respiratory protection	ABSL-2 facility plus: • Physical separation from access corridors • Self-closing, double-door access • Sealed penetrations • Sealed windows • Autoclave available in facility • Entry through ante-room or airlock • Negative airflow into animal and procedure rooms • Hand washing sink near exit of animal or procedure room
Additional regulations and work practices apply to specific types of research with potentially hazardous agricultural agents. More information is available from these sources: • USDA/APHIS: permit requirements for import, export, interstate transport, use; Select Agent regulations • The American Society of Tropical Medicine and Hygiene, *Arthropod Containment Levels* (ACL 1-4) • *NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules,* Appendix G (physical containment), and Appendix Q (large animals)				

Table 3. Summary of Plant Biosafety Levels

Sources:

- A Practical Guide to Containment, Plant Biosafety in Research Greenhouses, Virginia Tech, 2008.
- Report of the Trans-Federal Task Force on Optimizing Biosafety and Biocontainment Oversight. 2009.
- *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*, Appendix P.

Plant Biosafety Level	Agents	Work Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
BSL-1P	Plant-associated organisms and transgenic plants not able to survive and spread in the environment, or if released would not pose a risk to the environment.	• Access is limited or restricted when experiments are in progress. • Personnel read the written procedures prior to entry. • Records are maintained for all current projects. • Experimental organisms are autoclaved prior to disposal	None	Impervious walkways are recommended; no special barriers are required.
BSL-2P	Transgenic plants with entire genome of indigenous pathogen, and related organisms of negligible impact or such impact is manageable	• Greenhouse manual is developed or adopted • Accidents involving unintentional release or spill of microorganisms are reported to greenhouse director and BSO • Porous flooring (gravel or dirt) is periodically treated to inactivate organisms potentially trapped in flooring • Posted notification of restricted access • Viable materials transported into and out of the facility are contained in non-breakable containers	Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials PPE: Laboratory coats, gloves, safety glasses, face shield or goggles.	• Autoclave available • Entry doors have locks Floor composed • of impervious material • Window and intake fan openings are screened (30 – mesh or higher) to exclude arthropods and flying animals
Additional regulations and work practices have been developed for specific types of research with potentially hazardous agricultural agents. More information is available from these sources: • USDA/APHIS permit requirements for import, export, interstate transport, and use • The American Society of Tropical Medicine and Hygiene, Arthropod Containment Levels (ACL 1-4) • NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules, Appendix G (physical containment) and Appendix P (plants and plant-associated microorganisms)				

VIII. OVERVIEW OF SELECTED BIOSAFETY PROCEDURES AND TASKS

This section presents basic information about equipment and procedures that are commonly used in laboratories for cell biology, tissue culture, molecular biology, and microbiology activities.

AUTOCLAVES AND STEAM STERILIZATION

Principle

Supersaturated steam (water heated above 212° F) under pressure is an efficient and cost effective means of:

1) Sterilization of liquids and heat-stable solids and
2) Decontaminating viable organisms cultured on solid media, genetically modified plants, and related biohazardous waste.

Overview

At start-up, a generator delivers steam (superheated water) into the airtight "jacket" that surrounds the chamber of the autoclave. The chamber door is fitted with a gasket to ensure an airtight seal when the door is closed and the cycle engaged. Sterilization is achieved by applying heat to at least 250° F (121C) and pressure to15 psi and maintaining these conditions for at least 15 minutes.

The nature and quantity of the materials being autoclaved affects the total cycle run time. For instance a half hour timed cycle for twenty 500 ml bottles or ten 1.0-liter flasks may take 90 minutes to heat up, run for 30 minutes at temperature and pressure, and exhaust. Alternatively, the total time for twenty boxes of µl pipette tips (a dry load) to complete the sterilization cycle will be close to the 30 minutes scheduled for the cycle.

Similarly, a single, loosely packed bag of biohazardous waste will take less time to decontaminate than several bags of tightly-packed waste. Steam must penetrate throughout the over-wrapped implements, bags, or containers for the requisite contact time in order for the items to be decontaminated. If sufficient steam does not come into contact with the materials inside large containers or tall vessels, then microorganisms can survive the autoclave cycle.

Finally, the state Environmental Department regulates decontamination of laboratory waste under the New Mexico Administrative Code (NMAC), *Special Waste Requirements* (NMAC Title 20, Chapter 9, Part 8). Sterilization of lab ware, media, and supplies before use is not regulated. Per Solid Waste regulations, records that validate the waste decontamination process must be generated and maintained for each autoclave used to decontaminate laboratory waste.

Definitions

Autoclave: a steel chamber with an integral jacket constructed to withstand internal pressurization when charged with super-saturated steam; a "pressure cooker".

Sterilize: to make free from living bacteria, virus, and other microorganisms; the complete destruction of all forms of microbial life, including bacterial and fungal spores.

Decontaminate: a process to reduce the number of viable organisms so that the risk of disease transmission is eliminated.

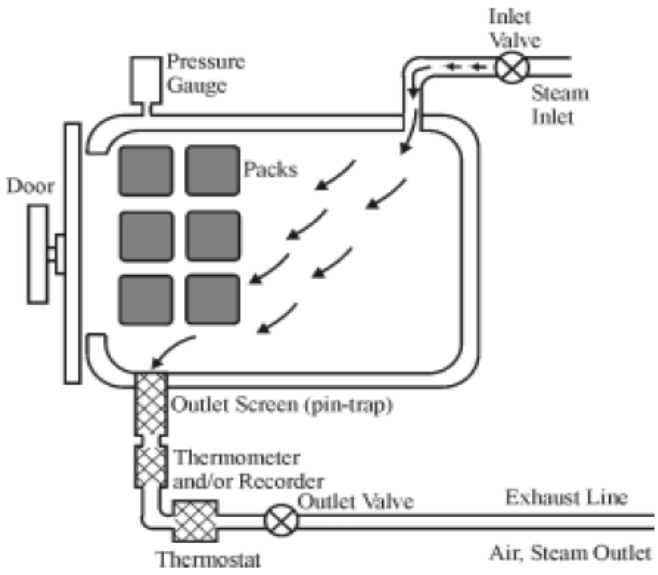

Figure 3. Autoclave diagram.

Autoclave Operating Parameters

Cycle settings: Review the autoclave manufacturer's instruction manual to select the proper cycle conditions for the solid or liquid materials to be sterilized. Autoclave users must be trained to operate the equipment and to understand the methods to verify the effectiveness of the decontamination treatment. Contact the building safety monitor or the biosafety officer for more information about the cycle settings, or if you observe problems related to the autoclave pressure, exhaust, or sterility of materials.

Cycles can be programmed to differ in the rate that the chamber is exhausted at the completion of the cycle. Typically, materials run on the "gravity" cycle are exhausted more rapidly than a "liquids" cycle. It is essential that vessels containing liquids are loosely capped during autoclaving to permit off-gassing during the exhaust cycle. Tightly capped vessels may explode or implode in the autoclave during the exhaust cycle or after the container is removed from the autoclave due to pressure or vacuum created in the vessel head-space (the space between the cap and liquid surface) as the container cools.

Temperature: Temperature is routinely set to 250° F (121C).

Pressure: The steam generator is set to provide 17 psi to the interior chamber of the autoclave. *Note: Chamber pressurization requirement increases at higher geographic elevations.*

Time: The cycle time is dependent on the load. Total run time depends on the load (liquid or solid) that in turn determines the appropriate exhaust setting (rapid or slow). Generally, a rapid exhaust is selected for sterilization of dry goods, and a slow exhaust is selected for liquids.

NOTE: The printed recorder tape should be retained as part of the autoclave records.

Monitoring Autoclave Operating Parameters

Autoclaves used to decontaminate laboratory waste prior to disposal in a New Mexico landfill must be tested to confirm adequate temperature and pressure are sustained during the cycle.

New Mexico regulations for infectious waste define the procedures for "sterilization by heating in a steam sterilizer so as to render the waste non-infectious", in *Special Waste Requirements*, Paragraph (2) of Subsection F of 20.9.8.13 NMAC:

1. the operator shall have available and shall certify in writing that she or he understands written operating procedures for each steam sterilizer including time, temperature, pressure, type of waste, type of container(s), closure on container(s), pattern of loading, water content, and maximum load quantity;
2. infectious waste shall be subjected to sufficient temperature, pressure and time to kill *Geobacillus stearothermophilus* spores or induce a complete color change in an approved steam sterilization integrator when either indicator is located in the center of the waste load being decontaminated;
3. unless a steam sterilizer is equipped to continuously monitor and generate a printed paper record of time, temperature and pressure during the entire length of each sterilization cycle, a chemical indicator shall be attached to each package of infectious waste that will visually demonstrate at the end of the autoclave cycle that each package was exposed to a temperature of at least 250 degrees Fahrenheit or 121 degrees Celsius in the presence of steam under pressure was reached during the process; the original printed record generated by the autoclave must be maintained for three years;
4. each sterilization unit shall be evaluated for effectiveness with spores of *Geobacillus stearothermophilus* or approved steam sterilization integrator at least once each 40 hours of operation; and
    a) a written log shall be maintained for each sterilization unit which contains:
    b) date, time and load number for each load;
    c) amount per load;
    d) duration of the cycle; and
    e) the operator's name

Operators must monitor autoclave operation in order to validate that waste was exposed to adequate steam under pressure for a sufficient time to achieve decontamination of the waste material. A biological or a chemical indicator is used to validate the decontamination process.

A **biological indicator (BI)** uses spores of a thermophilic bacterium to demonstrate a microbiological kill at the end of the autoclave decontamination cycle. A typical BI contains spores of a challenge organism suspended in an appropriate media that contains a pH-sensitive dye. Prior to autoclaving, the BI is placed inside the waste bag (integrated into the load). Use an "alligator" clip and a length of string, wire or small gauge chain to assist with post-cycle recovery of the strip or vial from the bag. After the decontamination cycle is complete, the BI is recovered from the load and incubated usually for 24 hours (sometime as long as 72 hours) and visually inspected for a color change. No color change indicates the spores were killed. A color

change indicates the spores were not killed and bacterial growth has caused the pH indicator to change color.

A typical **chemical indicator (CI)** is a strip of foil-backed bonded paper (approximately 4 inches by 0.5 inches) embedded with a chemical that migrates across a "window" when exposed to steam under pressure at 250 degrees F (121 degrees C) for the requisite 15 minutes (See figure 4). The chemical integrator reacts to steam under pressure for the duration of the autoclave cycle in a manner considered equivalent to a microbiological kill. It can be used in solid and liquid loads, and with gravity or vacuum cycles. Prior to autoclaving an indicator strip is placed inside of each bag or container of laboratory waste. Use an "alligator" clip and a length of string, wire or small gauge chain to assist with post-cycle recovery of the strip from the bag.

Figure 4. Chemical indicator strip display

If the indicator chemical fails to migrate the entire length of the strip, the decontamination cycle is considered unsatisfactory. The autoclave cycle is repeated with a new CI strip to confirm the result. A second event resulting in the indicator chemical failing to migrate across the entire length of the strip means the autoclave must be taken out of service and is not used until it is repaired and re-tested. The unit should be posted with an "out of service" or similar warning notice. Laboratory waste cannot be released for disposal to a landfill unless it has been decontaminated as part of a validated autoclave cycle.

Note: Autoclave indicator tape and biohazard bags with chemically embedded stripes or words (e.g., "AUTOCLAVED") that appear after exposure to 250 °F are not equivalent to a chemical integrator. Autoclave tape is useful for marking and labeling the exterior of items to be autoclaved, but the use of autoclave tape alone is not sufficient to demonstrate decontamination of the contents of the package. Autoclave tape may be used together with a chemical indicator strip but may not be used instead of a chemical indicator strip. Further, biohazard bags and containers that are red or orange indicate regulated waste, and if used cannot be disposed in the regular trash. Any item labeled with the biohazard symbol (except sharps containers and regulated medical waste) that has been treated by heat or chemicals to render the contents non-infectious must have the biohazard symbol defaced and be marked to indicate the method of sterilization before release to the general waste stream.

Each supervisor is responsible for providing and documenting training for autoclave users. A general explanation of autoclave principles, sterilization and recordkeeping is provided in the Biosafety Awareness training class. In addition to this general awareness training, a Standard Operating Procedure should be generated at the department or laboratory level and communicated to autoclave users for site-specific training. See Appendix E for a sample Autoclave Standard Operating Procedure template.

The Biosafety Officer and EHS&RM specialists conduct periodic reviews of log details and training records in order to certify that NMSU's sterilization processes are effective to render laboratory waste non-infectious.

Required Monitoring Procedure for Laboratory Waste Decontamination Run

1. Obtain a steam sterilization integrator strip, also known as a chemical indicator (CI). CIs can be purchased from scientific supply companies.
2. Place the CI test strip inside of each bag or container of laboratory waste.
3. Run the sterilizer cycle according to the autoclave manufacturer's instructions.
4. After the cycle is completed, allow the content to cool to room temperature.
5. Open the bag and retrieve the CI test strip.
6. Confirm that the CI dye has migrated completely from the "start" to "finish" zone.
7. If the dye fails to completely migrate across the strip, the test has failed to demonstrate that sufficient temperature and pressure was generated within the bag. The waste cannot be considered decontaminated. Replace the CI with a new strip and autoclave again. The test should be repeated. In the event of a second failed test, the autoclave should not be used until the reason for the failure has been resolved.
8. Record each load in the logbook. Include the date, start time, run number, load volume, load description, cycle duration, operator name, and the result of the CI test.
    - See Appendix E for a sample Autoclave Standard Operating Procedure template.

Personal Protective Equipment for Autoclaving

Use heat-resistant insulated gloves when loading and unloading the autoclave and when handling any hot items. At a minimum, use of a lab coat, closed-toed shoes, and eye protection are required when handling hot liquids. The extent of protection required (e.g., safety glasses or face shield) should be determined according to the contents, volume and handling steps.

Hazard Assessment for Autoclave Operations

Burn: Autoclaved materials (especially liquids and metals) remain hot long after removal from the chamber. Use thermal resistant gloves and exercise extreme caution when handling autoclaved materials.

Explosion/Implosion: Tightly sealed or stopped bottles will become pressurized during autoclaving and may explode/implode either during chamber de-pressurization or once the item is removed from the chamber. Make sure bottle caps are loosened prior to autoclaving.

Item failure: All solid materials (glass, plastic, fibers) are subject to damage from frequent exposure to steam under pressure. The integrity of these items should be checked routinely before and after each autoclave event.

Hearing: Steam generators can be noisy and pose a threat to hearing. Contact EHS&RM with any health or safety concern, including noise levels in the area.

High room temperature: Autoclaves and steam generators (and other heat-generating equipment) can elevate the room temperature to an uncomfortable level. A ventilated exhaust canopy installed above the autoclave door will mitigate excess heat buildup in the room.

Chemical Vapors: Autoclaving chemicals may generate noxious, toxic and caustic vapors that are likely to pose a respiratory hazard. Do not autoclave chemicals - especially halogens (chlorine, bromine) and halogen-containing solutions including bleach, strong acids or strong bases, or radioactive materials.

Spills: Bags and containers improperly prepared for autoclaving may spill or rupture while being loaded or unloaded from the autoclave. Evaluate and choose an appropriate bag or container to contain the material to be autoclaved. For collection of biohazardous waste and semi-solid media cultures of viable organisms, do not fill beyond 75% of the volume capacity of the bag or container. Inspect frequently autoclaved items to discover failures, or cracks.

Super-heated liquids: Autoclaved liquids may have temperatures well above boiling at one atmosphere. Vibration or stirring may cause super-heated liquids to boil rapidly, possibly causing a violent release of the super-heated material and projectiles.

Loading the Autoclave

For liquid materials, ensure the primary vessel is vented to facilitate steam access; i.e., bags and containers are loosely sealed. When possible, consider using foil or a porous material to plug the vessel instead of a plastic or metal cap. Plastic and metal caps may "settle" back down on the threads of a bottle and cause a vacuum. A vacuum is undesirable because contaminated air may be drawn into the vessel when the cap is loosened.

1. Place the bag, bottle or other container into a secondary container such as a metal or polypropylene pan.
2. Arrange material within the secondary container to not interfere with steam penetration, i.e., don't stack bags on top of one another.
3. Close and tighten the autoclave door.
4. Confirm that the proper cycle has been chosen for the load being run.
5. Start the autoclave and observe the pressure gauge to ensure that the cycle starts correctly.
6. Record the load description, cycle, date, time and your initials in the logbook.

