

NEW MEXICO STATE UNIVERSITY

*BIOLOGICAL*

*SAFETY*

*MANUAL*

*2005*

**ENVIRONMENTAL HEALTH & SAFETY**

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Date: November 7, 2005

To: NMSU Researchers with Programs That May Require IBC Oversight

From: John D. Kemp, Ph.D. Chair, Institutional Biosafety Committee

Subject: Biosafety Manual

Materials contained in the biosafety Manual were prepared by personnel in the NMSU Environmental Health and Safety Office and have been reviewed by members of the Institutional Biosafety Committee (IBC). Use of biohazardous materials at NMSU is regulated by federal, state and local regulations. The Vice Provost for Research has delegated the responsibility for ensuring compliance to the IBC. The IBC works with the Biosafety Officer (BSO) in maintaining the Biosafety Program. 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.

## NOTICE OF ACCEPTANCE

New Mexico State University Biosafety manual is intended to inform faculty, staff and students on regulatory requirements, University policy, sound work practices and essential biosafety information in support of teaching and research. This manual (including subsequent additions and revisions) is prepared by Environmental Health & Safety Biosafety Manager and is reviewed and approved by members of the Institutional Biosafety Committee.

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Katrina D. Doolittle, Ph.D., Director for Environmental Health & Safety

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John D. Kemp, Ph.D., Chair for Institutional Biosafety Committee

## IMPORTANT TELEPHONE NUMBERS

### EMERGENCY TELEPHONE NUMBERS

Fire, Police, Emergency Medical Service

- On-campus phone 911
- Off-campus phone 646-3311 or 911 for Mesilla Valley Central Dispatch

### ASSISTANCE TELEPHONE NUMBERS

- University Employee Health Center – 646-6600
- University Student Health Center – 646-1512
- Environmental Health & Safety – 646-3327
- Biological Safety Officer – 646-4463
- Biological Waste Pick-up – 646-3327
- Radiation Safety Officer – 646-5427

# 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 information 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 regulations and University policy.

While the Principal Investigator remains responsible for overall compliance with regulations and policy for activities conducted at their direction, Environmental Health and Safety (EH&S) is 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 research venues using biohazardous agents, and includes internships and work-study programs with a potential for exposure to biohazardous agents, in clinics, teaching and research laboratories, greenhouse and field studies of genetically modified plants and other bioengineered materials. The NMSU Colleges of Arts and Sciences, Agriculture, Engineering, Health and Social Services and the Dona Ana Branch 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 Application. See the form in Appendix A or on line at <http://www.nmsu.edu/safety/program-link.htm>. Projects involving humans, animals, radioactive materials, or radiation generating equipment require additional approval by the Institutional Review Board, Institutional Animal Care and Use Committee, 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 EH&S through submission of a completed and signed IBC application.

### 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 or tissues or cell lines, biological toxins, and recombinant DNA must submit a completed and signed IBC Application.
- Greenhouse and field research using genetically modified plants, plant pests, or pesticide-containing genes, and recombinant DNA must submit a completed and signed IBC Application.

### III DEFINITIONS

Biohazardous Agent are defined as:

1. 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:
  - 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. Also, 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 non-human animals, humans, or plants defined by:
  - a. CDC as biosafety level (BSL) 2-4 (BMBL 4th Edition), or
  - b. NIH as risk group (RG) 2-4 agent (NIH Guidelines April 2002)
  
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 US Department of Health & Human Services and United States Department of Agriculture agents (current as of 07-05).*
  
5. 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. And DNA molecules that result from the replication of those molecules described above.

Containment means the physical control of pathogens, infectious 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, such as a pathogenic virus, parasite, or bacterium that is capable of invading body tissues, replicating itself and causing disease.

Laminar Air Flow means unidirectional airflow at a constant velocity.

Pathogen means microorganisms (e.g., bacteria, viruses, or parasites) that can cause disease in humans, animals and plants.

Infectious waste under the New Mexico Environment Department Solid Waste Bureau regulations (Title 20, Chapter 9 Part 1) means solid waste that carries a probable risk of transmitting disease to humans or animals including but not limited to:

1. 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.
2. Human pathological wastes, including tissues, organs, body parts, and body fluids that are removed during surgery, autopsy, other medical procedures, or laboratory procedures, but not including hair, nails, or extracted teeth.
3. Human and body fluid waste, including but not limited to: liquid waste human blood, blood products, items caking, flaking, saturated or dripping 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 pharmaceuticals.
4. 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 but not limited to during research (including research in veterinary schools and hospitals), production of biologicals or testing of pharmaceuticals.
5. Discarded sharps, used or unused, generated at a facility 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.
6. Regulated waste as specified in 29 CFR Part 1910.1030, "Bloodborne Pathogens".

## 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 for activities being conducted or modify those activities accordingly 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 dissemination of policies and other safety-related information (safety policies).
6. Establishing procedures to implement policies.
7. Utilizing the system that will be established for assessing safety performance to evaluate their own areas of responsibility and report findings back to central administration.
8. Immediately notifying NMSU Environmental Health and Safety 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.

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 the University.
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 necessary preventive measures to control hazards.
4. Ensuring necessary support such as engineering and administrative controls, personal protective equipment and occupational medical examinations, 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 Office of Human Resources and the Biosafety Officer.
7. Reviewing incident and injury reports for their area(s).
8. Serving as a focal point for safety and health concerns.
9. Immediately notifying Environmental Health and Safety 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 Federal or State regulatory agency.