Unloading the Autoclave

Check that the chamber pressure gauge is zero (no dial deflection). There will be residual internal pressurization of the chamber generated by off gassing of the autoclave contents, especially with liquid loads. This residual pressurization is usually insufficient to deflect the needle on the autoclave pressure gauge.

1. Carefully loosen the door to release the residual pressure and allow the autoclaved load temperature to cool. Do not open the door more than one inch until the steam and hot air has dissipated. Wait 5-10 minutes before removing hot items from the chamber.
2. While wearing heat-resistant insulated gloves and eye or face protection, remove the contents and place on a cart, bin, or other firm, heat resistant surface.
3. Confirm the cycle temperature and duration on the autoclave chart or printout, and record the results of the CI test strip in the logbook.

BIOLOGICAL SAFETY CABINETS (BSCS)

Background

The design concept for what we now refer to as a biological safety cabinet originated at the National Institutes of Health and resulted in the development of a product specification that remains in use to this day. Colloquial terms such as “hood” and phrases like “tissue culture hood” and “laminar flow hood” are often used (incorrectly) to refer to biological safety cabinets. In a laboratory setting, the term “hood” may mean chemical fume exhaust hood, or it may refer to a component of a respiratory protection device. Similarly, the phrase “tissue culture hood” is inaccurate because it implies that the BSC may be restricted to use for tissue culture. And the generic phrase “laminar flow hood” is not specific because it refers to any device that moves air at a constant velocity in a uniform pattern and direction. Use of non-specific phrases may cause confusion between biological safety cabinets and clean air benches. There are two ways to clearly refer to biological safety cabinets; the term biosafety cabinet is a contraction of the proper name, and the other is the acronym BSC. The CDC/NIH publication *“Primary Containment for Biohazards: Selection, Installation, and Use of Biological Safety Cabinets”* is a good source of information on the operation, use, and certification of BSCs. This document is available in *Biosafety in Microbiological and Medical Laboratories* (BMBL), Appendix A, and can be accessed online at https://www.cdc.gov/safelabs/resources-tools.html.

Biological safety cabinets (BSCs) are primary containment devices designed to protect the product being manipulated, the operator, the environment, or all three. BSCs use high efficiency particulate air (HEPA) filters to remove airborne particles, bacteria, spores, and viruses from laboratory work areas. Equipment manufacturers may have more than one design for each type of BSC in their product line. The original NIH design evolved into the three classes of BSCs in use today, known as Class I, Class II and Class III. Class II BSCs are the most widely used in clinical, biomedical, and microbiological research and manufacturing applications.

Table 4 summarizes the differences in face velocity, airflow patterns and the acceptable biosafety level for each class and type of BSC.

Class II Type A1 & A2 BSCs are designed to exhaust the HEPA-filtered air from the BSC either into the laboratory or via a canopy exhaust connection to the building exhaust duct. A BSC canopy exhaust connection resembles the canopy used to capture the steam plume released when opening an autoclave door or a kitchen range hood. There are three benefits to installing a canopy exhaust: 1) The canopy connection assists with maintaining the negative pressurization of the BSL-2 laboratory; 2) the canopy will exhaust contaminated air in the event of a BSC exhaust HEPA filter failure; and 3) the canopy connection reduces the noise from the BSC blower motor.

Table 4. Summary of Biological Safety Cabinet Classes

Source: BMBL 5th Ed., Appendix A

Type	Face Velocity (Linear feet/minute)	Airflow Pattern	Lab Biosafety Level
Class I	75 lfm	Air flows in at front (not HEPA-filtered); exhaust through a HEPA filter into the room or to the outside through a canopy unit.	BSL 1, 2, 3
Class II A1	75 lfm	70% of HEPA-filtered supply air is re-circulated within the BSC and 30% is exhausted through a HEPA filter into the room or to the outside through a canopy unit.	BSL 1, 2, 3
Class II A2	100 lfm	70% of HEPA filtered supply air is re-circulated within the BSC and 30% is exhausted through a HEPA filter into the room or to the building exhaust through a canopy unit. Plenums are under negative pressure to the room.	BSL 1, 2, 3
Class II B1	100 lfm	30% of internal air is re-circulated within the BSC and 70% of HEPA filter-air is exhausted through a dedicated duct to the outside	BSL 1, 2, 3
Class II B2	100 lfm	No recirculation; total exhaust to the outside through a HEPA filter and dedicated duct	BSL 1, 2, 3
Class III	N/A	Supply air is HEPA filtered. Exhaust air passes through two HEPA filters in series and is exhausted to the outside via a hard connection to building exhaust system.	BSL 3 & 4

Testing and Certification of Biological Safety Cabinets

Every BSC used for work at BSL-2 must be tested and certified prior to initial use, at least annually thereafter, whenever a BSC is relocated, and after repairs that require accessing a contaminated plenum (blower motor and HEPA filter replacement are the most common events).

In 2012, the National Sanitation Foundation International in conjunction with the American National Standards Institute issued a revised NSF/ANSI Standard 49 on Class II (laminar flow) biosafety cabinetry. The NSF/ANSI Standard 49 applies only to Class II biological safety cabinets, as designed to minimize the hazards inherent in working with agents assigned to biosafety levels 1, 2, or 3. Contact the Biosafety Officer to obtain a copy of the current standard.

An NSF-accredited field certifier performs a battery of primary and secondary tests to measure the performance of a BSC in meeting the manufacturer's operating specifications. BSC containment is assessed by testing HEPA filters for leakage, the internal airflow pattern, measuring the down flow velocity of the HEPA filtered air, the in-flow velocity, and when appropriate, a cabinet leak test. Each of these parameters must meet the original equipment manufacturer (OEM) specifications in order for the unit to be certified.

Secondary tests include measurements of noise output, light intensity, electrical voltage supply, and ground resistance. The electrical connections are checked to ensure the proper polarity, the ground fault interrupter circuit, and any alarm is checked as well. At the conclusion of the testing, a report is issued to the laboratory director indicating that the BSC passed certification or failed certification. The certifier often applies a decal listing the BSC serial number, the date of the test, the certification expiration date, and the certifier's name. If the BSC fails certification testing, the report will provide recommended corrective actions (usually HEPA filter replacement) needed to pass certification. In each case a copy of the test report should be forwarded to the Biosafety Officer.

UV Lights and Biological Safety Cabinets

In a controlled environment and using a validated procedure, constantly emitted UV light of the appropriate wavelength and intensity is effective at decontaminating non-porous surfaces and in denaturing DNA. UV light does not decontaminate some organisms (non-replicating bacteria, some molds, and yeasts). Variables that adversely affect the efficacy of UV include failure to routinely wipe dust off of the UV lamp and a failure to routinely monitor the UV lamp to ensure output is of the appropriate wavelength and intensity. The tendency to store equipment and supplies results in at least a portion of the BSC work surface area being "shaded" from exposure to UV light, and there are areas that are inherently shielded from UV light by the design of the cabinet. Note that the lamp will emit a blue light long after the output has ceased to meet the requisite intensity and wavelength. And, even if the intensity and wavelength are appropriate, UV light does not penetrate surfaces and cannot penetrate covered surfaces like the underside of fittings (petcocks and outlets) and other places where potential contaminants may "hide".

Hazards associated with exposure to UV light include retinal irritation (prolonged exposure can lead to permanent retinal damage) and mild irritation of unprotected skin. These hazards are somewhat mitigated by an interlock incorporated into the design of newer models that requires the view screen to be fully closed in order for the UV light to work. Reflected UV light is also hazardous and UV protective eyewear must be used to mitigate hazard from reflective UV light.

The cost of operating a UV light over the serviceable lifetime of a BSC can be substantial and is not often considered. The cost of installing a UV lamp fixture in a new BSC (~$200.00) pales in comparison to the cost of UV lamp replacement ($15.00 - $40.00 each, depending on part number and vendor) and fees for disposal of the mercury-containing spent lamps as hazardous waste as required by EPA regulations. This is a significant expense over the life of a BSC. The CDC, NIH, and the American Biological Safety Association do not recommend the use of UV as the primary sterilizing procedure in BSCs.

Finally, it is critical to note that experimental manipulations occur in the absence of UV radiation, because the lamp is turned off during culture work. That means success in experiments is entirely dependent on the operator's aseptic technique and not the use of UV light. The IBC requires each laboratory to develop a chemical decontamination procedure and ensure that every user follows the procedure at start up and shut down of the BSC.

Proper Use of Biological Safety Cabinets

Proper use of a biological safety cabinet is based on the user's training and understanding of the operating parameters of the BSC, the experimental procedures to be performed, and most importantly, activities and events that reduce the effective functioning of a BSC. The following description of the proper use of a BSC at BSL-2 is based on the laboratory door being closed, the unit having been certified within the past year, and the unit being located away from high traffic areas and away from any potential source of disruption to the internal BSC air curtain (primarily doors and room air supply ducts).

Using the BSC properly includes these steps:

1. If the BSC blower is off, turn on the blower motor and let the unit run for 5-10 minutes to allow the cabinet to purge ambient air and establish an internal equilibrium. Check the airflow gauge or display to ensure the BSC is operating correctly.
2. After the unit has equilibrated, wipe down the interior (work surface, side and back walls, and interior of the view screen) with an appropriate chemical solution (for example, Wescodyne®, a bound iodine solution, or 70% ethyl alcohol) prior to placing equipment or supplies into the BSC. Decontaminating solutions intended for use inside a BSC should be made using sterile water.
3. Similarly, wipe down each piece of equipment (automatic pipettors, power supplies, racks, etc.) as it is brought into the BSC.
4. Consider the tasks to be performed and place only necessary equipment and supplies inside the BSC. This will minimize the number of times the operator's hands will need to "enter" and "exit" the BSC and subsequently minimize the opportunity for contaminants to "draft" into the work area.
5. Place equipment and supplies toward the back wall of the BSC. Be aware that the laminar flow within the BSC "splits", meaning that half of the HEPA filtered air flows to a slot in the back wall of the cabinet and half flows toward the front intake grill. Keep the front and rear grills clear.
6. Biological safety cabinets are designed for continuous operation. Although energy conservation may suggest BSC operation only when needed, especially if the cabinet is not used routinely, room air balance is an overriding consideration in the decision to operate the BSC 24 hours a day or to shut down the blower when not in use. The air discharged through ducted BSCs is considered as part of the overall air balance to maintain "negative pressure" in the laboratory. Instruct users on startup/shutdown procedures, including guidance if the laboratory ventilation system requires continuous operation of the BSC.

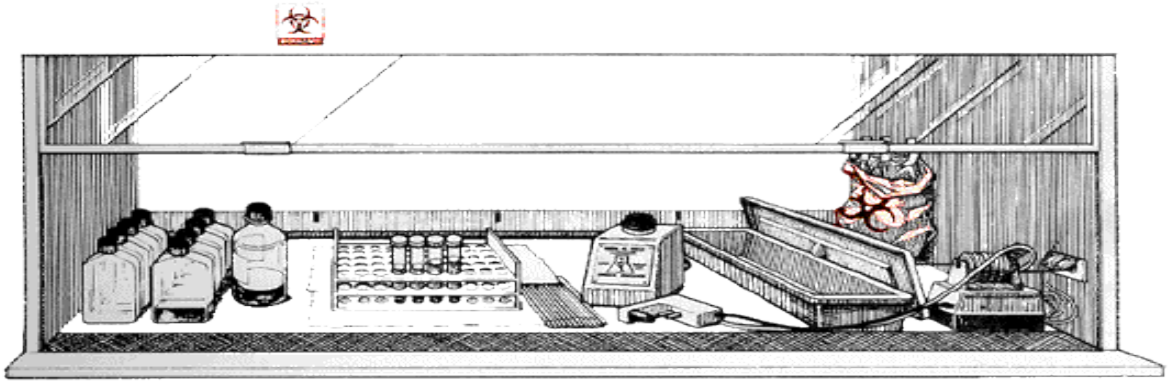

Figure 1. Arrangement of materials in a BSC, with "clean" items separated from "dirty" materials. Source: *BMBL, 5th Edition*

The use of flammable or explosive materials within BSCs is prohibited. Most BSC manufacturers apply a decal on the front of each unit warning against use of flammables and explosive materials. Since HEPA filters remove only particulates from the air that is recirculated inside the BSC, flammable and volatile vapors may build up inside the cabinet creating a fire or explosion hazard. Furthermore, the use of chemicals in a BSC that is vented to the laboratory may create an inhalation hazard for other people in the room or building area.

In the past, the two most common flammables used in BSCs were an alcohol burner and a Bunsen burner. Flames disrupt the airflow and contribute to the heat load inside the BSC. Flames have burned holes through HEPA filters and have caused explosions in BSCs.

Disposable plastic lab ware (pipettes, & loops for streaking agar plates) should be used instead of metal implements. If there is no alternative to re-useable implements, a "Bacti Cinerator"® (a portable, electric furnace) may be used to sterilize loops, needles, and scalpels instead of alcohol or Bunsen burners.

Figure 2. Bacticinerator®

BSCs are precision-engineered primary containment devices and should be serviced only by NSF-accredited technicians (or by someone supervised by an NSF-accredited technician)

qualified to perform testing and repair of BSCs. **Under no circumstances should laboratory users attempt electrical repair or part replacement of a BSC.** For units less than three years old, improper use or maintenance by anyone other than a qualified service provider is likely to invalidate the warranty. The Biosafety Officer can provide a list of service contractors for annual certification testing and repair.

Improper Use of Biological Safety Cabinets

Consider the following examples to avoid improper use of a BSC.

- Avoid keeping excess supplies like pipette tips, paper towels, and other lab ware in the BSC. Clutter inside the BSC may affect proper airflow and the level of protection provided.

- Do not place equipment or supplies on the air intake grill. Any obstruction to the supply air volume will adversely affect the function of the BSC.

- Do not work in a BSC while a warning light or alarm is signaling.

- The UV lamp should never be on while an operator is working in the cabinet.

- Avoid drafts from doors, air conditioning, and excess movement around the BSC operator.

- Avoid keeping loose paper towels and Kim Wipes™ on the BSC work surface while the blower motor is running. Paper towels can be entrained in the interior exhaust through the slot in the back of the cabinet and will lodge against the exhaust HEPA filter, creating an annoying fluttering noise, and reducing the surface area of the HEPA filter. Paper towels may or may not “fall off” of the HEPA and be recovered from the rear exhaust slot when the blower motor is turned off, but this is not routinely successful. Usually, the BSC must be decontaminated and the exhaust plenum accessed by a trained technician in order to remove the paper towel.

- Drilling, grinding or cutting into an internal or external surface of the cabinet for any reason is prohibited. BSC design and performance is rigorously tested to meet NSF International “listing” criteria. Any change to the physical structure of a BSC is considered an adulteration of the original equipment manufacturer specification and invalidates the NSF listing of the adulterated unit. BSC certification technicians are taught to evaluate each BSC for intentional and unintentional damage and note these observations on the test report document. The reason for prohibiting drilling and grinding is that holes will affect the internal air balance by creating a leak of potentially contaminated air from the pressurized plenum. There is also a risk of damaging electrical wiring. For newer BSCs, any user changes to the physical structure or internal electronic systems of a BSC will void the manufacturer’s warranty.

- Do not attempt to access the motor or HEPA filter areas of the BSC. The internal housing is considered contaminated with infectious material trapped in the HEPA filters. The BSC must be chemically sterilized before filter changes, maintenance and repair.

- Using a biosafety cabinet as a chemical fume exhaust hood when manipulating large quantities (above 250 ml) of volatiles, acids or bases is prohibited. Only the relatively small volume of chemicals typically used in molecular and microbiological protocols is permitted. As mentioned previously, HEPA filters act on particulate matter only and do not capture chemical fumes. The electrical systems and front sash of biosafety cabinets are not designed to provide protection from sparks or chemical explosions, and Class II BSCs are not constructed to be gas-tight. The airflow of many biosafety cabinets is exhausted back into the laboratory. Flammable, combustible, or toxic vapors and gas should be handled only in a chemical fume hood.

- Do not place boxes or other objects on top of the BSC that exhausts into the room, as this will block the exhaust airflow, and will damage the exterior surface of the HEPA filter.