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

1. Participating in mandated training programs provided by Environmental Health & Safety, 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, environmental health hazards, as well as injuries and illnesses to the cognizant supervisor or program director.
5. Giving due consideration to personal safety and the safety of others while performing assigned tasks.
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. 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 CDC or NIH Guidelines on Research Involving Recombinant DNA Molecules and other 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 DNA research or biohazardous materials.
7. Providing advice on laboratory security.
8. Providing technical advice to Principal Investigators, staff, the IBC and IACUC on research biosafety procedures.
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.

## 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, EH&S training records and the Proposal Award Form from the Grants & Contracts Office. Other documents that may have a Biosafety component are the IACUC and IRB application forms. The IBC application is addressed in Section VI of this manual. The IACUC application is reviewed for use of rDNA and infectious or pathogenic agents.

Additionally, there are government and vendor-generated documents related to regulatory and policy compliance. For example, purchasing research materials from a commercial vendor, shipping environmental samples, or other biological materials necessarily creates a paper trail of the transaction. Documents related to the acquisition of, permit applications for, transfer of, and use of some research materials is legally significant to the Principal Investigator and the University. In some instances there are statutory requirements for archiving these documents, DOT requires copies of Shipper's Declaration of hazardous materials be held for 375 days. The Compliance Office and the Grants and Contracts Office, the Principal Investigator, and EH&S must work together to ensure the University maintains accurate records of research regulatory compliance documentation. Faculty and staff are not authorized to sign any document purporting to "bind the University" to terms and conditions for any purpose. The Office of the Vice Provost for Research is responsible for signing all agreements related to research activities.

Examples of legally binding research-related documents include, but are not limited to commercially or privately tendered Material Transfer Agreements (MTAs), a "Customer Acceptance of Responsibility" form (e.g., ATCC Form 62), a U.S. Department of Defense Facility Site Safety Plan and Certificate of Environmental Compliance, Air Way Bill (Air bill) and if applicable, the Shipper's Declaration of a hazardous material. Many persons are not aware of the record-keeping requirements for these documents that are critical to demonstrating NMSU and PI regulatory compliance. Generally, records for obtaining and transfer of research materials should be kept for three years, financial records must be kept for seven years, and records for employee health should be kept for 30 years after separation of employment. Recent regulations such as *The USA PATRIOT Act of 2001*, *Public Health Security and Bioterrorism Preparedness and Response Act of 2002* and *The Select Agent Regulations (HHS; 42 CFR Part 73 Possession, Use and Transfer of Select Biological Agents and Toxins & USDA; Agricultural Bioterrorism Protection Act of 2002)* have inventory control, access, training, biosafety and biosecurity requirements. Faculty and staff must not sign any legally binding document on behalf of the University. All such documents must be forwarded to the Office of the Vice Provost for Research or EH&S for completion.

### **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, biohazardous agents, hazardous chemicals, radiation, and recombinant DNA. The PI is responsible for obtaining review and approval from each respective NMSU oversight committee and compliance with NMSU safety policy. For example, the hazardous chemical inventory must be updated at least annually.

PIs must submit an IBC application for any research involving biohazardous agents or recombinant DNA (as indicated by markings boxes in item 14 on the Proposal Award Review form). All applications are administratively reviewed. Potential results of 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.

The Office of the Vice Provost for Research has established the following internal review to ensure compliance with the regulations and NMSU safety policy. The Vice Provost for Research forwards the PI name, grant title, and nature of materials intended to be used in the proposal (animals, biohazardous agents, hazardous chemicals, radiation, and recombinant DNA) to EH&S. EH&S reviews records to ensure that necessary committee approvals (IACUC, IBC, RSC) have been obtained and are in good standing. EH&S notifies the PI if a necessary approval is lacking and must be obtained. EH&S notifies the Vice Provost for Research on the status of compliance as incomplete, complete, or pending.

## Permits

Depending on the nature of the material (proprietary rDNA, genetically modified organism, product licensed under the Virus-Serum-Toxin Act, and some commercially available materials) and the type of experiment (laboratory, greenhouse, single site field trial or multi-site field trial), 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 Grants & Contracts Office, EH&S and other appropriate NMSU Administrative Offices. Examples of permit issuing agencies are the NM Agriculture Department, NM Fish and Wildlife Department, the U.S. Department of Agriculture (APHIS, VS, Fish and Wildlife), the U.S. Department of Health and Human Services (CDC), 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). The importation of animal or crop, or foodstuffs may require an import permit from one of the USDA offices (Plant Protection & Quarantine, Veterinary Services, or Biotechnology Regulatory Services), the EPA or the FDA. Materials and technologies listed on the Commerce Control List of items are prohibited from export. 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. 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. 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 with the HHS (CDC) or USDA (APHIS) to transfer, possess, or use **Select Agents or High Consequence Livestock Pathogens** is available on the internet at <http://www.cdc.gov/od/sap/downloads2.htm>. 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 Facility 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, EH&S will submit the Select Agent permit application for NMSU on behalf of a Principal Investigator interested in conducting research using Select Agents or Toxins.

There may be permit requirements for organisms and toxins that are not select agents and toxins that are exotic to New Mexico or otherwise pose a threat to plants, animals, or the environment. The NM Department of Agriculture (NMDA), USDA APHIS, (Veterinary Services, or Plant Product Quarantine and Biotechnology Regulatory Services) regulations should be contacted for details. These agencies also regulate the import of agricultural materials, and organisms into the United States. The Principal Investigator is responsible for discovery of import restrictions and permit requirements. Discovery of applicable regulations can be resolved by contacting the appropriate agency directly. A copy of the permit must accompany all IBC applications involving permitted materials. In the case where a permit requirement is unknown or uncertain, contact the EH&S Biosafety Officer 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 (US Department of Commerce, USDA, HHS, US Postal Service, commercial shipping companies and the airline industry) 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 primary cell lines, and other bacterial stocks, viral stocks, and cDNAs. The ATCC requires each purchaser to enter into an MTA for each product ordered 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 “commerce control list” of materials that may pose a risk to the public health, or have a potential for “dual use” and are 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 Provost for Research for acceptance by NMSU.