BIOHAZARD SPILL CLEAN UP

Overview

Spills will occur with any task involving liquids. This section describes the proper way to clean up cell or tissue culture spills in a BSL-1 and BSL-2 laboratory. The PI must identify and provide a safe and effective compound for decontamination. For many BSL-1 organisms, a solution of commercial anionic or non-ionic detergent (household dish soap) is an appropriate decontaminating agent. For BSL-2 organisms, a 10% (v/v) dilution of 6.0% sodium hypochlorite (household bleach) is an appropriate decontaminating agent. A routine formula is 100 mls of bleach per 900 mls of distilled or de-ionized water. The solution is discarded and replaced by a fresh-made solution at least every other day.

Typically cell and tissue culture procedures involve anywhere between 10 ml and 500 ml of liquid, or higher volumes for pilot plant or scale up projects. Propagation of agents on semi-solid media involve upwards of 10^6 colony forming units (cfu). Persons cleaning up spills must always wear gloves and a lab coat for protection during the clean-up procedure.

Spill Risk Assessment

Assess all spills before beginning the clean-up. Lab users must be trained to recognize the hazards, and must be able to obtain the appropriate personal protective equipment and spill kit materials. Consider these items in planning the Emergency Action procedures for spill clean-up in research and teaching laboratories:

1. Is the agent known to be infectious via exposure to aerosols and if so what is the infectious dose? This consideration is significant for deciding if the laboratory (or other affected area) should be vacated after the spill for a period of time to permit aerosols to settle out of the atmosphere.
2. What is the largest volume of liquid culture manipulated and what is the highest titer of the organism per vessel (or colony population per plate) attained in your protocol?
    a. A large volume spill must be contained from uncontrolled spread throughout the laboratory. It is essential to have sufficient paper towels or other absorbent material on hand to contain a large volume spill.
    b. Large volume cultures (>10 L) of recombinant organisms (even if non-infectious) must be approved by the IBC, and spills of these materials must be reported to the Biosafety Officer.
    c. A high titer of organisms in a culture may enhance the risk of exposure if the vessel or plate(s) hits the floor. This is not usually a concern for work at BSL-1 but must be considered for cultures of BSL-2 organisms, particularly for spills outside of a BSC.
3. For spills inside of the biosafety cabinet: while laboratory occupants should be notified, a spill contained within a BSC represents less of a risk to uninvolved laboratory occupants since the BSC will contain aerosols generated within the unit. However, the operator must take care to avoid panic that will likely result in rapid hand movements into and out of the BSC. Rapid motions will affect the airflow and permit aerosols to escape from the BSC.

Under no circumstances should an operator put their head inside of a BSC while cleaning up a spill. The risk is obvious. Once the work surface is decontaminated and cleaned, it should be lifted up carefully to inspect the underside and cleaned if necessary. Do not remove the work tray from the BSC until it is properly decontaminated.

4. For spills outside of biological safety cabinet: liquid spills outside of the BSC should be contained to a minimum area and not permitted to spread. Plates may or may not open when dropped or they may shatter spreading cultures and shards of plastic across the floor. The surrounding floor and work surface areas where splashes or larger aerosols may have settled around the spill should be included in the clean-up.
5. Major spills of chemicals must be immediately reported to EHS&RM via 911 or direct call.
6. Major spills, incidents, or illnesses involving BSL-2 materials must be immediately reported to the Biosafety Officer via 911 or direct call. After the crises, send an email with details of the incident to biosafe@nmsu.edu.
7. Spills or accidents occurring in Biosafety Level 2 laboratories with recombinant materials resulting in an overt exposure must be immediately reported to the National Institutes of Health (NIH) Office of Biotechnology Activities (OBA). Contact the Biosafety Officer for assistance. The NIH template for reporting incidents can be accessed online from Research Integrity & Compliance webpage or the NIH OBA website.

Liquid Spill Clean Up Procedure

1. Alert other persons in the vicinity that a spill has occurred, especially for large volume spills, so that others do not walk through the contaminated area. Based on hazard of the materials and the Emergency Action Plan, determine if evacuation is necessary.
2. Don necessary protective equipment: gloves, eye protection and lab coat at a minimum; a face shield and/or a filtering facemask respirator may be needed.
3. Cover the spill with paper towels or other absorbent material to confine the spill to as small an area as possible and absorb the liquid.
4. **Apply decontaminating solution (usually a bleach solution of 10% or higher, to reach a final concentration of at least 0.5-0.6% sodium hypochlorite) to the absorbent material. Let stand for 10 minutes or the recommended contact time for the chemical disinfectant.**
5. Discard the disinfectant-soaked materials into the wastebasket. The resulting waste, paper towels, and other materials do not require autoclaving for most BSL-2 biohazards. Consult with the Biosafety Officer or EHS&RM for disposal of mixed wastes or other special hazards.
6. Repeat the application of disinfectant solution to the work surface (step 4 and step 5).

- **Do not autoclave materials containing bleach or toxic chemicals**, as this will result in the release of vapors into the room and can corrode the interior of the autoclave.

Note: For information on chemical spill clean-up, see the chemical Safety Data Sheets and Hazard Communication Plan in your department, and consult with safety specialists at EHS&RM.

BLENDING, MIXING, SONICATING AND CELL DISRUPTION

Overview

This section identifies risks associated with blending, mixing, sonicating, and disrupting cells and tissue. Potentially hazardous aerosols are likely to be generated by blending, grinding, mixing, stirring, shaking, or disrupting cells, tissues, blood, and environmental samples. Each of these actions applies force (mechanical or sound waves) to manipulate the material of interest. When available, use laboratory-grade equipment designed to contain aerosols of potentially infectious or pathogenic cells, tissues, or similar materials. Overall, laboratory-grade equipment is designed to contain liquids, and any aerosol likely to be generated during its use. For example the Warring Blender can withstand autoclaving, the motor bearings are made of Teflon®, the agitator is fabricated into the lid, and the screw-cap lid is fitted with an O-ring. Additionally, the blender has built in access ports that allow adding or removing materials without opening the blender. As a group, magnetic stirrers, incubator shakers, and water baths impart a less vigorous action on the materials but are not without risk of aerosol generation.

In the absence of a laboratory-grade device, use of an engineering control such as a biological safety cabinet, or fume hood is recommended to contain or ventilate aerosols generated during manipulations of these materials.

Finally, it is important to disassemble and thoroughly clean these devices between uses to prevent cross-contamination of subsequent processes.

Personal Protective Equipment

Lab coat and eye protection are required. A risk assessment of the procedures must be done to evaluate the need for additional precautions, such as a full face shield, particulate facemask respirator, and/or hearing protection.

Hazard Assessment

Aerosol generation is a constant by-product of these activities. Failure to contain the aerosol will lead to dispersal throughout the workplace.

Electric shock hazard is possible when using electric-powered equipment with liquids.

CENTRIFUGATION

Overview

Centrifugation is a common step in a multitude of laboratory procedures. Centrifugal force applied to a solution will result in separation of solution components according to their respective mass. A number of different centrifuge and rotor designs have evolved for specific applications, but the principle of operation remains constant. Older tabletop centrifuges (and some super speed) models are not fitted with an airtight seal and will not contain aerosols. The nature and volume of the material to be recovered influences the type of rotor and velocity chosen for a particular run. There are four classifications of centrifuges loosely based on the range of operating speeds.

Low speed centrifuges typically operate in the range of 100 rpm to ~1000 rpm.

High-speed centrifuges typically operate from 1000 rpm to ~5000 rpm.

Super speed centrifuges typically operate from 5000 rpm to ~20,000 rpm.

Ultra-centrifuges typically operate up to ~100,000 rpm.

The user is responsible for cleaning, decontamination and visual inspection of centrifuges on an as-needed basis. Due to the increased risk inherent in their operation, high-speed and ultra-centrifuges (along with their rotors) must be routinely inspected and maintained by manufacturer-qualified service persons. Rotors are subject to the cumulative effects of metal fatigue and corrosion experienced over prolonged use. Based on a visual inspection, and the total run time, the maximum operating velocity of a rotor is reduced, or "de-rated". Information recorded in the centrifuge logbook is used in making this determination.

Bottles and tubes intended to be re-used should be monitored over multiple runs, and inspected for leaks. These observations provide the basis for assigning a maximum number of uses after which these tubes and bottles should be replaced. It is essential that bottles and tubes are properly capped or sealed prior to being centrifuged. If available, caps fitted with O-rings are preferable to plastic or rubber-lined caps. Generally, bottles and tubes threaded on the outside and fitted with a screw cap provide a more reliable seal than non-threaded stoppers or plugs. Aluminum foil or "parafilm" should not be used to seal bottles or tubes, especially for cell cultures.

Definitions

Centrifuge safety cups are containers that fit around rotor buckets and provide containment of tubes or bottles holding potentially infectious agents while transporting the bucket from the biosafety cabinet to the centrifuge, during the centrifuge run, and while transporting the materials to the biosafety cabinet for further processing.

Rotor is a container or container-holder that rotates about the drive shaft of a centrifuge.

Over-speed occurs when a rotor accelerates beyond its maximum rated velocity.

Trunnion is a cup that holds bottles or tubes and is placed on opposing arms of a centrifuge rotor, and "swings" outward during the centrifuge run. Some types are fitted with caps.

Personal Protective Equipment for Centrifuge Operations

- Use gloves when handling potentially hazardous materials.
- Use eye protection when there is a potential for a splash.
- Wear close-toed shoes.

Hazard Assessment

Aerosol release may result when tubes or bottles fail during centrifugation or if integral tubes and bottles are handled in an unsafe manner, i.e., not observing good laboratory practices.

Drive shaft failure can result in catastrophic consequences, especially if the centrifuge is operating near maximum velocity at the time of failure.

Oil leak from the vacuum pump or motor that escapes from the centrifuge will result in a slip-hazard, and if not promptly wiped up, will damage the floor surface or finish.

Rotor imbalance can damage the centrifuge drive shaft, and if allowed to accelerate uninterrupted, may result in the centrifuge "walking" across the room.

Rotor failure has several causes. For example an improperly maintained and inspected rotor can disintegrate during a run, or if an improperly balanced rotor is permitted to accelerate beyond a certain speed, or if a rotor (properly maintained and balanced) accelerates beyond its rated maximum velocity. Depending on the speed at the time of failure, a disintegrating rotor can destroy the protective chamber and "spray" fragments around the room.

Loading the Centrifuge

Check the rotor or buckets to ensure absence of residue or debris. Check the tubes, caps, bottles, O-rings, and chamber seals for damage. Use a biosafety cabinet to contain aerosols when loading tubes or bottles with potentially infectious cultures. Over-filling the tube or bottle will contaminate the tube closure. Ensure that tubes and bottles are balanced and that balanced pairs are inserted at opposing positions in the rotor or trunnion. Confirm that the run speed does not exceed the rating of the rotor, bottles or tubes. Exercise care in placing the rotor on the drive shaft to ensure the unit is properly seated on the spindle. For ultra-centrifugation, ensure that the proper over-speed decal is installed. Record the run data; rotor number, speed setting, date, material description, operator initials. Start the centrifuge.

Unloading the Centrifuge

Allow the rotor to stop completely before opening the centrifuge lid or door. Practically, this requires waiting at least ten minutes (longer for higher run speeds) after the run timer has expired before opening the centrifuge. After opening, check the chamber for leaks or other abnormalities. Remove the rotor (or rotor content) containing potentially infectious cultures and place it in a biosafety cabinet. Do not open vessels containing potentially infectious cultures on the open bench. Once the centrifuge is empty, decontaminate any liquid that leaked inside the chamber. Decontaminate the entire centrifuge chamber with a dilute bleach, iodophor or quaternary ammonium compound, ensuring the exposed surfaces experience a 10-minute contact time with the solution.

DISPOSAL PROCEDURES FOR BIOLOGICAL LABORATORY WASTES

In accordance with the state regulations for special waste in New Mexico Administrative Code (NMAC), materials identified as infectious waste must be handled with special consideration. For laboratory-generated biological waste, Principal Investigators are responsible for preparing a written procedure for steam sterilization or another method used to disinfect infectious wastes, and for training laboratory personnel in those procedures.

All material that has been rendered non-infectious is not subject to the handling requirements of 20.9.8.13 NMAC, provided:

(1) if it is an otherwise regulated, hazardous, special, or radioactive waste, it shall be handled according to regulations applicable to that type of waste;

(2) any person that processes or transforms infectious waste shall certify in writing on at least an annual basis, or upon any change that could affect the efficacy of the treatment that the waste has been rendered non-infectious by sterilization, incineration or another method approved by the secretary;

(3) a certification that the waste has been rendered non-infectious shall be provided to the generator, transporter, and disposal facility; the generator, processing or transformation facility, and disposal facility shall maintain copies of certifications for a period of three years and the records shall be made available to the department upon request. (Source: *Special Waste Requirements,* Subsection B of 20.9.8.13 NMAC)

Information from this manual can be used to develop the laboratory-specific procedures and training. A sample standard operating procedure (SOP) template for autoclave use is given in Appendix E.

Biohazard (Infectious) Waste

1. Segregate biohazard waste from ordinary trash. Biohazard waste includes microorganisms that are infectious to humans, animals, or plants; blood, blood products and internal body fluids from humans and animals; cells and unfixed tissues; and recombinant materials that are non-exempt from the *NIH Guidelines*.

2. Sharps (razors, glass, scalpels, needles, etc.) should not be placed in bags of other waste. Provide rigid, puncture-proof sharps containers that are clearly marked according to biohazard or chemical contamination.

3. All materials from activities which utilize culture plates or other biological growth media regardless of use must be sterilized by autoclaving or chemical decontamination before disposal.

4. Maintain a written log for each sterilization unit used to autoclave infectious waste. See the autoclaving section of this manual for instructions on recordkeeping and training.

5. Certify in writing (using the TRAINING RECORD template in this section or a similar form) that the personnel using the autoclave understand the written operating procedures for each steam sterilizer including time, temperature, pressure, type of waste, type of

container and closure, and use of a steam sterilization indicator. A copy of the training records must be maintained with the sterilization log.

6. Solid laboratory waste must be placed inside a biohazard-labeled container that is puncture-resistant, leak-proof, and closed when not in use. Reusable rigid containers can be lined with an autoclave-safe bag. Place temperature sensitive autoclave tape diagonally across the biohazard symbol on marked bags before autoclaving. Container sizes should be limited to small volumes in order to ensure effective autoclave sterilization.

7. Materials for autoclaving should be placed in a plastic or metal pan to contain spillage. Autoclave at sufficient temperature, pressure and time to render all contents non-viable. Waste shall not be considered sterilized if either the steam indicator or tape fails to demonstrate that a temperature of at least 250 degrees Fahrenheit (121 degrees Celsius) was reached during the process.

8. All material certified as non-infectious by steam sterilization and not otherwise regulated as hazardous, special or radioactive can be disposed by landfilling. Enclose sterilized waste in a regular trash bag to ensure that contents do not leak or spill during transport through public areas.

9. Many liquid cultures can be chemically treated by adding household bleach to a final concentration of 10% (v/v). Let stand for at least 30 minutes, then pour down the sanitary drain with running water. Contact the biosafety officer or EHS&RM if information is needed.

Non-Infectious Waste

Ordinary non-sharp waste can be disposed of in the general trash.

“Look-alike” waste (i.e., gloves, culture dishes) from BSL-1 and BSL-2 laboratories should be autoclaved.

Broken glass that is not contaminated with biohazards or chemicals should be placed in a rigid container that is clearly marked with the word “GLASS”, such as a cardboard box lined with a plastic bag. When full, the box should be taped closed and disposed of in the dumpster.

Sharps

- Glass or plastic supplies that have the potential to break or puncture, such as microscope slides, serological pipettes, test tubes, culture plates or thin-walled vials should be placed in a rigid container lined with a plastic bag for autoclaving before disposal.

- Never discard sharps into ordinary trash or bags of biological waste.

- Place sharps containers within arm’s reach of the location where sharps will be used. Immediately after use, discard the entire sharps device (whether contaminated or not) into puncture resistant sharps containers. Do not attempt to recap, bend, or remove the needle.

- Separate sharps that are contaminated with chemicals from other biologically-contaminated sharps. Chemically-contaminated sharps must be placed in rigid, non-biohazard sharps containers (thick plastic, metal). Environmental Health, Safety & Risk Management (646-3327) can provide non-biohazard sharps containers for free, and will pick up the containers for disposal at no cost to the laboratory.

- Biologically-contaminated medical sharps (i.e., syringes, needles, scalpels) must be placed in a rigid, puncture proof container manufactured for use in sharps disposal (i.e., red sharps container labeled with the biohazard symbol). Biohazard sharps containers can be purchased from laboratory supply distributors.