### **Required EH&S Training.**

The following are descriptions of training sessions provided by EH&S 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 by the BSO for all persons listed on the IBC application, including the PI. Attendance at appropriate training sessions is a condition of IBC approval and all IBC applications are administratively reviewed for compliance with OSHA and NMSU training requirements. Persons 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.

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 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 and graduate teaching assistants that supervise teaching and research laboratories and provides information on compliance with the regulations. This is a one-time requirement for each employee and student whose work meets the above criteria. Completion of Hazard Communication is prerequisite to registering for Laboratory Standard training.

Bloodborne Pathogen (BBP) Exposure Control training is required for persons who have a routine potential for exposure to human blood, internal body fluids, and unfixed tissues, and 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. The “routine potential for exposure” at NMSU is defined as working with these materials more than once per month.

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

## VI THE IBC APPLICATION

PIs must submit an IBC application for any research or teaching involving biohazardous agents or recombinant DNA. The IBC Application is used to document the “who, what, where, and how” for all teaching and research projects involving biohazardous agents at NMSU and NMSU-affiliated locations. (See Section III for the definition of biohazardous agents.) From a regulatory compliance perspective (primarily the rDNA Guidelines, but also the BMBL and other federal and state agencies), research projects can be divided into two classes, those that are exempt from the regulations and those that are non-exempt from the regulations. As a matter of NMSU policy, the distinction between exempt and non-exempt research rests with the IBC. Exempt research is usually administratively approved and non-exempt research is reviewed and voted on by the IBC.

The ability of EH&S and 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 required in Sections III, IV, & V prompts the applicant to submit questions or solicit advice on matters related to procedural or facility biosafety for the proposed project to EH&S or 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 PI and staff in the EH&S training database, and a survey of the laboratory facility. If necessary, the 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 and 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, PI's 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 at [http://www.nmsu.edu/safety/programs/bio\\_safety/IBC/IBC\\_Activity\\_mod.doc](http://www.nmsu.edu/safety/programs/bio_safety/IBC/IBC_Activity_mod.doc) and is Appendix B of this manual. Detailed descriptions of the modifications are discussed later in this section under the Principal Investigator Statement. The IBC application also supports the Vice Provost for Research “Proposal Review Form” used to evaluate compliance.

### **Section I: Administrative Information**

The information requested in this section identifies the Principal Investigator, and co-Principal Investigators, the project title, the funding source and proposes a 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 expired 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 subject volunteers (or organs and tissue from identifiable persons) in a research project at NMSU can begin without IRB approval.

Federal Permits: Acquisition, possession, transfer (interstate, intrastate, import or export) and use of *certain* bacteria, virus, rickettsia, parasites, biological or plant toxins, plants and plant pests, and whole or parts of their respective genetic elements and genetically modified organisms require obtaining a permit. Permitting agencies include the USEPA, Public Health Service-CDC, USDA Animal and Plant Health Inspection Service (APHIS), the NM Department of Agriculture (NMDA), or 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), and 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 BSL-2 laboratory, preparation room, and shared equipment space or rooms, off-campus and satellite campus locations, or greenhouses 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.

- If a biological safety cabinet will be used for experiments with the listed organisms,
- 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.
- Strain or type of bacteria, cell line, or virus.

Responses to box B (rDNA) and C (Proposed Biosafety Level) will demonstrate that the PI is familiar with the *NIH Guidelines on Research Involving Recombinant DNA Molecules*, April 2002, and the CDC/ Public Health Service Publication *Biosafety in Microbiological and Biomedical Laboratories*, 4<sup>th</sup> Edition, May 1999. The applicant identifies recombinant materials, their source (collaborator, commercial vendor, or other) and proposes a Biosafety level for the work.

## Section V: Description of Activity

There are four parts to Section V, Parts A, B, C, and D. 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, 2<sup>nd</sup> 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 expectation”.

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), 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 microbial spore challenge test.

## 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” 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 EH&S training dates on the application form. The PI must provide safety training appropriate for the specific work to be performed.

## Section VII: Safety Plans

Each applicant must generate a Laboratory Safety Plan and ensure that they train 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. Name, and after hours phone contact info should be entered into the secure Hazard Communication inventory database at [www.nmsu.edu/safety](http://www.nmsu.edu/safety). Contact name and business phone should be posted at the laboratory entrance.

The laboratory safety plan answers the following.

1. What types of hazards are present in the lab? (biological, chemical, radiological)
2. What are the safety training requirements for persons entering the lab? (Hazard Communication, Bloodborne Pathogens, Laboratory Biosafety Awareness, Radiation Safety)
3. What personal protective equipment is required? Specify minimum equipment and task-specific requirements.
4. Description of spill response procedure. (biological, chemical, radiological)
5. Who is to be notified in the event of an emergency? Provide contact information.
6. Describe laboratory security (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 at [http://www.nmsu.edu/safety/policies/policy\\_emergency\\_action\\_plan.htm](http://www.nmsu.edu/safety/policies/policy_emergency_action_plan.htm). 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 on the following requirements.

1. Conduct research and teaching activities in compliance with the *NIH Guidelines on Research Involving Recombinant DNA Molecules*, April 2002 (and subsequent revisions) and the Public Health Service publication *Biosafety in Microbiological and Biomedical Laboratories*, 4<sup>th</sup> Edition May 1999 (and subsequent revisions) and other applicable guidelines and regulations.
2. Provide for staff training about laboratory biosafety, emergency procedures, and the risk of occupational exposure.
3. Provide staff with necessary personal protective equipment.
4. Report to the IBC through EH&S of all instances of
  - a. Occupational injury and exposure to biohazardous agents or rDNA (needle sticks, inhalation, and ingestion).
  - b. Events (known or likely) resulting in environmental release of biohazardous agents or rDNA.
  - c. Instances of containment equipment breakdown and facility system failures.
5. Submit an Activity Modification Report (APPENDIX B) 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 tissue), infectious or pathogenic organisms, or rDNA
6. Submit a new IBC Application for the following MAJOR modifications.
  - a. Change in PI.
  - b. Research project expands to include work with live animals (animal cells or tissue), infectious or pathogenic organisms, or rDNA.
  - 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 rDNA construct) or initial acquisition of new organisms or toxins. Submit description of changes to BSO. The IBC Chair will review on a case-by-case basis.
  - e. Submission of the signed Principal Investigator statement binds the signatory to the above terms.

## VII BIOSAFETY LEVELS AND WORK PRACTICES

This section references the CDC/ NIH publication *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), 4<sup>th</sup> Edition, May 1999, the *NIH rDNA Guidelines*, April 2002, and *A Practical Guide to Containment – Greenhouse Research with Transgenic Plants and Microbes*, 2001, Information Systems for Biotechnology, Virginia Tech. Research at NMSU is properly classified at BSL-1 and BSL-2. Research at BSL-3 must have a laboratory specific Biosafety manual and therefore is not included here. This section reviews the biosafety levels for laboratory and field research conducted at NMSU, biological (including molecular and microbiological techniques), animal, and plant procedures.

BSL-1 and BSL-2 describes standard practices, special work practices, safety equipment and (for BSL-2) facility requirements used for biomedical, microbiological and molecular biological laboratory research as defined in the Public Health Service publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), 4<sup>th</sup> Edition.

ABSL-1 and ABSL-2 describes standard practices, special work practices, safety equipment and facility requirements for use of laboratory animals in research as defined in the Public Health Service publication, BMBL. Note that the NIH Guidelines for Research Involving Recombinant DNA Molecules references the BMBL descriptions of facilities and work practices as appropriate for work with rDNA.

BSL-1P and BSL-2P refers to greenhouse plant containment in research as briefly defined in Appendix P of the NIH Guidelines for Research Involving Recombinant DNA Molecules, April 2002.

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

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

***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 a biological safety cabinet is generally not required for manipulations of agents assigned to Biosafety Level 1.
2. It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.
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 is 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. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

**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 that are of minimal potential hazard to laboratory personnel and the environment.

***A. Standard Practices for ABSL-1***

1. The animal facility director establishes policies, procedures, and protocols for emergency situations. Each project is subject to pre-approval by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee (IBC). Any special practices are approved at this time.
2. Only those persons required for program or support purposes are authorized to enter the facility. Before entering, persons are advised of the potential biohazards and are instructed on the appropriate safeguards.
3. An appropriate medical surveillance program is in place.

4. A safety manual is prepared or this manual is adopted. Personnel are advised of special hazards, and are required to read and follow instructions on practices and procedures.
5. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use should only be done in designated areas and are not permitted in animal or procedure rooms.
6. All procedures are carefully performed to minimize the creation of aerosols or splatters.
7. Work surfaces are decontaminated after use or after any spill of viable materials.
8. All wastes from the animal room (including animal tissues, carcasses, and contaminated bedding) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional or local requirements. Incineration is recommended.
9. Policies for the safe handling and disposal of sharps are instituted. Sharps are disposed of in red puncture proof containers labeled with biohazard symbols and manufactured for sharps disposal.
10. Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.
11. A biohazard sign must be posted on the entrance to the animal room whenever infectious agents are present. The hazard warning sign identifies the biosafety level, 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).

**B. *Special Practices for ABSL-1*** None.

**C. *Safety Equipment (Primary Barriers) for ABSL-1***

1. The wearing of laboratory coats, gowns, and/or uniforms in the facility is recommended. Laboratory coats remain in the animal room. Gowns and uniforms are not worn outside the facility.
2. Persons having contact with non-human primates should assess their risk of mucous membrane exposure and wear appropriate eye and face protection.

**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.
2. External facility doors are self-closing and self-locking. Doors to animal rooms open inward, are self-closing, and are kept closed when experimental animals are present. Cubicle room inner doors may open outward or be horizontal or vertical sliding.
3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) are water resistant.
4. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas.
5. Windows are not recommended. Any 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.
6. If floor drains are provided, the traps are always filled with water and/or an appropriate disinfectant.
7. Ventilation should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*, latest edition. No re-circulation of exhaust air should occur. It is recommended that animal rooms maintain negative pressure compared to adjoining hallways.