- Do not overfill sharps containers. Close completely when 3/4 full, and request pickup from Environmental Health, Safety & Risk Management. Never place red sharps containers in ordinary trash, even if the sharps and/or containers have been autoclaved.

- In the event of a needle stick or other injury from sharps or glass, wash the area thoroughly with soap and water. Notify your supervisor and go to Aggie Health & Wellness Center immediately for evaluation.

Preserved Biological Waste

1. Segregate waste into dry solid waste and bulk liquid waste.

2. Package all waste to prevent spills, leaks, or breaks during transportation. Use containers strong enough to support the contained waste.

3. Dry Solid Waste: Use a plastic liner inside the bucket/drum supplied by EHS&RM.

4. Place dry carcasses into lined container. No free standing liquids are acceptable.

5. Liquid Waste: Use sturdy transport boxes with cardboard separators for bulk liquid waste bottles.

6. Complete the waste tracking form and secure to outside of container and/or box.

7. Call Environmental Health and Safety, 646-3327, to schedule pick-up or request re-usable bio-waste containers.

Samples from Animals

Fresh biological material (must not contain preservative, biohazardous, or regulated material) shall be taken to the City Landfill. A limited amount of carcass disposal can be arranged through the Animal Care Facility. For more information, contact the Biosafety Officer, the IACUC Chair, or EHS&RM.

STEAM STERILIZATION TRAINING RECORD

The following personnel have read and understand the written operating procedure for steam sterilization in compliance with New Mexico Administrative Code requirements for special waste (NMAC 20.9.8). The trainee has been specifically instructed on the use of the autoclave(s) listed below.

Investigator:	Autoclave ID:
Building/Room:	Autoclave Location:

Trainee Name	Trainer Name	Date

EXPOSURE AND EXPOSURE CONTROL

A primary goal of every safety program is to enhance worker protection by providing information and instruction on the risks associated with research and how to reduce those risks. The level of risk is never zero. For laboratory experiments, a risk assessment process must be conducted to identify the characteristics of the hazardous materials and the procedure, and the controls that will be used to minimize the hazards. Laboratory exposures to potentially infectious agents, recombinant DNA, and toxins are reportable to the laboratory director, Aggie Health & Wellness Center, NMSU EHS&RM, and the IBC, under NMSU policy and Federal and State regulations.

A Laboratory Safety Plan is generated as part of the IBC registration process (see Chapter VI. *The IBC Application* in this Biosafety Manual), and consists of the Hazard Communication elements for all chemical, biological, and radiological activities that are conducted. Note that many of these hazard communication elements, such as the Bloodborne Pathogen Exposure Control Plan, have a requirement for an annual review of the plan and annual refresher training to update personnel on changes to procedures or equipment specific to the laboratory activities. Lab-specific training given by the laboratory director should be documented on a signature page and retained with the laboratory records.

A biosafety risk assessment considers the routes of transmission for infectious disease and the prevention measures that must be established. Additional consideration should be given to physical hazards associated with equipment or methods, individual health factors that may affect susceptibility, and emergency action planning. **The risk assessment and risk management information must be communicated to all personnel, and should be provided to health professionals in the event of an injury or exposure**.

The classical path to a disease state is composed of three distinct steps; an exposure event that leads to an infection and if the infection persists, the condition progresses to a disease. This section describes the potential routes of exposure to biohazards, along with risk reduction measures.

Ingestion

- Risk: Occupational exposure by ingestion occurs when an individual consumes food or drink that is contaminated with any research material, or when contaminated hands, utensils, or other objects are placed in the mouth.

- Risk reduction: Exposure by ingestion is substantially reduced by keeping food and drink out of the laboratory, not applying lip balm in the lab, and not touching the mouth with pens or other objects. Wash your hands frequently, especially after handling research materials, after removing gloves, and prior to leaving the laboratory.

Skin Contact

- Risk: Exposure of unprotected skin (hands, face and arms) occurs when handling or manipulating a biohazard without wearing gloves, protective clothing, face protection, or close-toed shoes.

- Risk reduction: Minimize the area of exposed skin when in the laboratory; wear protective gloves and a lab coat. Cover broken skin, wounds and abrasions with a clean bandage or other protective coverings prior to entering the laboratory.

Mucous Membrane Contact

- Risk: Exposure of eyes, mouth, or nasal membranes may occur when handling cell/tissue culture flasks, chemical solutions, and materials derived from animals and humans, including blood, internal body fluids and unfixed tissues.

- Risk reduction: Always wear eye protection in the lab when hazardous chemicals are in use, and when biological procedures may create splashes. Use full-face shield when handling liquid nitrogen, viable cultures, acids or bases in quantity outside of containment. Chewing gum and applying cosmetics (including lotion and lip balm) in the laboratory are prohibited.

Sharps Injury

- Risk: Exposure via a sharp injury (laceration, needle-stick, or scrape) with a contaminated needle, razor blade or other tool, or broken glass represents a serious threat of infection due to the the potential for the agent, compound or toxin to be introduced directly into the bloodstream.

- Risk reduction: Reduce the use of needles and razors, and when possible, substitute plastic lab ware for glass lab ware. Train staff and practice safe sharps use, access, storage, and disposal procedures for your laboratory.

Inhalation

- Risk: To an extent, every manipulation (vortexing, sonicating, pipetting, centrifugation) of a liquid material in an open vessel (culture flasks, conical tubes) generates an aerosol or droplet nuclei. Similarly, the act of opening capped tubes or flasks may release an aerosol. Exposure to animal dander or urine and feces present in spent cage bedding is cumulative and is known to cause allergies in some persons.

- Risk reduction: Proper techniques to minimize aerosols must be emphasized. Whenever procedures have a potential for creating infectious aerosols, the procedure must be

conducted in a properly maintained BSC (inspected and certified annually), or with other appropriate personal protective equipment or physical containment devices.

1. Most standard pipetting and plating protocols, if done properly and with lower risk materials, do not generate aerosols such that a biological safety cabinet (BSC) is necessary. In these cases, eye protection and proper handling of materials are sufficient. For example, when pipetting, hold the pipet tip against the edge of the culture tube to allow the liquid to run down the inside of the tube (rather than dripping liquid into the tube) and stop before the final drop of culture is blown out of the pipet. When using heat to sterilize loops, separate sterile plates with agar media for students to use as a "sizzle plate." In this case, hot loops always touch the sterile agar sizzle plate before touching the working culture.
2. Laboratory procedures that are expected to create aerosols include centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, and some pipetting. These procedures must be conducted in a properly maintained BSC (inspected and certified annually), or using other appropriate personal protective equipment or physical containment devices. In addition, the BSC is required when using high concentrations or large volumes of infectious agents, or when opening sealed containers of organisms in a BSL2 lab that become depressurized upon opening and can result in the release of concentrated stock culture.
3. Use engineering controls (BSCs, ventilated animal cage racks and change stations, dedicated exhaust systems in animal holding rooms) to reduce inhalation hazards associated with animal dander, excreta, and spent cage bedding.

Reporting Incidents, Injuries, and Exposures

Personnel must be informed of the signs and symptoms of illness or infections associated with the specific biological materials in use. Since an infection may not be apparent until days or weeks after an incident, it is important that personnel recognize exposures and report the circumstances to the supervisor for documentation. Every injury, and exposures such as splashes to the eye, nose, or mouth involving potentially infectious materials, should be reported whether or not medical care is needed.

All on-the-job injuries and safety concerns must be reported immediately to the responsible supervisor by completing and submitting the **Notice of Incident** form whether or not medical care is needed. The Notice of Incident form can be accessed online at www.safety.nmsu.edu/risk-management/submit-claim/.

For any injury of NMSU employees, including student employees, the supervisor completes an **Employer's First Report of Injury or Illness Form** and a **Supervisor's Investigation Report Form** whether or not medical care is needed. For guidance on selection of the correct form, please review **Reporting Safety Issues, Injuries or Property Damage** on the EHS&RM "Risk Management" webpage.

All three forms are to be sent to the Workers' Comp Coordinator within 24 hours of an incident involving injury. The forms and contact information can be accessed from the Aggie Health & Wellness Center webpage**, "Work Related Injuries and Illnesses".**

If you are injured at work, you do not need to be on the NMSU health plan to receive medical care at the Aggie Health & Wellness Center All employees (including student employees) are protected under the provisions of the Workers' Compensation Law of the State of New Mexico. On-the-job accidents and occupational diseases incurred while working for the university are eligible for coverage. NMSU recommends that an injured employee see an Aggie Health & Wellness Center provider first for all medical care and for any specialist referrals for a work related injury.

Exceptions are:

- Need for emergency hospital care
- Serious injury that occurs after CHC clinic hours
- Injury that occurs outside Las Cruces area

If any of the above work related situations occur, contact the NMSU Workers' Comp Coordinator (phone 575-646-7375) and the Aggie Health and Wellness Center (phone 575-646-1512) within 24 hours to coordinate all follow-up medical care.

INTEGRATED PEST MANAGEMENT AT NMSU

Integrated Pest Management (IPM) is a decision-making process that uses all available pest management strategies to prevent economically damaging pest outbreaks while reducing risks to human health and the environment. IPM is a continuum along which there are many levels of treatments. The IPM program takes advantage of all pest management options including but not limited to the judicious use of pesticides. Pest control ranges from simple monitoring to properly timed pesticide use, or even bio-intensive IPM in which there is total elimination of synthetic pesticides. Pests are managed in order to reduce any potential human health hazard, to protect against a significant threat to public safety, to prevent loss of or damage to university property and to enhance the quality of life for faculty, students, staff, and visitors.

IPM is an important part of managing a research facility. Many pests, such as flies and cockroaches, can mechanically transmit disease pathogens and compromise the research environment.

NMSU Facilities and Services (FS) maintains an IPM Program managed by Facilities Maintenance and the Grounds Department that is compliant with EHS&RM policy on pesticide use. The use of pesticides at NMSU is subject to the OSHA Hazard Communication standard. Safety Data Sheets (SDS) are readily available from NMSU Facilities and Services.

Building occupants should report pest infestations to NMSU Facilities and Services by submitting a work order. Indoor applications are routinely scheduled when the building or room is unoccupied. Personnel should be notified in advance and may vacate the building or room depending on the pesticide used for treatment.

Many pest problems can be prevented or corrected by ensuring proper sanitation, reducing clutter and pest habitat, and by performing repairs that exclude pests. Laboratory records should be maintained to track repairs and interventions, and determine if corrective actions were effective. Monitor for pests using traps, visual inspections, and staff interviews. Record the results of monitoring in a logbook to support quality control assessments for the research operations and submit recommendations for improvement if needed.

SHIPPING RESEARCH MATERIALS

Contact the Biosafety Officer for current guidance and assistance with shipping biological materials from your laboratory.

A condensed version of the shipping requirements for infectious substances can be found in the CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories*. **The information provided here is introductory, and is not intended to substitute for the comprehensive training requirements specified in the regulations.**

Overview

The efficient and safe transport of research materials requires good coordination between the sender, the carrier and the receiver. From a research perspective, there are several preliminary determinations that must be made prior to shipping research materials. Review the following considerations for responsible and successful transportation:

- Do any proprietary restrictions apply to the material, e.g., do you own the rights to the material or do you have written permission from the owner to transfer the material? For example, the ATCC material transfer agreement (MTA) explicitly prohibits subsequent transfer of cell cultures and other purchased materials without the company's written permission. Review the purchase documents or contact the source's Intellectual Property office for details. Conversely, if the material to be shipped is proprietary to NMSU or an NMSU PI, the Office of Technology Transfer should be consulted to determine if an MTA is necessary prior to shipping the material.

- Are permits needed to transfer, transport, or possess the material? Many permits restrict the possession and use of the material to the permit holder and prohibit subsequent transfer to a third party (who may or may not hold a permit for the material). In any case, the applicable permit conditions must be accounted for prior to shipping any permitted material.

- Materials exported to a destination outside the United States may require an export license or reporting to the U.S. Department of Commerce, using the Export Classification and Control Number (ECCN). This practice prevents export to restricted countries and controls the export of "dual use" materials so that research items are not used for unintended purposes. Contact the office of the Vice President for Research and the Graduate School for information and guidance.

- Many research materials are considered hazardous materials, defined by the U.S. Department of Transportation (DOT) as a substance or material which is "**capable of posing an unreasonable risk to health, safety, and property when transported in commerce**". This definition is found in the requirements for transporting hazardous materials known as the ***Hazardous Materials Regulations*** (HMR), issued by DOT's Pipeline and Hazardous Materials Safety Administration (PHMSA). The HMR are published in Title 49, Code of Federal Regulations (49 CFR), Parts 171-180.

- In addition to DOT requirements, shipping research materials via air is guided by the International Air Transport Association (IATA) ***Dangerous Goods Regulations*** (DGRs).

- Commercial carriers are likely to have additional requirements for transporting packages containing research materials. Not all shipping entities accept every type of package, and the requirements for shipping documentation may vary by carrier.

- The DOT and Federal Aviation Administration regularly review the records of shipping companies such as FedEx, and have the authority to perform unannounced inspections of facilities (including research laboratories) that ship dangerous goods. Regulatory inspections can assign civil and criminal liability to the organization and the shipper and result in substantial fines for violations.

- Ensure adequate communication between the sender, the contracted carrier service (e.g., FedEx), and the receiver.

  The shipper (sender, consignor)

  - Makes advance arrangements with the receiver, including investigating the need for import/export permits, letters of authorization, or other documents;
  - Makes advance arrangements with the carrier to ensure that the shipment will be accepted for transport and delivery, and be shipped by the most direct routing;
  - Prepares necessary documentation according to the proper classification of the material, IATA packing instructions, carrier requirements and applicable permits;
  - Notifies the receiver of transportation arrangements and the expected arrival time.

  The carrier provides advice to the sender about correct packaging, shipping documents and instructions, and confirms the routing and delivery.

  The receiver (consignee)

  - Obtains the necessary authorizations to receive the material (i.e., import permit, institutional approval) and provides these to the sender in advance if needed;
  - Should inform the sender upon receipt of the material.

Classification Process

For packaging and shipping purposes, research materials are classified according to the DOT *Hazardous Materials Regulations* and IATA *Dangerous Goods Regulations.* Dangerous goods classification is a mandatory three-step process.

Dangerous goods are assigned **UN numbers** and **proper shipping names** according to their hazard classification and their composition. Proper shipping names are used to clearly identify the dangerous article or substance. Classification allows the shipper to select the proper IATA **packing instructions** and directions to use, and provides information necessary to complete **documentation** (i.e., the Shipper's Declaration).

Classification step 1: Determine the IATA-specified Class of dangerous goods (see Table 5).

Table 5: IATA-defined Classes of Dangerous Goods *Source: 49 CFR 173.2*		
Class	Substance	49 CFR reference
1	Explosives	173.50
2	Gasses	173.115
3	Flammable liquids	173.120
4	Flammable solids	173.124
5	Oxidizing Substances and organic peroxides	173.127, 173.128
6	Division 6.1 (poisonous material) Division 6.2 (infectious substance)	173.132 173.134
7	Radioactive material	173.403
8	Corrosive material	173.136
9	Miscellaneous hazardous material (e.g., dry ice)	173.140

Classification step 2: Determine the Division within the IATA-specified Class

- Biological research materials typically are classified as Class 6.2 (infectious substances).

Classification step 3: Determine the IATA-specified Type of infectious substance.

- Class 6.2 Infectious substances are assigned a proper shipping name and corresponding UN number: UN 2814, UN 2900, UN 3291 or UN 3373. See 49 CFR 173.199 for details of these classifications. You can refer to the *Transporting Infectious Substances* brochure online at www.phmsa.dot.gov for a summary of the classification process, guidance list of infectious substances, and packing and marking requirements.

Category A: the material is known or reasonably expected to contain a pathogen that is capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals. Specialized training is required before packaging and shipping Category A infectious substances. Contact the Biosafety Officer or EHS&RM for assistance.

PROPER SHIPPING NAME AND IDENTIFICATION NUMBERS:

Infectious substances, affecting humans, UN 2814

Infectious substances, affecting animals *only*, UN 2900

Category B: the material is known or reasonably expected to contain an infectious substance not in a form generally capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals.

PROPER SHIPPING NAME AND IDENTIFICATION NUMBER:

Biological substance, Category B, UN 3373

Regulated Medical Waste, UN 3291

Note: RMW that contains or is suspected to contain Category A substance must be identified as a Category A infectious substance UN 2814 or UN 2900.

There are differences in the packaging, paperwork, and container requirements for each respective material to ensure the package arrives at its destination on time and without breakage or leaks. The following information assumes the package contains non-infectious biological materials (i.e., cDNAs with less than ~50% of the genome) or Category B infectious material. The following briefly describes the components that make up the UN standard **triple packaging** system for infectious substances, and is appropriate for any package of biological research materials:

- Leak-proof primary receptacle and secondary packaging, that prevents any loss of contents that might be caused in transportation by vibration, changes in temperature, humidity, or pressure , AND
- with absorbent material sufficient to absorb the entire contents of all primary receptacles that are packaged together, AND
- An outer container (usually a box) manufactured according to regulatory specification (i.e., capable of passing a 1.2-meter (~4 feet) drop test), AND
- Is properly marked (with orientation arrows: ↑ ↑, and the code of manufacturing specifications "i.e., 4G/X 8/S/04" or other as appropriate, screened onto the box) AND
- Is labeled with the proper shipping name for the material (e.g., Biological substance, Category B), AND
- Is labeled with the correct markings containing the UN identification number of the materials (e.g., a diamond-shaped marking containing the number, "UN 3373"), AND
- Is free of extraneous markings, labels, stains or discoloration AND
- Clearly identifies the sender/consignor, AND
- Clearly identifies the receiver/consignee, AND
- Is accompanied by the correct paperwork (i.e., air waybill) that contains the name, address, and telephone number of a person knowledgeable about the material.

The Shipping Process

To send biological materials for shipment through Fed Ex, shipping personnel must have specialized training and ensure that specific packages, labels, and paperwork are properly used. Lab supervisors and departmental personnel should review procedures periodically and consult with the Biosafety Officer and EHS&RM technical experts to ensure that shipping preparations are appropriate.

<u>To ship hazardous materials from the NMSU – Las Cruces campus</u>:

1. Environmental Health, Safety & Risk Management is designated as the central hazardous materials shipping center. For outbound shipping of "hazardous materials" or "dangerous goods" by ground vehicle or air, the EHS&RM "Hazardous Materials Shipping Form", available on the EHS&RM webpage under Chemical and Waste Management, must be completed three (3) days in advance of the shipment and submitted to EHS&RM. The Biosafety Manager will assist you with shipping IBC-related infectious substances, and EHS&RM specialists help with packing for biological, chemical and radiological packages.
2. Contact the Biosafety Officer for assistance with classifying the materials and selection of the proper packaging, labeling, and shipping requirements for your materials.

3. For security purposes, all hazardous materials must be out of eyesight in a **locked location** when not in use. Stolen, lost, or missing hazardous materials, or suspicious activities involving hazardous materials need to be immediately reported to Environmental Health, Safety & Risk Management (575-646-3327) and the NMSU Police (575-646-3311).

To ship hazardous materials from a location outside the Las Cruces area:

1. The NMSU employee responsible for shipping must be trained before performing hazardous materials shipping duties and retrained every three years (49 CFR 172, Subpart H). The course must provide security and general awareness and function specific training for those involved in hazardous materials transportation compliance. Training and testing must be documented.
2. The shipper must have proper packaging materials according to DOT 49 CFR 172.
3. All radioactive materials shipments must be approved by and coordinated through NMSU Radiation Safety Manager.

Hazardous materials transported by NMSU employees in approved vehicles

Small quantities of hazardous materials transported by an NMSU employee for official business are often exempt from the HMR, as long as requirements are met for the DOT Materials of Trade (MOT) exemption. An example would be a NMSU researcher transporting a package from campus to a research location or to a shipping center for assistance. Although formal DOT training is not required in this situation, specific packaging and marking requirements must be followed. Refer to the EHS&RM website for the MOT policy and instructions. Note: the MOT exemption does not apply to Biological Substance, Category A materials.

Shipping Materials on Dry Ice

While the use of dry ice to preserve the integrity of research materials in transit is necessary, it's use is not without risk. There are three hazards when using dry ice, 1) packing dry ice in an air tight container may cause the container to explode, 2) sublimation of dry ice in poorly ventilated or confined areas (like aircraft cargo holds and courier vehicles) may generate a suffocating concentration of CO2 in the atmosphere, and 3) dry ice can burn exposed skin. Both DOT and IATA have specific packaging requirements for packages containing dry ice. When dry ice is the only hazardous material in the package (e.g., no infectious agents) the package must be labeled with a Class 9 decal that lists the quantity of dry ice contained in the package. Note: Between 5 and 10 lbs of dry ice will preserve temperature for 24 hours.

Dry ice is a hazardous material and is regulated under the DOT. Federal law requires appropriate training for anyone wanting to ship dry ice. Contact EHS&RM to review the training guidance and specific procedures that must be followed. The EHS&RM training enables lab personnel to be certified to ship dry ice as long as no other hazardous materials are involved.

Shipping Guidance

In summary, there are specific requirements for determining what forms are necessary for a particular package, how to complete the form(s), and the record keeping requirement for these forms.

All persons who offer a package for shipment must be trained in the regulations within 90 days of being assigned to perform shipping duties, and training must be repeated every 2 years under IATA, and every 3 years under DOT. The supervisor is responsible for arranging the required training, and communicating with Environmental Health, Safety and Risk Management to ensure the package conforms to carrier-specific requirements for the material being shipped.

Websites for Shipping Guidance

1. CDC Laboratory Training
   https://www.cdc.gov/labtraining/training-courses/packaging-shipping-division-6.2-materials.html

2. CDC Import Permit Program (IPP)
   https://www.cdc.gov/cpr/ipp/index.htm

3. DOT Transporting Infectious Substances:
   https://www.phmsa.dot.gov/transporting-infectious-substances/transporting-infectious-substances-overview

4. FedEx Hazardous Materials Shipments:
   https://www.fedex.com/en-us/service-guide/hazardous-materials/how-to-ship.html

5. International Air Transport Association
   https://www.iata.org/publications/dgr/Pages/index.aspx

6. United Parcel Service Hazardous Materials Guide:
   https://www.ups.com/us/en/help-center/packaging-and-supplies/special-care-shipments/hazardous-materials.page

7. U.S. Postal Service Hazardous, Restricted, and Perishable Mail (Publication 52):
   https://www.usps.com/ship/shipping-restrictions.htm#

8. WHO Transport of Infectious Substances
   https://www.who.int/ihr/publications/guidance_infectious_substances/en/

9. ASM 2012. Sentinel Level Clinical Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases. Packing and Shipping Infectious Substances. Accessed on 4/4/2013 from www.asm.org

APPENDIX A. THE IBC APPLICATION

Biosafety PO Box 30001 / MSC 3RES Las Cruces, NM 88003 Phone: (575) 646-4463 biosafe@nmsu.edu	NEW MEXICO STATE UNIVERSITY INSTITUTIONAL BIOSAFETY COMMITTEE **IBC Application**	FOR OFFICE USE ONLY Application No. Receipt Date: Status: Approval Date:

SECTION I: ADMINISTRATIVE INFORMATION

PRINCIPAL INVESTIGATOR INFORMATION	
Name:	Date:
Academic Title:	Department:
Email address:	Campus Mail Stop Code:
Phone:	Lab Location:

PROJECT INFORMATION	Proposed Biosafety Level: BSL-	← As entered in Section IV Part C
Title of Research Project:		

CATEGORY OF APPLICATION

This application is: ☐ Initial (New) ☐ Updated- Existing IBC approval will expire.

This application contains proprietary or confidential business information. ☐ Yes ☐ No

FUNDING INFORMATION
Name of PI on Grant:
Funding Agency/Source:
Grant Title:
Approval of this protocol is needed for grant application deadline? ☐ Yes ☐ No Grant deadline date:

SECTION II: INSTITUTIONAL & REGULATORY APPROVALS / REGISTRATIONS

OTHER INSTITUTIONAL REVIEWS/APPROVALS/PERMITS		
A. USE OF VERTEBRATE ANIMALS Does this biosafety activity involve the use of animals?	☐ Yes ☐ No Registration with the NMSU IAUCC is required for all work with live animals	If Yes, please provide IACUC approval date: _____, and expected project completion date or IACUC approval expiration date (whichever is later) : _____. To obtain IACUC approval see: http://compliance.research.nmsu.edu/IACUC
B. USE OF RADIATION Does this biosafety activity involve the use of radioactive materials?	☐ Yes ☐ No A permit from the RSC is required for all work with radioactive materials	If Yes, please provide Radiation Safety Committee (RSC) approval date: _____ and check appropriate box if you are: ☐ currently using radioactive materials ☐ not currently using radioactive materials. To obtain the application for Use of Radioactive Materials, contact thr Radiation Safety Manager, https://safety.nmsu.edu
C. USE OF HUMAN SUBJECTS Does this biosafety activity involve the use of human subjects?	☐ Yes ☐ No Permission to use human subjects in research must be granted by the NMSU IRB	If yes, please provide the IRB approval date: and the approval expiration date: _____. For IRB policy and forms see: https://compliance.nmsu.edu/IRB
D. FEDERAL PERMITS Does this biosafety activity require any Federal permits that are not included in A, B, or C above?	☐ Yes ☐ No Permits from Federal agencies (e.g. APHIS, CDC) are required for handling of toxins, pests, certain biological organisms, or to import exotic agents and organisms.	If yes, list issuing agency; _____ , permit number: _____ and expiration date:_____. Please forward a hard copy of the permit to Biosafety at MSC 3RES Contact the Biosafety Officer (575) 646-4463 with questions or for assistance with permit application. For USDA permits see: APHIS / Permits and Certifications

SECTION III: LOCATION OF ACTITIVITIES

LOCATION Approval of the proposed activity is given only for the locations listed below.			
NMSU Indicate which NMSU locations are used for this activity?	For each Yes, Complete the table below. Building/Site, Greenhouse or Field Location	Room number(s)/site identifier	Name and phone number of contact person at this site
NMSU main campus ☐ Yes ☐ No			
NMSU greenhouses ☐ Yes ☐ No			

NON-NMSU
Does any part of this activity occur at a non-NMSU facility or site? ☐ Yes ☐ No
If Yes, complete all of the following information for each non NMSU facility

Name and address of non-NMSU Facility	Contact Name, Title, Phone Number

Do any NMSU personnel associated with this activity physically participate in the activity at this non-NMSU facility?
☐ Yes ☐ No If Yes, write in the name(s) of these personnel in this space.

Are any biohazardous or recombinant DNA materials transferred to your lab from this non-NMSU facility for this activity?
☐ Yes ☐ No If Yes, write the materials transferred in this space.

Do you have the necessary permits required for transfer of these materials? ☐ Yes ☐ No

Does this facility/or specific area where the work is conducted have an IBC approval for work at the appropriate biosaftey level?
☐ Yes ☐ No If Yes, what is the approved BSL? ☐ BSL-1 ☐ BSL-2 ☐ BSL-3

Have you attached a copy of the non NMSU facility IBC approval to this form ☐ Yes ☐ No (If NO, the NMSU IBC requires a copy of the non-NMSU facility IBC approval if the work involves recombinant DNA or BL containment of BL2 or higher)

SECTION IV. TYPE OF BIOLOGICALS AND BIOSAFETY ACTIVITY

A. BIOHAZARDOUS AGENTS: Check all boxes that apply to this project.

1. ☐ Arthropod (e.g., mosquitoes, ticks)
2. ☐ Bacteria
3. ☐ Cells or tissues (Animal source)
4. ☐ Cells or tissues (Human or non-human primate source), blood, or body fluids, unfixed tissue including immortalized cell lines.
5. ☐ Fungi
6. ☐ Mold
7. ☐ Parasite (e.g., Plasmodium spp.)
8. ☐ Recombinant or synthetic nucleic acid molecules; refer to the *NIH Guidelines*
9. ☐ Toxin (☐ chemical or ☐ biological product)
10. ☐ Use of expression vectors
    - ☐ Yes viral vector
    - ☐ Yes cosmid, phagemid, plasmid vector
11. ☐ Virus - Animal (☐ exotic or ☐ endemic to NM)
12. ☐ Virus – Plant (☐ exotic or ☐ endemic to NM)
13. ☐ Yeast
14. ☐ Other, list material below (e.g. *dura mater* from human, non-human primate, livestock, rickettsia, etc.)

_____

_____

Check the appropriate boxes to indicate the sources of the materials listed above.

☐ A commercial vendor (ATCC or as part of a kit, i.e., Stratagene, Promega, etc.) If using a "kit", provide the vendor or manufacturer and product number ______________________________.

☐ Isolation from environmental samples (water, soil, etc.) ☐ Isolated from a plant or animal ☐ Hospital or clinic

☐ Colleagues and collaborators in: ☐ Academia or ☐ Industry ☐ Other (specify) ____________.

For each box checked in (A) provide the COMMON NAME, SCIENTIFIC NAME (genus & species, strain and/or Manufacturer's kit name and product number). Check each box that applies in the "SPECIFICS" column. Add more spaces as needed.

COMMON NAME	SCIENTIFIC NAME (Genus & species, strain)	Yes	No	SPECIFICS
		☐ ☐ ☐ ☐	☐ ☐ ☐ ☐	Certified BSC will be used for propagation / manipulation of agent. Vaccine is available (☐ licensed or ☐ IND). Vaccination recommended. Special precautions to be used? (If yes, list below):
		☐ ☐ ☐ ☐	☐ ☐ ☐ ☐	Certified BSC will be used for propagation / manipulation of agent. Vaccine is available (☐ licensed or ☐ IND). Vaccination recommended. Special precautions to be used? (If yes, list below):
		☐ ☐ ☐ ☐	☐ ☐ ☐ ☐	Certified BSC will be used for propagation / manipulation of agent. Vaccine is available (☐ licensed or ☐ IND). Vaccination recommended. Special precautions to be used? (If yes, list below):

B. RECOMBINANT DNA

Does this research involve the use of recombinant DNA molecules? ☐ Yes ☐ No

Provide details below; add more lines or a separate page if needed.

SOURCE: What is the origin of DNA or nucleic acid sequence of interest? (Give the common name, scientific name, and how obtained):

What is the nature of the nucleic acid sequence of interest?

What host organism(s) will be used? (If using a commercial product, please specify the vendor and product name, e.g., Invitrogen *E. coli* Top10):

Vector name(s) (add additional spaces or attach a separate page if necessary)	Specify selection markers. (Provide a gene map or give a reference from literature or commercial supplier if applicable.)	Will the foreign gene be expressed? If yes, indicate the protein that will be produced and identify promotors

C. PROPOSED BIOSAFETY LEVEL	
What is the proposed Biosafety Level for this activity? ☐ BSL-1 ☐ BSL-2 ☐ BSL-3 CDC BMBL Section II CDC BMBL Section III NIH Guidelines Section II NIH Guidelines Appendix G NIH Guidelines Appendix I	• Biosafety Level 1 involves well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. • Biosafety Level 2 involves work with agents of moderate potential hazard to personnel and the environment. • Biosafety Level 3 involves clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents that may cause serious or potentially lethal disease as a result of exposures by the inhalation route. • Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease. BSL-4 is not allowed at NMSU facilities. Sources: NIH Guidelines and CDC BMBL 5th Edition

D. CLASSIFICATION OF RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES (*NIH Guidelines)*
Insert the applicable classification from Section III of the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, online at https://osp.od.nih.gov/biotechnology/nih-guidelines/

SECTION V: DESCRIPTION OF ACTIVITY

A. LAY SUMMARY
In lay language, use this page to describe the experimental design and research objectives of the activity, with specific mention of the materials LISTED IN SECTION IV. Provide details that will allow a non-scientist to understand your work and assess the hazards and risks. Please define all acronyms at first use.
INSERT LAY SUMMARY HERE

B. PROCEDURES
Research Methods/ Procedures *Use the space below to describe the procedures that you use for this activity. Provide this description with the intent of providing the IBC with a clear understanding of what you are doing IN TERMS OF THE MATERIALS LISTED IN SECTION IV.* *Include any activities which may produce aerosols, or which may increase the hazard of working with the biohazardous agent(s).* *Include both standard procedures (referred to by common names such as PCR), and novel procedures or significant modifications to standard procedures (which should be clearly described and/or a reference should be provided)*
INSERT PROCEDURES HERE

C. SUBSTANCE DISPOSAL AND DECONTAMINATION PROCEDURES

Review the template language below describing the method of disposal of biohazardous substances and recombinant DNA transformed organisms (e.g., incineration, autoclaving, chemical disinfections). Check the box for all methods that are used.