8. The facility has a sink for hand washing.
9. Cages are washed manually or in a cage washer. The mechanical cage washer should have a final rinse temperature of at least 180<sup>0</sup> F.
10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

### **Plant Biosafety Level 1 (BSL-1P) Greenhouse Containment**

The principal purpose of plant containment is to avoid the unintentional transmission of a recombinant DNA-containing plant genome, including nuclear or organelle hereditary material or release of recombinant DNA-derived organisms associated with plants. The containment principles are based on the recognition that the organisms that are used pose no health threat to humans or higher animals (unless deliberately modified for that purpose), and that the containment conditions minimize the possibility of an unanticipated deleterious effect on organisms and ecosystems outside of the experimental facility, e.g., the inadvertent spread of a serious pathogen from a greenhouse to a local agricultural crop or the unintentional introduction and establishment of an organism in a new ecosystem.

Four biosafety levels are defined in the Public Health Service publication, *Biosafety in Microbiological and Biomedical Laboratories (BMBL, 4<sup>th</sup> Edition May 1999)* as Biosafety Level (BSL) -1Plants (P), BSL-2P, BSL-3P, and BSL-4P. BSL-1P through BSL-4P distinguishes between Biosafety levels for plants in the absence or presence of other experimental organisms that contain recombinant DNA. These biosafety levels, in conjunction with biological containment conditions provide flexible approaches to ensure the safe conduct of research. Containment for work with rDNA in plants or plant pathogens includes the use of plant tissue culture rooms and growth chambers within laboratory facilities, or experiments performed on open benches. The greenhouse director and the IBC may add additional biological containment practices 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.

The BSL-1P designation provides for a low level of containment for experiments involving transgenic plants in which there is no evidence that the modified organism 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 BSL-1P.

BSL-1P 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 BSL-1P 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 BSL-1P**

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 BSL-1P 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 (BSL-1P)***

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)***

1. Experiments involving other organisms that require a containment level lower than BSL-1P may be conducted in the greenhouse concurrently with experiments that require BSL-1P containment, provided that all work is conducted in accordance with BSL-1P greenhouse practices.

#### ***D. Facilities (BSL-1P)***

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.

### **Biosafety Level II (BSL-2) for Agricultural, Molecular and Microbiological**

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 directed by scientists with experience in the procedures; (2) access to the laboratory is limited when work is being conducted; (3) explicit precautions are taken with contaminated sharp items; and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment. In addition to other 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 and applying cosmetics are not permitted in the work areas. Food and cosmetics for human use are stored outside the work 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 labeled with the biohazard symbol and manufactured for the purpose of sharps disposal.
6. All procedures are performed carefully to minimize the creation of splashes or aerosols.
7. Work surfaces are decontaminated on completion of work and at end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern.
8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by autoclaving or being picked up by EH&S for disposal by incineration. 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.

**B. *Special Practices for BSL-2***

1. Access to the laboratory is restricted when work with infectious agents is in progress. 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. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections. 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 whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.
3. A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Essential information to be posted includes the biosafety level, the required immunizations, 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, and the procedures required for exiting the laboratory.
4. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB testing). Contact the Employee Health Center at 646-6600.
5. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel may be collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.
6. Biosafety procedures are incorporated into standard operating procedures or into a biosafety manual prepared specifically for the laboratory by the laboratory director and key laboratory personnel. Personnel are advised of special hazards and are required to read and

- follow instructions on practices and procedures.
7. The Biosafety Officer in cooperation with the laboratory director ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary or as procedures change. A record of training for all laboratory personnel is kept by Environmental Health & Safety and the laboratory director and is updated as training is completed. Personnel who have not completed the necessary training are not allowed to work in the laboratory.
  8. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Micro liter pipette tips are not considered to be sharps.
    - a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plastic ware should be substituted for glassware whenever possible.
    - b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
    - c. Syringes that re-sheathe the needle, needle-less systems, and other safety devices are used when appropriate.
    - d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated by autoclaving or other means prior to contacting EH&S 646-3327 for pick up.
  9. Cultures, tissues, body fluid specimens, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.
  10. Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant at least daily, after work with infectious materials is finished and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated before it is sent for repair or maintenance or packaged for transport before removal from the facility.
  11. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the laboratory director and BSO. A physician provides medical evaluation, surveillance, and treatment. The BSO maintains records on the investigation of reported incidents.
  12. Animals not involved in the work being performed are not permitted in the lab.

### ***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 in a biological safety cabinet. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious

- materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or
- b. embryonate eggs.
  - c. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.
2. Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.
  3. 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 university; personnel do not take laundry home.
  4. 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 must not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves and after removal of gloves and lab coats prior to leaving the laboratory.

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

1. Provide lockable doors for facilities that house restricted agents (as defined in Title 42 Part 73.11 Possession, Use and Transfer of Select Biological Agents and Toxins).
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. Carpets and rugs in laboratories are inappropriate.
4. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the 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. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
6. Install biological safety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Locate biological safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the biological safety cabinets' air flow parameters for containment.
7. An eyewash station is readily available within 50 feet of the work area.
8. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
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. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

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

Animal Biosafety Level 2 involves practices for work with those agents associated with human disease. It addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure. ABSL-2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1.