☐ Inappropriate disposal of waste poses a potential for adverse environmental impact and regulatory enforcement action. We will follow NMSU procedure and NMED Solid Waste Bureau regulations on disposal of solid lab waste, viable organisms and waste DNA and rDNA.

☐ All personnel will be trained by EHS&RM in OSHA Hazard Communication, Laboratory Standard, and at least one person in our lab will be trained in NMSU Hazardous Waste Disposal.

☐ We decontaminate all solid waste (transformation products, spent agar plates) by autoclaving for 60 minutes.

We record autoclave use in a logbook containing the following information for each load:

The date and time the cycle is engaged.

The operator's initials.

Content (waste for decontamination, implements being sterilized, liquids being sterilized.)

Volume of the load, (e.g., bag size X number of bags).

Cycle duration and type of load, i.e. 30-minute/liquid, 60-minute/dry.

☐ We decontaminate liquid wastes and surfaces contaminated with liquid waste or cultures using a 10% volume/volume dilution of 6% sodium hypochlorite (household bleach) prior to disposal down the sanitary sewer. The resulting concentration is 0.6% sodium hypochlorite. We understand that the hypochlorite solution will break down within days, so we make up a fresh dilution at least every other day. We track expiration by labeling the container with the date the solution was made.

☐ If chemical disinfectant other than a 10% dilution of 6.0% sodium hypochlorite is used, state chemical and concentration here:

☐ We practice good housekeeping and package sharps in puncture-resistant containers manufactured for the purpose of sharps disposal and contain our waste in lined, rigid containers. We contact NMSU EHS&RM (6-3327) for pickup of chemical waste and full sharp containers.

In the space below, specify additional waste handling, decontamination, and disposal operations beyond those described above. (For example, household dish detergent may be used for some BSL-1 organisms instead of sodium hypochlorite.)

INSERT ADDITIONAL PROCEDURES HERE

D. EQUIPMENT

Do you use a biological safety cabinet (BSC) for this activity? ☐ Yes ☐ No

Do you use a clean air bench (CAB) for this activity? ☐ Yes ☐ No

Do you use an autoclave for decontamination of laboratory waste? ☐ Yes ☐ No

If Yes to above, please check the appropriate box and fill in the information requested below.

	BUILDING	ROOM	ID INFO (ie. MANUFACTURER, MODEL, SERIAL NUMBER)	CERT / TEST DATE
☐ BSC ☐ CAB ☐ Autoclave				
☐ BSC ☐ CAB ☐ Autoclave				
☐ BSC ☐ CAB ☐ Autoclave				

SECTION VI: PERSONNEL

A. PERSONNEL
Provide the names and title (faculty, staff, post-doc, grad student, undergrad student, technician, or other person) working on this project. Also for each person provide the following: *(1) Experience relevant to the proposed research; list equipment, methods, procedures and experience at BSL-1 or BSL-2; If no prior experience, provide a brief description of how training will be given;* *(2) Where obtained (NMSU or other), and (3) Number of years worked in a laboratory at biosafety level 1, 2, or 3.*
1. Name and Title (1) (2) (3) 2. Add Additional Personnel as needed (1) (2) (3)

The BSO will review records of EHS&RM training attendance for all personnel listed above (including the PI) and record training dates in the table below. Disputes on training attendance must be accompanied by a hard copy of training documentation to prove completion of training. Personnel must at least be enrolled in the required training prior to release of IBC approval.

NAME	BIOSAFETY AWARENESS	HAZ COMM	HAZ WASTE	LAB STANDARD	BBP
Personnel who will work on this project.	Required for BSL-2; Recommended for BSL-1	Required for all personnel	Required of one person per lab	Required for all personnel	Required for use of human and non-human primate cells/blood

SECTION VII: SAFETY PLANS

Our lab has reviewed the NMSU Biosafety Manual and has adopted the manual for use in our work. ☐ Yes ☐ No

Our lab has additional lab-specific standard operating procedures and emergency plans. ☐ Yes ☐ No

Established Exposure Response Procedures	
Accidental Exposure*: Indicate that you agree with the text supplied below by marking the appropriate box ☒ OR delete the text below and describe your alternative exposure response.*	
Material (rDNA, Bacteria, Virus etc.)	Response Procedure
	Available evidence suggests that materials used in this research ☐ are / ☐ are not (mark the appropriate box with an "X") implicated in occupational illness due to exposure in a research environment. Our response to an occupational exposure to DNA, rDNA, or *E. coli* K-12 derivatives, pathogens or potentially infectious agents via splash to exposed skin is to wash the affected area with mild soap and warm water. For mucosal exposures (eyes, mouth or nose) our response is to flush with warm water. All exposure events, including sharps exposures and other occupational injuries are reported to EHS&RM. Injured persons who need or want medical treatment will report to the Aggie Health & Wellness Center (646-1512).
Per NIH rDNA Guidelines IV-B-7-e-(2) and NMSU Policy, the Principal Investigator will report occupational exposures and spills of research materials to the Biosafety Officer and applicable authorities.	

SECTION VIII: Principal Investigator Statement

PRINCIPAL INVESTIGATOR STATEMENT

I agree to comply with all requirements pertaining to the use, handling, storage and disposal of biohazardous agents and recombinant DNA molecules. I also agree to follow the current *Research Involving Recombinant or Synthetic Nucleic Acid Molecules*, referred to as the *NIH Guidelines,* and the CDC recommendations from the CDC/NIH handbook, *Biosafety in Microbiological and Biomedical Laboratories*, referred to as the BMBL 5th Edition.

I have ensured that all laboratory workers under my supervision have received or will receive biosafety training and that they are familiar with the hazards and symptoms of exposure relevant to the biological materials used within the lab. These individuals have been briefed on emergency procedures, good laboratory work practices, and the safe operation of laboratory equipment prior to the initiation of the experimental work.

I will select and provide personal protective equipment to all lab workers as necessary for the procedures required in the experiment. If I must use a biosafety cabinet while working with biological materials, then I will ensure that it is certified annually and maintained properly. Any vaccinations or medical surveillance requirements as determined by the IBC will also be met prior to the initiation of experimental work.

I will notify the IBC through the Biosafety Officer (biosafe@nmsu.edu) in the event of any of the following:

1. Any accident that results in inoculation, ingestion, and inhalation of biohazardous agents or recombinant DNA or any incident causing serious exposure of personnel or danger of environmental contamination.
2. Any problem pertaining to the operation of biological and physical containment safety equipment such as a biosafety cabinet or facility failure such as a power outage which may compromise building engineering controls and subsequently, the safety of the workers in the lab.
3. The experimental work has been completed and/or I am leaving New Mexico State University. In either instance, a closeout decommissioning inspection will need to be conducted by the Biosafety Officer. I will contact the Biosafety Officer at 646-4463 to arrange for the inspection.

If this work involves other Institutional or Federal Regulatory Review/Approvals/Permits, or involves the use of any CDC or USDA Select Agents and Toxins, or materials that are regulated under the OSHA Bloodborne Pathogen Standard, I will not proceed with the activity until I have submitted all relevant documentation, and have received an official notice of approval from the IBC.

I acknowledge that the IBC approval granted by this application is not transferable to any other New Mexico State University faculty member. If the PI on a project changes, a new application form must be submitted to the IBC. I also acknowledge that I will not transfer or receive biological materials from vendors, other NMSU faculty members or collaborators until IBC approval is granted. The IBC will verify that the receiving faculty member has appropriate IBC approval to use the agents. I further understand that the duration of IBC approval of an application is for three years with annual administrative review (review of facilities by the BSO), and that I must submit an activity modification report to the IBC if and when the project changes significantly in terms of experimental activities, facilities; or for any personnel change, during the approval period. It is my responsibility to submit an activity modification report in a timely manner to avoid research delays.

To the best of my knowledge, the information contained in this application is accurate and complete.

Principal Investigator Signature_____ Date_____

If submitting this application as an electronic copy, print name and date above and check here: ☐

If this is a revised version of a submitted application, please date this copy. Date_____

SECTION IX: IBC APPROVAL

The content of this signed form represents the final application reviewed and approved by the NMSU IBC for a period of three years. A new application must be submitted prior to expiration of this approval in order to continue these activities.

_____ _____

Chair, Institutional Biosafety Committee Date

Changes to any component of the research described above (including personnel) must be communicated to the IBC using the "Activity Modification Report".

APPENDIX B. THE IBC ACTIVITY MODIFICATION REPORT

NMSU INSTITUTIONAL BIOSAFETY COMMITTEE
MSC-3RES, P.O. Box 30001, Las Cruces, NM 88003
Phone: 575-646-4463 Fax: 575-646-2480
biosafe@nmsu.edu

ACTIVITY MODIFICATION REPORT

File within 60 days of proposed change

NAME OF PRINCIPAL INVESTIGATOR: _____

APPLICATION NO: _____

APPLICATION TITLE: _____

RESEARCH TYPE (check all that apply):

- ☐ Cell Culture
- ☐ Environmental samples
- ☐ Plant (Greenhouse)
- ☐ Molecular Biology
- ☐ Animal husbandry
- ☐ Plant (Field)
- ☐ Recombinant DNA (animal or plant)
- ☐ Wildlife/Arthropods
- ☐ Other:_____

Minor Modifications:

☐ This project involves proprietary or confidential business information.

☐ Personnel added _____
(List new personnel & attach training and experience record form)

☐ Personnel terminated (List personnel no longer involved) _____

☐ Change in biological materials - project no longer uses:
☐ live animals ☐ potentially infectious agents or pathogens ☐ use of recombinant DNA

☐ Laboratory is being renovated and the project is suspended until completion of renovation.
Prior to renovation: Decommissioning by EHS&RM is required; Post-renovation: Biosafety inspection is required.

☐ Laboratory is moving to another floor or building. Please identify new location. _____.
Prior to move: Decommissioning by EHS&RM is required. New lab area: Biosafety inspection is required.

☐ Project is voluntarily suspended (due to sabbatical, minor lab renovation, or project awaiting funding).

☐ Project is no longer active. End Date _____

☐ Change/addition of funding: Funding Agency _____OGC or Grant/Contract # _____

Title: _____

☐ Minor changes in procedures _____

☐ Other: _____

Major Modifications

☐ Substantial changes in the IBC-approved procedures (new technology or novel R-DNA construct) or initial acquisition of new organisms or toxins. Submit description of changes to Biosafety. IBC Chair will review on a case-by-case basis. A new IBC application may or may not be required.

☐ Change in Principal Investigator. The new PI must submit an IBC Application for the project.

☐ Research project expanded to include live animals. Submit a new IBC application.

☐ BSL-1 research project expanded to include acquisition of potentially infectious agents or pathogens for use at BSL-2. Submit a new IBC application.

☐ Project expanded to include recombinant DNA. Submit a new IBC application

☐ Other: _____

Verify that the content of this document is correct. Send the signed form via campus mail to Biosafety, MSC 3RES.

PI Signature _____ Date _____

APPENDIX C. ANNUAL LABORATORY SURVEY FORM

PI Name:	Ph. Office:		Ph. Lab:	
Mail Stop:	Application No.:			
Dept:	IBC Expiration Date:			
Lab Location: BSL-	Prior Survey Date:			
Secondary Location:	Facility: BSL- Practices: BSL-			

Project Title:	
Biohazardous Material	___ Arthropod (e.g., mosquitoes, ticks) ___ Bacteria ___BSL- ___ Virus: Plant ___ or Animal ___ (exotic ___ or endemic ___ to NM) ___ Cells or tissues (Animal source) ___ Cells or tissues (Human or non-human primate source), blood or internal body fluids ___ External body fluids (Human or non-human primate source), including urine, saliva, fecal matter ___ Fungi, mold or yeast ___ Parasite (e.g., Plasmodium spp.) ___ Toxin (biological product) ___ Recombinant DNA ___ Expression vectors(plasmid, cosmid, phage, viral, other) ___ Other, list material below (e.g. *dura mater* from human, non-human primate, livestock, rickettsia, etc.) _____

PERSONNEL	BIOSAFETY AWARENESS	HAZ COMM	HAZ WASTE	LAB STANDARD	BBP
Training dates will be filled in by the BSO.	Recommended for BSL-1 Required for BSL-2	Required for all personnel	Required of one person per lab	Required for all personnel	Required for use of human and non-human primate cells/blood

Y/N	SURVEY ITEM	COMMENTS
	ADMINISTRATIVE CONTROLS AND DOCUMENTATION	
	Are Laboratory Safety Plans <u>updated</u> and <u>available</u>? a. Emergency Response/Action Plan b. Biosafety Manual & lab-specific info (e.g., risk assessments, specialized training) c. Chemical Hygiene Plan and MSDS d. Bloodborne pathogen exposure control plan, for labs using human-derived materials (annual requirement for update and refresher training)	
	Biohazard sign posted at the entrance to BSL-2 lab a. Includes emergency contacts: PI & an alternate b. Notice of potential hazards and entry requirements	
	Controlled access (door closes and locks, authorized personnel only)	
	Refrigerators in lab are marked "No Food or Drink". Food that is stored as lab materials is marked "Not for human use"	
	Other Documentation (list location or contact person): a. Decontamination of equipment before repair, disposal, or transport b. Shipping of biohazardous materials c. Inventory of biohazardous materials d. Reports of injury or exposures	
Y/N	**STANDARD MICROBIOLOGICAL PRACTICES**	**COMMENTS**
	Policies: Are the following PROHIBITED in the lab? - Open-toed shoes or sandals - Food or drink (for eating or for storage) - Handling contact lenses and applying cosmetics - Mouth pipetting	
	PPE is available - Lab coats, gowns or other coverings - Gloves (appropriate to hazard) - Safety glasses, goggles, face shield	
	Glove use (observed) - Gloves are removed prior to exiting the laboratory and when handling "common" items like doorknobs, telephones, and computer keyboards. - Personnel wash their hands after handling viable materials, after removing gloves, and before leaving the lab.	

Y/N	SURVEY ITEM	COMMENTS
	Sharps - Are rigid containers used for storing supplies (razor blades, syringes)? - Are sharps containers available for disposal of biohazardous sharps? - Are rigid containers available for disposal of broken glass? - Is broken glass decontaminated before disposal?	
	Decontamination practices - Work surfaces are cleaned at least after completion of work and after spills **Identify disinfectant used, frequency:** - Other items (door handles, telephones, pens) - Floors - Laundering of lab coats - Small equipment (pipetters, spatulas) - Large equipment (centrifuges, freezers)	
	Sterilization/disposal of used materials (identify method) - Liquids - Solids	
Y/N	**LABORATORY FACILITIES & SAFETY EQUIPMENT**	**COMMENTS**
	Hand washing sink, hand soap, paper towels available?	
	Eye wash station is located within 50 ft of lab benches?	
	BSL2 lab is negative pressure to corridor	
	Vacuum lines are protected with liquid traps and/or HEPA filters.	
	Lab is designed for easy cleaning (e.g. appropriate bench tops, no carpets or cloth furniture)?	
	Windows that open to the outside are screened	
Y/N	**SPECIAL PRACTICES**	**COMMENTS**
	Biological safety cabinets - primary reason for use: - properly located & certified annually (requirement for BL2 labs) **Location, ID, and Test date**:	
	Centrifuges use sealed rotors or safety cups, and capped tubes?	
	Liquid nitrogen used in lab? - SOPs include use of thermal gloves and face shield?	
	Autoclave - Location and ID: - Is operating procedure posted? - Who trains personnel in autoclave use? - Has printer that records sterilization cycle parameters? - Is Log book maintained?	
	Validation of waste sterilization - Method: steam integrator strip, spore vial - Frequency: - Records kept by:	
	Are biohazardous materials transported between locations? (e.g., Infectious cultures, Human/primate cells, Recombinant DNA) - Are durable, leak-proof containers available as secondary containment?	
	Pest Management - Is the lab free of insects/rodents?	
	Good housekeeping and sanitation practices are evident.	
	The areas surveyed are free of imminent hazards to life and property.	
	Other:	

ACTION ITEMS:

Action items identified during this survey:

-

If safety issues are noted above, please respond by e-mail (biosafe@nmsu.edu) or in writing within 30 days of receipt on how and when the deficiencies will be corrected. Contact Biosafety at 646-4463 with any questions or concerns.

APPENDIX D. PROTECTIVE GLOVE USE POLICY

Original Memorandum issued March 24, 2004

To: NMSU Faculty, Staff & Students

From: NMSU Institutional Biosafety Committee

Subject: Glove Use Policy

It is the policy of the NMSU Institutional Biosafety Committee and EHS&RM that "Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves". The previous is a direct quote from the U.S. Department of Health & Human Services publication Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, May 1999, pp 25.

The policy applies to laboratory and other research environments. The following is offered as interpretive guidance for the glove use policy. Contact with potentially infectious materials means physically obtaining vials, tubes and other containers of stocks, cultures, and other specimens of bacteria, mold, fungi, yeast, virus (plant and animal), viral constructs (plant and animal), toxins, animal, and human tissues, and materials derived from animal and human tissue (including human blood) in the laboratory or the environment (as in field procedures that present risks i.e., trapping mosquitoes or feral animals in the field). Gloves are to be worn during each step of an experimental procedure until the material is used up, decontaminated, or otherwise rendered biologically inactive and the procedure is completed. Gloves are not to be worn while handling doorknobs, telephones, or office equipment in the laboratory or outside of the laboratory or in areas of public access like elevator lobbies, lounges and offices. Each laboratory will designate common equipment and work areas or activities where gloves will always be worn and where gloves are prohibited from being worn. Each supervisor shall provide training on area-specific glove use.

Contact the Biosafety Manager at 575.646.4463 with questions.

APPENDIX E. SAMPLE TEMPLATE: AUTOCLAVING PROCEDURE

Standard Operating Procedures - Autoclave Steam Sterilization

PURPOSE

The purpose of this document is to provide standard operating procedures for common-use autoclaves. Autoclaving is a process used to destroy microorganisms using a combination of heat and pressure for a time duration that is sufficient to provide a 4Log10 reduction in bacterial spores of *Geobacillus stearothermophilus* or induce a complete color change in an approved steam sterilization integrator.

SCOPE AND APPLICATION

Autoclaves within the _____ Department are inspected routinely by the NMSU Biosafety Officer for compliance with federal, state, and local regulations. Training of users is required and is performed by Faculty advisor/employer or departmental safety personnel. It is the responsibility of the Faculty supervisor and Principal Investigators to limit operations only to trained personnel and to record such trainings.

EQUIPMENT

Autoclaves are located as listed below:

Room	Manufacturer/Model	Purpose	Contact Name

REQUIRED SUPPLIES

- Autoclave bag, clear (with or without the biohazard symbol), small to medium sizes.
- Autoclave log book
- Stainless steel pans or autoclavable polypropylene trays.
- Chemical Indicator Strips for Steam Sterilization (aka Chemical Integrator, or “CI”)
- Personal Protective Equipment
    - Heat resistant gloves
    - Eye Protection (Safety Goggles, glasses)
    - Lab coat

SAFETY AND HEALTH

1. Physical Hazards

Burns can occur with contact of outside of equipment, contact with autoclaved items, and contact with pressurized steam. Consider posting signage near the autoclave to alert users to burn hazards.

Warning! Burn Hazards Clogged lines, equipment malfunction or a failure in the steam supply may cause the autoclave chamber to fill with scalding water. If water leaks from the front of the autoclave, DO NOT open the chamber door. Burns from scalding water may result.

2. Health Hazards

Potential exposure to infectious agents through either airborne, droplet or contact transmission; and Potential exposure to corrosive, toxic, or noxious vapors if chemical compounds are autoclaved.

PREPARATION, LOADING AND UNLOADING

Preparation for Daily Operation

1. Check to determine if paper is in the chart recorder.

2. Replace the recorder chart paper if necessary. Additional paper can be obtained from _____. Return filled chart paper to _____.

3. Weekly: check the drain screen at the bottom of the chamber and clean if blocked. If the sieve is blocked with debris, a layer of air may form at the bottom of the autoclave, preventing efficient operation.

4. Each load of waste requires the use of a Chemical Sterilization Indicator Strip for Steam. Additional strips can be obtained from _____. Use of the strip must be noted in the Autoclave Log Book.

Lab Preparation of Autoclave Waste

1. Wear a lab coat, eye protection, gloves and closed toe shoes.

2. Place biowaste into appropriate autoclave bag.
   - Sharp objects should not be placed in bags (razors, glass, scalpels, needles, etc.)
   - Obtain sharps containers for collecting sharps and for autoclaving them.

3. Gather the top of the biowaste bag loosely and secure with a twist tie, a large rubber band or autoclave tape. Leave autoclave bags loosely secured to allow steam to penetrate inside the bag. Do NOT overfill bags; the contents may fail to heat to the necessary temperature for sterilization.

4. Use caution when handling an infectious waste autoclave bag in case sharp objects were inadvertently placed in the bag. Do not lift from bottom.

5. Transport the bagged biowaste to the autoclave room in a secondary container (e.g., an autoclave-safe tray or bucket), using a cart if needed to prevent injury and spills.

Lab Preparation for Non-waste

1. Liquids being sterilized should be in containers that are 100% larger (e.g. 1 L of liquid in 2 L flask) and placed in a secondary container such as an autoclave tray. DO NOT TIGHTEN LIDS; the opening must allow for the escape of gas or overboiling. Flasks with vented caps or loosely covered with foil or cotton plugs are recommended.

2. Use of Chemical Indicator Strips or autoclave tape to verify proper sterilization is at the discretion of the user for any non-waste autoclaving. Waste decontamination requires the use of a Chemical Indicator Strip in every waste package, and results must be recorded in the autoclave log book.

3. Transport items to and from the autoclave room by appropriate means (for example, use a cart to transport a tray filled with hot liquids).

LOADING - Autoclave Room

1. Log in your cycle with the appropriate Autoclave Log Book. If book is full or if a pen is absent, contact _____ Immediately. Do NOT run a cycle without logging in. Fill in all blanks. This information is used for safety purposes and in identifying problems with the machine.

2. Place autoclave bag or non-waste load in autoclave tray.

3. Attach the chemical sterilization indicator. If more than one bag is used, each bag needs its own indicator. DO NOT re-use indicators.

4. Open autoclave chamber door by turning the chamber wheel door counter clockwise.

5. Place tray into center of autoclave. Do NOT overfill the autoclave or steam may not penetrate to all of the items being sterilized.

6. Lock chamber door by turning chamber door wheel clockwise.

Note: DO NOT leave an operating autoclave unattended for longer than 45 minutes. Leaving an autoclave engaged after a cycle is completed may result in a vacuum making it very difficult to open the autoclave door.

Cycle, Temperature and Time Selection

1. Select the cycle needed. Common cycles are shown below, but you must verify the settings that are used on your equipment.
    a. Trash - 45 minutes of sterilization at 121C with a fast exhaust
    b. Liquids - 20 minutes of sterilization at 121C with a slow exhaust
    c. Wrapped - 20 minutes of sterilization at 121C with a 15 minute dry cycle.
    d. Unwrapped - 20 minutes of sterilization at 121C with no dry cycle.

2. Push number of cycle twice to start.

UNLOADING

1. Wear a lab coat, eye protection, heat resistant gloves and closed toe shoes. Transport hot items using a cart to prevent burns or accidental spills.

2. Once the cycle is completed, verify that the indicators for the chamber pressure show no pressurized steam (zero psi) is left in the chamber. The jacket pressure should show normal pressure (often 20 psi).

3. Stand behind the door on the hinge side and open the door slowly by rotating handle. A burst of steam will evacuate the chamber as you open the door.

4. Autoclaves will require a 10 minute break after the cycle has ended. It is recommended that user wait 10 minutes with the door only 1 inch open prior to pulling contents out of any autoclave.

5. After checking the Chemical Sterilization Indicator for full sterilization, securely close the autoclave bag if it contains autoclaved waste. Sterilized waste can be thrown immediately into the garbage if the indicator shows proper sterilization. Be sure to record the result of the indicator in the autoclave log book. If the indicator does not show full sterilization, record the failed result, attach a new indicator and run the autoclave cycle again.

6. Use care when transporting autoclaved materials. You are responsible for cleaning spills immediately in public areas such as common rooms, elevators, and hallways. Autoclaved materials can have an unpleasant odor, and these materials should be transported to the dumpster immediately, using a cart or secondary container to prevent leakage from bags.

7. Do not pour hot agar into the sink drain. Place the autoclaved agar on a cart or countertop to cool. Once the material has solidified, it can be placed in a regular trash bag for discard in the dumpster.

MALFUNCTION, MECHANICAL PROBLEMS and **EMERGENCY PROCEDURES**

MALFUNCTION, MECHANICAL PROBLEMS, AND MAINTENANCE

A component failure or lack of full sterilization should be reported to _____ immediately. If needed, post a sign on the autoclave to prevent usage until repairs can be made. DO NOT use the autoclave if a sign is posted by another user, the Building Monitor, or a repair tech. Questions can be directed to _____.

EMERGENCY PROCEDURES
In the event of an emergency, call 911.
In the event of an employee injury during regular working hours, go to the Aggie Health & Wellness Center Center at the corner of Breland and Stewart St.

SPILLS

- If there is a spill inside the autoclave chamber, turn the autoclave off and allow the unit to cool before attempting to clean up the spill. If you are unsure of proper clean-up procedures, contact the building monitor or another person with knowledge of the autoclave.
- If glass breaks in the autoclave, use a mechanical device such as tongs or forceps to recover fragments. Do not use bare hands or gloved hands to pick up broken glass.
- DO NOT leave spilled material in the autoclave since it will adhere to the chamber bottom and walls, and can cause imperfections and/or perforations in the chamber if improperly removed. Most of the cost of an autoclave is in the integrity of the chamber to provide the proper conditions for steam pressure build-up to ensure sterilization.
- When cleaning up spills, DO NOT use bleach. Bleach can damage the chamber surfaces. A 10% solution of vinegar in water is the best way to clean the chamber after a spill.
- Check the drain screen when cleaning is completed to remove any debris. A clogged drain will cause a component failure in the autoclave, and result in costly repairs.

Posted _____(date)

By: _____
(Name of contact, room #, phone)

Sterilization Report

Instructions: Fill out the top and bottom of this form with identical information. After autoclaving, attach one half to the sterilized bag, and keep the other half in the waste record files. Do not leave untreated waste in public areas after 5 pm or on weekends. Do not fill bags more than half-full.

Contact _____ with any questions.

Principal Investigator _____ Date _____

Location _____ Your initials: _____

Items enclosed _____

Circle One: BSL I or BSL II

Sterilized Completely (Indicated by results of Chemical or Biological Indicator) on _____ (date)

Autoclave Cycle Details: Temperature _____ Sterilization Cycle Time: _____

Notes

For questions or comments, please contact NMSU Biosafety Manager (575) 646-4463

Sterilization Report

Instructions: Fill out the top and bottom of this form with identical information. After autoclaving, attach one half to the sterilized bag, and keep the other half in the waste record files. Do not leave untreated waste in public areas after 5 pm or on weekends. Do not fill bags more than half-full.

Contact _____ with any questions.

Principal Investigator _____ Date _____

Location _____ Your initials: _____

Items enclosed _____

Circle One: BSL I or BSL II

Sterilized Completely (Indicated by results of Chemical or Biological Indicator) on _____ (date)

Autoclave Cycle Details: Temperature _____ Sterilization Cycle Time: _____

Notes

For questions or comments, please contact NMSU Biosafety Manager (575) 646-4463

AUTOCLAVE LOG for Building and Room #:

Autoclave Mfr: NMSU Property ID:

Model No.: Serial No.:

* If the autoclave chart or printer is not used or is broken, a chemical indicator must be attached to each bag of laboratory waste. RECORD RETENTION: Records of sterilization must be retained for three (3) years (NMAC Title 20, Chapter 9, Part 8).

Run Date (mm/dd/yy)	Run Start Time	Load Description (waste, media/reagent, other)	Load Volume (3 X 20gal bags)	Cycle Duration (30 min, 60 min)	*Cycle Verification Record: Pass/Fail if chemical indicator is attached AND Cycle temp & sterilization time from autoclave chart or printer	Operator (Name & room or phone #)

APPENDIX F. OCCUPATIONAL HEALTH ENROLLMENT FORM
NMSU Preventing Occupational Exposures for Personnel

The goal of these services is to promote a safe and healthy workplace, by limiting opportunities for exposure, promptly detecting exposures, and offering prompt and appropriate treatment for exposures. **Individuals who may be exposed to human pathogens as the result of performing their job duties include plumbers, custodians, laboratory researchers and teaching assistants, athletic trainers, emergency care providers, and others as determined by the job hazard analysis.** Medical services should be designed by the healthcare provider in consultation with the supervisor, the research biosafety officer, Environmental Health and Safety Staff, and/or Human Resources.

INSTRUCTIONS: This is a two part form. Part 1 is worker's contact information and a description of the work hazard. Part 2 is authorization to use department funds. Both parts **must** be signed by the supervisor.

Part 1 Occupational Health Services Assessment

1. Complete the contact information in the boxed area below. Provide a description of the workplace hazard(s), i.e., specific biological agent, or the procedure associated with a potential for exposure. Attach a separate page if more space is needed.
2. The supervisor must sign this form to confirm the hazard assessment is accurate for this individual.
3. Call Aggie Health & Wellness Center (646-1512) to make an appointment for immunizations. Relevant, commercially available immunizations may be indicated, based upon an understanding of the potential workplace health hazards and the individual's history of prior immunizations.

AGGIE HEALTH & WELLNESS CENTER NOTIFICATION

<table>
<tr><td colspan="2">Name:</td><td colspan="2">Date:</td></tr>
<tr><td colspan="2">Job Title or Category of Exposure:
☐ Plumber (Tetanus, Hepatitis A/B)
☐ Bloodborne PathogensExposure (Hepatitis B)
☐ Other – as recommended by Medical Professional based on the job hazard</td><td colspan="2">Brief description of hazard associated with this work:
☐ Contact with blood, body fluids, tissues or cells from humans
☐ Other:</td></tr>
<tr><td>Department:</td><td>Campus Mail MSC:</td><td>Supervisor:</td><td>Supervisor's Phone or Email:</td></tr>
<tr><td>Employee's Email:</td><td>Employee's Phone #:</td><td colspan="2">Supervisor's Signature:</td></tr>
</table>

THIS PORTION OF FORM IS TO BE COMPLETED BY A MEDICAL PROFESSIONAL

This certifies that an Occupational Health review has been completed, and the individual:

☐ has received the recommended services/procedures

☐ has declined these services/procedures _____

☐ does not require services/procedures at this time but will need services/procedures in _____ (year)

Signature _____ Date _____

Health Center Medical Professional

RECORD RETENTION:

1) **Medical records for this individual are maintained on file at NMSU Aggie Health & Wellness Center.**
2) **EHS&RM will provide acknowledgement of the completion of this process through Training Central and it will be documented in the Employee Training Record.**

AHWC: Please deliver this form to EHS&RM: MSC-3578 or fax 646-7898 or email to ehs@nmsu.edu

Part 2 Occupational Health Services Interdepartmental Voucher

AUTHORIZATION TO USE DEPARTMENT FUNDS

The completed form will be used as the basis and documentation for an Interdepartmental Voucher (IDV) charging the requesting department for the Occupational Health services rendered.

PATIENT NAME: _____

Date: _____

Services(s) Requested:

- ☐ Plumbers – Tetanus, Hepatitis A/B
- ☐ Bloodborne Pathogens – Hepatitis B
- ☐ Other – as recommended by Medical Professional based on job hazard

Servicing Department: Aggie Health & Wellness Center

Requesting Department: _____MSC_____

Supervisor: _____ Phone Number: _____

Use of department funds is

Approved by: _____ (Director/Department Head)

Index Number: _____ Fund Number: _____

Aggie Health & Wellness Center

_____

Front Office Staff: Print and Sign

APPENDIX G. NMSU IBC OPERATING CHARTER

NMSU INSTITUTIONAL BIOSAFETY COMMITTEE OPERATING CHARTER (2012)

GENERAL CHARGE

The New Mexico State University (NMSU) Institutional Biosafety Committee (IBC) reviews all institutional activities involving the use of **Biohazardous Agents** and **Recombinant DNA Molecules** that require approval for "biosafety activities" as described by current governmental agencies. These agencies include but are not limited to:

- U.S. Health & Human Services (HHS) Centers for Disease Control (CDC)
- U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS)
- U.S. Occupational Safety and Health Administration (OSHA) regulations and compliance directives as adopted and adhered to by the New Mexico Occupational Health and Safety Bureau (NMOSHB)
- U.S. National Institutes of Health (NIH) Office of Science Policy

In recognition of the large amount of information on biohazardous agents, recombinant DNA technologies and changing regulatory environment, the IBC requires the support of the Research Biosafety Manager and may need additional specialists for technical consultation. As health risks, new technologies and new regulations emerge, the NMSU IBC Operating Charter will be revised accordingly.