### ***A. Standard Practices for ABSL-2***

1. The facility director and Biosafety Officer establish standard policies, procedures, and protocols for emergency situations. The Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee (IBC) develop special policies and procedures as needed and approved.
2. Access to the animal room is limited to the fewest number of individuals possible. Personnel who must enter the room for program or service purposes when work is in progress are advised of the potential hazard.
3. An appropriate medical surveillance program is in place. All personnel receive appropriate immunizations or tests for the agents handled or potentially present (e.g., hepatitis B vaccine, TB skin testing). When appropriate, a serum surveillance system should be implemented.
4. The PI may prepare an entirely unique biosafety manual or adopt this NMSU biosafety manual. If a new manual is prepared the Biosafety Officer must review it. In either case, the manual is required reading for instruction on work practices and procedures and must advise personnel of special hazards. A record is maintained listing the names of persons who read the manual and the date they read the manual.
5. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food and cosmetics for human use should only be done in designated areas and are not permitted in animal holding or procedure rooms.
6. All procedures are carefully performed to minimize the creation of aerosols or splatters.
7. Equipment and work surfaces in the room are decontaminated at least daily with an effective disinfectant after work with the infectious agent, and especially after overt spills, splashes, or other contamination by infectious materials.
8. All infectious samples are collected, labeled, transported, and processed in a manner that contains and prevents transmission of the agent(s). All wastes from the animal room (including animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse) are transported from the animal room in leak-proof, covered containers labeled with the biohazard symbol for disposal in compliance with NMSU procedures. The outer surface of the primary container is disinfected prior to moving the material and is subsequently contained in a secondary leak-proof container for transport out of the facility.
9. Policies for the safe handling of sharps are instituted:
  - a. Needles and syringes or other sharp instruments are restricted to use in the animal facility and used only when there is no alternative, such as for parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
  - b. Syringes that re-sheath the needle, needle-less systems, and other safe devices should be used when appropriate.
  - c. Plastic ware should be substituted for glassware whenever possible.
10. Personnel wash their hands after removing gloves and just before leaving the animal facility.
11. A biohazard sign must be posted on the entrance to the animal holding room whenever

etiologic agents are in use. Essential information to be posted includes the biosafety level, the required immunizations, 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, occupational health requirements (e.g., the need for immunizations or use of a respirator) and the procedures required for exiting the laboratory.

### ***B. Special Practices for ABSL-2***

1. Animal care, laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary due to changes in experimental procedures or policy. The PI is responsible for maintaining protocol-specific training records. All training is documented. EH&S is responsible for maintaining hazard communication and biosafety awareness training records. Finally, records on medical surveillance of persons working with animals are maintained by Employee Health Center. Only animals used in the experiment(s) are allowed in the room.
2. All equipment must be appropriately decontaminated prior to removal from the room.
3. Spills and accidents resulting in overt exposures to infectious materials must be immediately reported to the facility director and the BSO. If warranted, medical evaluation, surveillance, and treatment are provided by Employee Health Center.

### ***C. Safety Equipment (Primary Barriers) for ABSL-2***

1. Gowns, uniforms, or laboratory coats and protective gloves are worn while in the animal room. Gloves are worn when handling infected animals and when skin contact with infectious materials is unavoidable. Gowns, uniforms, and laboratory coats are removed before leaving the animal facility.
2. Personal protective equipment is used based on risk assessment determinations and documented in the protocol.
3. Biological safety cabinets, other physical containment devices, and/or personal protective equipment (e.g., respirators, face shields) are used whenever conducting procedures with a high potential for creating aerosols. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, or intranasal inoculation of animals.
4. When needed, animals are housed in primary biosafety containment equipment appropriate for the animal species. Ventilated micro isolator cages and stand-alone filter top cages are always handled in properly designed and operating animal biocontainment cabinets recommended for rodents.

### ***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.
2. Secure, locked doors limit access to the facility. External doors are self-closing and self-locking. Doors to animal rooms open inward, are self-closing, and are kept closed when experimental animals are present. Cubicle room inner doors may open outward or be horizontal or vertical sliding.
3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) are water resistant.

4. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas.
5. Any 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.
6. If floor drains are provided, the traps are always filled with an appropriate disinfectant.
7. Exhaust air is discharged to the outside without being recirculated to other rooms. Ventilation should be provided in accordance with criteria from the *Guide for Care and Use of Laboratory Animals*, National Academy Press, 1996. The direction of airflow in the animal facility is inward; animal rooms should maintain negative pressure compared to adjoining hallways.
8. Cages are washed manually or in an appropriate cage washer. The mechanical cage washer should have a final rinse temperature of at least 180<sup>0</sup> F.
9. An autoclave is available in the animal facility to decontaminate infectious waste.
10. A sink is present in the animal room where infected animals are housed, and in procedure rooms as well. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

### **Plant Biosafety Level 2 (BSL-2P) Greenhouse Containment**

BSL-2P is assigned to experiments with transgenic plants and associated organisms, which, if released outside the greenhouse, could be viable in the surrounding environment but would have a negligible impact or could be readily managed. BSL-2P 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 BSL-2P because sunflower is capable both of hybridizing with wild relatives, and becoming established as a volunteer weed.

BSL-2P 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 BSL-2P 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 (BSL-2P)**

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 BSL-2P 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 BSO 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, 301-496-9839 (fax). Documentation of any such accident shall be prepared and maintained.

#### ***B. Decontamination and Inactivation (BSL-2P)***

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 (BSL-2P)***

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 a containment level lower than BSL-2P may be conducted in the greenhouse concurrently with experiments that require BSL-2P containment provided that all work is conducted in accordance with BSL-2P greenhouse practices.