DEFINITIONS

Biohazardous Agents:

- Any microorganism (including but not limited to bacteria, viruses, fungi, rickettsiae, or protozoa), or infectious substance, or naturally occurring, bioengineered, or synthesized component of any such microorganism or infectious substance that is capable of causing: **1**. death, disease or other biological malfunction in a human, an animal, a plant or another living organism; **2**. deterioration of food, water, equipment, supplies, or materials of any kind; or **3**. a deleterious alteration of the environment.
- Any toxic material or product of plants, animals, microorganisms (including but not limited to bacteria, viruses, fungi, rickettsiae, or protozoa), or infectious substances, or a recombinant or synthesized molecule (whatever the origin and method of production), which includes any poisonous substance or biological product that: **1**. may be engineered as a result of biotechnology; **2**. produced by a living organism; or **3.** is an isomer or biological product, homologue, or derivative of such a substance.
- Infectious or pathogenic biological agent defined by: **1**. CDC as biosafety level (BSL) 2-4 (BMBL 5th Edition), or 2**.** NIH as risk group (RG) 2-4 agent (*NIH Guidelines*) (also see Additional Definitions on page 5 of this Charter document).
- Regulated biological agent or toxin as identified by **1**. HHS 42 Code of Federal Regulations (CFR) Part 73 (Select Agents Program); **2**. USDA-APHIS lists of Biological Agents and Toxins that pose a severe threat to "animal health or animal products" (9 CFR Part 121); or to "plants health or plant products" (7 CFR Part 331) (Federal Register 9CFR. 121 7CFR 331).

Recombinant DNA Molecules:

- Nucleic acid molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecules that can be replicated in a living cell.
- DNA molecules that result from the replication of those molecules described above.

IBC RESPONSIBILITES AND SCOPE

- The IBC is responsible for reviewing all NMSU-IBC application forms submitted by research investigators and their laboratory staff members, teaching faculty, and visiting scientists (collectively defined as PI for Principal Investigator) whose activities involve:
  1. any biohazardous agent as defined above which can cause disease in humans
  2. any biohazardous agent which will be introduced into any animal
  3. any non-exempt recombinant DNA molecules (Exempt experiments are defined by *NIH Guidelines* Section III-F)
  4. any large scale production of viable organisms containing recombinant DNA, or with the potential to produce toxic or hazardous substances (as defined by *NIH Guidelines* Section III-D-6 and Appendix K)
  5. any possession, use, or transfer of HHS Select Agents and Toxins (42 CFR Part 73) or USDA Biological Agents & Toxins (9 CFR Part 121) or listed Plant Pathogens (7 CFR Part 331)
- The IBC will ensure that to the fullest extent practical, all risks to the health, safety, and well-being of laboratory employees, the public, and the environment regarding the use of biohazardous agents, non-exempt recombinant nucleic acid molecules, synthetic nucleic acid molecules, and large-scale production of recombinant DNA molecules, will be minimized.
- The IBC recommends policies to guide PIs, the University Biosafety Officer (BSO) and Environmental Health, Safety & Risk Management (EHS&RM) in the administration of NMSU's Biosafety Program with regard to the acquisition, use, transfer, storage, disinfection, disposal of agents, and emergency response procedures for all biosafety activities. The IBC shall ensure that such activities meet standards of good practice consistent with safety of personnel, the general public, and the environment in ways that best facilitate relevant research or teaching activities at NMSU.
- The IBC is vested with the authority to comprehensively review, and approve research applications with or without modifications, or withhold approval of all or any part of an application with regard to biological aspects of the research or activity. The IBC may make recommendations for corrective action on protocols.
- If a BSO review of a suspected or alleged violation of any University policy or external regulation that involves "biosafety activities" indicates that the violation is of a serious or continuing nature, the BSO will report such to the IBC. The IBC holds the authority to suspend any project in which serious or continuing violations have been reported. The IBC will notify the affected PI(s) and will proactively interact with the PI to rectify the situation. If further action is needed, the IBC will inform the Vice President for Research and the Graduate School.
- Upon request, the IBC shall review and comment on proposed biosafety regulations, including but not limited to federal, state, and local policies. When appropriate, the IBC will formulate draft policies and procedures for approval by the Vice President for Research and the Graduate School and other institutional officials as needed.

- The IBC shall periodically review the effectiveness of the Biosafety Program and make recommendations for improvements.
- The IBC shall ensure that "Biosafety activities that fall within the responsibility and scope of the IBC" which are official NMSU business conducted by an NMSU employee at a non-NMSU facility have been approved by the non-NMSU facility and adhere to the NMSU biosafety requirements.

IBC APPOINTMENTS AND COMPOSITION

- The IBC is appointed by the Vice President for Research and the Graduate School upon recommendation from but not limited to the Director of EHS&RM and the IBC Chair.
- The IBC Chair is appointed by the Vice President for Research and the Graduate School and serves as the link between the Office of the Vice President and the IBC.
- A Vice Chair should be appointed to conduct business in the absence of the Chair, or in place of the Chair if and when the Chair has an application before the committee.
- The composition of the IBC should include at least 8 NMSU members and 2 members not affiliated with NMSU.

    1. Individuals, either associated with NMSU or extra -institutional, with the following expertise and/or job duties may be appointed to the IBC:
        - recombinant DNA technology
        - molecular biology
        - biological safety
        - public health and epidemiology
        - virology
        - microbiology
        - infectious diseases
        - animal scientist
        - plant pathogen or plant pest containment principles
        - laboratory technician/non-doctoral
        - facilities management
    2. The community members should represent the interests of the surrounding community with respect to health and protection of the environment and should be knowledgeable in the basic principles of microbiology and recombinant DNA technology, or capable of assimilating these principles within the context of their applicability to the surrounding community and the general public. Individuals with the following expertise and/or job descriptions should be considered:
        - officials of state or local public health or environmental protection agencies
        - persons involved in medical, occupational health or environmental concerns in the community

- The IBC may also include ex-officio non-voting members who may be invited to serve when their expertise is required and can supplement the deliberations of the IBC. These members shall include but not be limited to additional representatives, usually administrative, of the following departments: Environmental Health, Safety & Risk Management, Employee Health Services, Research Administration, University Council, Facilities and Services and/or Planning Design and Construction, and biosafety expert consultants external to NMSU. All other

members of the IBC appointed by the Vice President for Research and the Graduate School will be voting members.

TERMS OF SERVICE

- The term of membership on the IBC is a 12 month renewable period. In general, members will serve 2-3 years. The IBC Chair and the Director of EHS&RM will make a recommendation for renewal of membership on the committee to the Vice President for Research and the Graduate School.
- The IBC Chair is a continuous appointment by the Vice President for Research and the Graduate School, with an annual confirmation from the committee to the Vice President for Research and the Graduate School.
- The BSO is a continuous position appointment. The BSO is a professional position which reports to the Executive Director of Research Administration Services.

IBC GUIDELINES AND PROTOCOL REVIEW PROCEDURES

- The IBC shall meet quarterly or as needed to ensure timely review of applications.
- All biosafety application/registration forms shall be available for review by any member of the IBC. The BSO shall maintain records of research application reviews, minutes of IBC meetings, including records of attendance and IBC deliberations.
- If requested, the minutes of meetings are available to the public under the open records law.
- Applications submitted by PIs for work that falls within the IBC responsibility and scope must be reviewed and approved by the IBC prior to the initiation of that work.
- Approval for biosafety activities is granted for three years after the initial review by the IBC, and is contingent upon the affirmative vote of the majority of a quorum. (The quorum for the NMSU IBC is defined under Additional Definitions on page 5 of this Charter document).
- An activity modification report must be submitted by the PI to the IBC if and when the project changes significantly in terms of experimental activities, facilities; or for any personnel change, during the approval period. If the PI on a project changes, a new application form must be submitted to the IBC.
- The BSO will conduct annual inspections of facilities of approved projects, and initial inspections of facilities of new projects, and report to the IBC. The following guidelines are established to aid the IBC in the exercise of its responsibilities:

    1. Biohazardous Agents
        - Research applications involving RG 1 and/or BSL 1 materials that do <u>not</u> involve recombinant DNA, <u>do not</u> require review by the IBC.
        - Dictated by the lack of facilities at NMSU, research using any RG 4 agents or any materials that require BSL 4 containment will not be considered by the IBC for work at any NMSU location or facility.
    2. Toxins
        - The routine use of most toxins will not require IBC review and approval. However, the possession, use, or transfer of any toxin which is included in 1. HHS Select Agents and Toxins (42 CFR Part 73), 2. the USDA-APHIS Biological Agents and Toxins severe threat to animal health or animal products list (9 CFR Part 121), or 3. USDA-APHIS Biological Agents and Toxins severe threat to plants health or plant products

list (7 CFR Part 331), will require IBC review and approval prior to initiation of the project. The BSO will notify the IBC if any experiments involve the isolation and production of toxins included in the aforementioned CFRs.

3. Recombinant DNA
    - Projects using recombinant DNA (that are not exempt) require IBC review and approval before initiation.
    - Experiments described as "Exempt" in Section III-F of the *NIH Guidelines* do not require IBC review and approval – but will require registration via the IBC application/ registration form for tracking and review by the BSO.
    - Planned release of any organism (e.g. transgenic plants, animals, bacteria) outside of the approved laboratory environment requires registration with the appropriate Federal regulatory agency and must be filed with the IBC.

REPORTING LINE AND ADMINISTRATIVE SUPPORT

- The IBC reports to the Vice President for Research and the Graduate School at New Mexico State University. The BSO is the administrator of the IBC and is also responsible for the day-to-day operation of the Biosafety Program. The BSO reports to the Executive Director of Research Administration Services and provides the necessary administrative support for the functions and business of the IBC.

ADDITIONAL DEFINTIONS

- Biosafety Level (BSL). A description of the degree of physical containment to be employed for managing infectious materials in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community. The essential elements of the four biosafety levels defined by the CDC for activities involving infectious microorganisms and laboratory animals are summarized in Sections III and IV of the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition.

- Risk Groups (RG). Agents are classified into four Risk Groups (RGs) according to their relative pathogenicity for healthy adult humans by the following criteria: (1) Risk Group 1 (RG1) agents are not associated with disease in healthy adult mans, (2) Risk Group 2 (RG2) agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available, (3) Risk Group 3 (RG3) agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available, (4) Risk Group 4 (RG4) agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available. Reference: *NIH DNA Guidelines* Section II-I-A, Appendix B.

- Quorum for the NMSU IBC. A quorum is defined as the number of members required to be present for business to be legally transacted. For the purpose of the NMSU IBC, a minimum quorum shall consist of the IBC Chair, the Biosafety Officer (BSO), a committee member representing the department or the research area of the proposed "biosafety activity", a committee member whose expertise is necessary to address all safety issues of the proposed "biosafety activity", and a committee member or members to meet the criteria of specific guidelines (such as the *NIH Guidelines*) when relevant.

APPENDIX H. REFERENCES

1. Environmental Health, Safety & Risk Management in NMSU Regents Policy Manual Title 16

2. Research Oversight and Risk Management in NMSU Administrative Rules and Procedures (ARP) Chapter 11.01

3. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, accessed from https://osp.od.nih.gov/biotechnology/nih-guidelines/

4. Laboratory Safety Monograph: A Supplement to the NIH Guidelines for Recombinant DNA Research (1979), accessed from https://archive.org/details/laboratorysafety00nati

5. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition (December 2009) , accessed from https://www.cdc.gov/labs/BMBL.html

6. Institute for Laboratory Animal Research. Guide for the Care and Use of Laboratory Animals, Eighth Edition. Washington, DC: National Academy Press; 2011.

7. A Practical Guide to Containment: Plant Biosafety in Research Greenhouses. Adair, D. and R. Irwin. 2008. Information Systems for Biotechnology, Virginia Tech, Blacksburg, VA, accessed from https://vtechworks.lib.vt.edu/handle/10919/78423

8. Primary Containment for Biohazards: Selection, Installation and Use of Biosafety Cabinets. In BMBL 5th Edition, Appendix A, accessed from at https://www.cdc.gov/safelabs/resources-tools.html

9. NSF/ANSI 49 – 2012. Biosafety Cabinetry: Design, Construction, Performance, and Field Certification. NSF International, 11/27/2012. This standard is available in the NMSU Library at http://libcat.nmsu.edu/vwebv/holdingsInfo?bibId=1585881

10. National Select Agent Registry: accessed 11 Jan 2019 from www.selectagents.gov)

11. USDA Animal and Plant Health Inspection Service Permits and Certifications, accessed from https://www.aphis.usda.gov/aphis/resources/permits

12. NM Department of Game & Fish, accessed at http://www.wildlife.state.nm.us/enforcement/special-use-permits/

13. CDC Import Permit Program, accessed from https://www.cdc.gov/cpr/ipp/

14. New Mexico Administrative Code Title 20, Environmental Protection, Chapter 9 Solid Waste, accessed from http://164.64.110.134/nmac/T20C009

15. Department of Transportation Hazardous Materials Regulations (49 CFR Parts 171-180), accessed from https://www.phmsa.dot.gov/transporting-infectious-substances/transporting-infectious-substances-overview

16. World Health Organization, “Guidance on Regulations for the Transport of Infectious Substances 2019-2020”, accessed from https://www.who.int/ihr/publications/guidance_infectious_substances/en/

APPENDIX I.
SELECT AGENTS AND TOXINS LIST (9/24/2018)
7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73

The following biological agents and toxins have been determined to have the potential to pose a severe threat to both human and animal health, to plant health, or to animal and plant products. An attenuated strain of a select agent or an inactive form of a select toxin may be excluded from the requirements of the Select Agent Regulations. The list of excluded agents and toxins can be found at: http://www.selectagents.gov

HHS SELECT AGENTS AND TOXINS

1. Abrin
2. *Baacillus cereus* Biovar *anthracis* *
3. Botulinum neurotoxins *
4. Botulinum neurotoxin producing species of Clostridium*
5. Conotoxins #
6. *Coxiella burnetii*
7. Crimean-Congo haemorrhagic fever virus
8. Diacetoxyscirpenol
9. Eastern Equine Encephalitis virus #
10. Ebola virus *
11. *Francisella tularensis* *
12. Lassa fever virus
13. Lujo virus
14. Marburg virus *
15. Monkeypox virus #
16. Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
17. Ricin
18. *Rickettsia prowazekii*
19. SARS-associated coronavirus (SARS-CoV)
20. Saxitoxin

South American Haemorrhagic Fever viruses
21. Chapare
22. Guanarito
23. Junin
24. Machupo
25. Sabia

26. Staphylococcal enterotoxins A, B, C, D, E subtypes
27. T-2 toxin
28. Tetrodotoxin

Tick-borne encephalitis complex (flavi) viruses
29. Far Eastern subtype
30. Siberian subtype

31. Kyasanur Forest disease virus
32. Omsk hemorrhagic fever virus
33. Variola major virus (Smallpox virus) *
34. Variola minor virus (Alastrim) *
35. Yersinia pestis *

OVERLAP SELECT AGENTS AND TOXINS

*36. Bacillus anthracis**
*37. Bacillus anthracis** Pasteur strain
38. Brucella abortus
39. Brucella melitensis
40. Brucella suis
*41. Burkholderia mallei **
42. *Burkholderia pseudomallei* *
43. Hendra virus
44. Nipah virus
45. Rift Valley fever virus
46. Venezuelan Equine Encephalitis virus #

USDA SELECT AGENTS AND TOXINS

47. African horse sickness virus
48. African swine fever virus
49. Avian influenza virus #
50. Classical swine fever virus
51. Foot-and-mouth disease virus *
52. Goat pox virus
53. Lumpy skin disease virus
54. Mycoplasma capricolum #
55. *Mycoplasma mycoides* #
56. Newcastle disease virus #
57. Peste des petits ruminants virus
58. Rinderpest virus *
59. Sheep pox virus
60. Swine vesicular disease virus

USDA PLANT PROTECTION AND QUARANTINE (PPQ) SELECT AGENTS AND TOXINS

61. Coniothyrium glycines (formerly *Phoma glycinicola and Pyrenochaeta glycines*)
62. Peronosclerospora philippinensis (Peronosclerospora sacchari)
63. Ralstonia solanacearum
64. Rathayibacter toxicus
65. Sclerophthora rayssiae
66. Synchytrium endobioticum
67. *Xanthomonas oryzae*

* Denotes Tier 1 Agent
# Refer to the Federal Select Agent Program for numbered notes