#### ***D. Facilities (BSL-2P)***

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. BSL-2P 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 Biosafety Levels**

Biosafety Levels	Agents	Work Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
BSL-1	Not known to consistently cause disease in healthy adults	Standard Microbiological Practices	None required	Open bench top, and a sink is required
BSL-2	Associated with human disease, hazards are 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	*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; face protection as needed.	BSL-1 plus: *Autoclave available

**Table 2. Summary of Biosafety Levels for Infected Animals**

Animal Biosafety Level	Agents	Work Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
ABSL-1	Not known to consistently cause disease in healthy adults	*Standard animal care and management practices.  * Medical surveillance program in place.	As required for normal care of each species.	*Standard animal facility  *No recirculation of exhaust air  *Directional airflow recommended  *Hand washing sink recommended
ABSL-2	*Associated with human disease  *Hazard: percutaneous injury, ingestion, mucous membrane exposure	ABSL-1 plus: *Limited access *Biohazard sign *Sharps precautions *Biosafety manual  *Decontamination of infectious waste, and animal cages and bedding prior to washing	ABSL-1 equipment plus *Containment equipment appropriate for animal species  *PPE: Lab coat, gloves, face and respiratory protection as needed	ABSL-1 facility plus  *Autoclave available  *Hand washing sink in animal rooms  *Mechanical cage washer used

**Table 3. Summary of Plant Biosafety Levels**

Source: *A practical Guide to Containment, Information Systems for Biotechnology*, Virginia Tech, 2001  
*NIH rDNA Guidelines, Appendix H*

Plant Biosafety Level	Agents	Work Practices	Safety Equipment (Primary)	Facilities (Secondary Barriers)
BSL-1P	Transgenic plants not able to survive and spread in the environment, or if released would not pose a risk to the environment Examples of DNA-modified organisms e.g., <i>Agrobacterium</i> or <i>Rhizobium</i>	<ul style="list-style-type: none"> <li>*Access is limited or restricted when experiments are in progress.</li> <li>*Personnel read the written procedures prior to entry.</li> <li>*Records are maintained for all current projects.</li> <li>*Experimental organisms are autoclaved prior to disposal</li> </ul>	None	<ul style="list-style-type: none"> <li>*Impervious walkways are recommended, no special barriers are required.</li> </ul>
BSL-2P	Transgenic plants with entire genome of indigenous pathogen and related organisms that if released are viable in the surrounding environment but of negligible impact or such impact is manageable	<ul style="list-style-type: none"> <li>*Greenhouse manual is developed or adopted</li> <li>*Accidents involving unintentional release or spill of microorganisms are reported to greenhouse director and BSO</li> <li>* Porous flooring (gravel or dirt) is periodically treated to inactivate organisms potentially trapped in flooring</li> <li>*Posted notification of restricted access</li> <li>* Viable materials transported into and out of the facility are contained in non-breakable containers</li> </ul>	<ul style="list-style-type: none"> <li>*Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials</li> <li>*PPE: laboratory coats, gloves, safety glasses, face shield or goggles.</li> </ul>	<ul style="list-style-type: none"> <li>*Autoclave available</li> <li>*Entry doors have locks</li> <li>*Floor composed of impervious material.</li> <li>*Window and intake fan openings are screened (30 – mesh or higher) to exclude arthropods and flying animals.</li> </ul>

## **SECTION VIII: OVERVIEW OF SELECTED BIOSAFETY PROCEDURES AND TASKS**

### **AUTOCLAVING**

#### **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 heating the load to at least 250° F (121C) and pressurized to 15 psi and maintaining these conditions for at least 15 minutes. The nature and quantity of the materials being autoclaved affect 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 biohazardous waste. For each event the steam must be able to penetrate throughout the over wrapped implements, bags, or containers to afford the requisite steam contact time in order for the items to be sterilized or decontaminated. If sufficient steam does not come into contact with the materials then microorganisms can survive the autoclave cycle.

Finally, the NM Environment Department Solid Waste Bureau regulates decontamination of laboratory waste. Sterilization of lab ware 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 six-log reduction in the number of viable organisms.

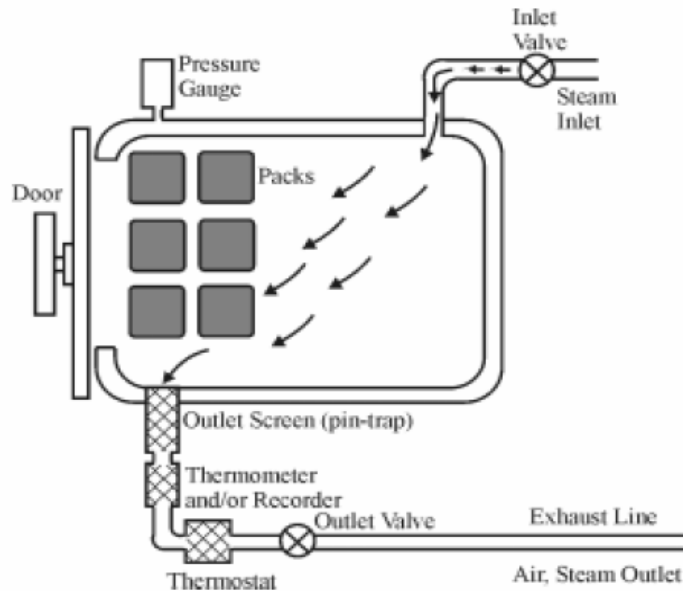


Figure 3. Autoclave diagram.

### Autoclave Operating Parameters

**Cycle settings:** Select the liquid cycle for liquids. Select the gravity cycle for solid materials. The main distinction between gravity and liquid cycles is the rate that the chamber is exhausted at the completion of the cycle. Materials typically run on the gravity cycle are exhausted more rapidly relative to the liquid 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.

**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:**
1. Chamber pressurization requirement increases at higher geographic elevations.
  2. Recorder tape should be retained as part of the autoclave record.

### 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. Further, the NMED requires on-going performance evaluation of autoclaves (every 40 hours of operation) used to decontaminate laboratory waste. For every forty hours of operation the unit is challenged with a biological or chemical indicator. Add all run times (decontamination and sterilization cycles) when calculating the 40 hour operating time. 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 autoclave decontamination of waste.

A biological indicator (BI) uses spores of a thermophilic bacterium (either *Geobacillus sterothermophilus*, *Bacillus atrophaeus*, or *B. globigii*) 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, usually yellow in color. 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 from the bag. After the decontamination cycle is complete, the BI is recovered from the load and incubated usually for 24 hours (sometimes as long as 72 hours) and visually inspected for a color change. No color change indicates the spores were killed and a color change indicates the spores were not killed and bacterial growth has caused the pH indicator to change color – usually from yellow to red.

A typical chemical indicator 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° F (121C) for the requisite 15 minutes. (See figure 4.) The chemical compound embedded in the 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 with gravity and liquid cycles. Prior to autoclaving an indicator strip is placed inside of each bag or container of laboratory waste prior to the autoclave run.



Figure 4. Chemical indicator strip display.

If the indicator chemical fails to migrate the entire length of the strip, the test is considered negative for decontamination. Negative tests are repeated 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 is 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.

A log must be maintained for each autoclave used to decontaminate laboratory waste prior to disposal in a landfill. The log must contain the following data. Date, start time, run time, load volume, run number, load description and operator name. Each supervisor is responsible for providing and documenting training for each autoclave operator. Each operator must understand the written operating procedures for each steam sterilizer they use including correct cycle selection for the load, temperature and pressure requirements, type of waste, type of container(s) and closure(s), pattern of loading, water content, and maximum load quantity.

### **Required Monitoring Procedure for Laboratory Waste Decontamination Run**

1. Obtain a steam sterilization integrator (source: Castle/ Getinge 800-950-9912, catalogue number 61301603658)
2. Place the integrator in the center of each load and process according to the sterilizer manufacturer’s instructions.
3. After the cycle is completed, allow the content to cool to room temperature.
4. Open the bag and retrieve the integrator test strip.

5. After the decontamination process is complete, the integrator chemical will advance along the wick from the “start” to the “finish” across the strip.
6. Confirm that the dye has migrated completely from the “start” to “finish” zone.
  - a. 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 and waste cannot be considered decontaminated.
  - b. 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.
7. Record each load in logbook including date, start time, run time, load volume, description and operator name.

**Note:** Indicator tape with the word “AUTOCLAVED” chemically embedded that appears after exposure to 250° F for 15 minutes is not equivalent to a chemical integrator. Autoclave tape is useful for marking and labeling items to be autoclaved but does not show decontamination. Autoclave tape may be used together with a chemical integrator strip but may not be used *instead* of a chemical indicator strip. Use of autoclave tape alone is **not accepted** by the NM Environment Department to demonstrate decontamination of regulated waste. See non-biological integrator products at <http://www.biosure.com/rpages/integratoB.html>

### Personal Protective Equipment for Autoclaving

Use elbow-length insulated gloves when handling any hot items. Use of a lab coat, closed-toed shoes, eye and face protection are required when handling hot liquids. The extent of protection required (safety glasses or face shield) depends on the volume being handled.

### 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 EH&S 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 build up 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 causing a violent release of the super-heated material and, possibly, projectiles.

### **Loading the Autoclave**

For reusable items (scalpels, clamps, filter housings, etc) check the item to be autoclaved to ensure the item is free of residues and otherwise clean, wrapped and ready for use at the completion of cycle.

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 cheesecloth or other 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 – 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 gage to ensure cycle is engaged.
6. Record the load description, cycle, date, time and your initials in the logbook.

### **Unloading the Autoclave**

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

1. Carefully loosen the door to release the residual pressure and allow the autoclaved load temperature to cool. (Recommend at least 10 minutes.)
2. While wearing elbow-length gloves, and eye or face protection, remove the contents and place on a cart, bin, or other firm, heat resistant surface.
3. Confirm and record results for temperature and cycle duration (on the autoclave chart or printout) in the logbook.

## **BIOLOGICAL SAFETY CABINETS (BSCs)**

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 and NIH publication *Primary Containment for Biohazards: Selection, Installation, and Use of Biological Safety Cabinets*, 2<sup>nd</sup> Edition, September 2000 is a good source of information on the operation, use, and certification of BSCs. The booklet is available on the internet at <http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm>.

The original NIH design evolved into the three classes of BSC in use today. They are designated as Class I, Class II and Class III. Consider that each manufacturer may have more than one design for each type of Class II BSC in their product line. Biological safety cabinets are primary containment devices designed to protect the product being manipulated, the operator, the environment, or all three. Class II BSCs are sub-divided into types A1, A2, B1, B2, and B3. Class II Type A1 & A2 BSCs are the most widely used in clinical, biomedical, and microbiological research and manufacturing applications. The differences between an A1 and an A2 BSC is that the exhaust from a Type A1 BSC is vented into the laboratory, while the exhaust from a type A2 BSC leaves the room via a canopy exhaust connection to the building exhaust duct and the A2 BSC requires an intake velocity of 100 FPM vs. 75 FPM for an A1 cabinet. Table II summarizes the differences in face velocity, airflow patterns and the acceptable biosafety level for each Class and type of BSC. 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**

Type	Face Velocity Linear Ft/min	Airflow Pattern	Lab Biosafety Level
Class I	75 lfm	In at front, rear and top through a single HEPA filter	BSL-2 & BSL-3
Class II A1	75 lfm	70% of HEPA filtered supply is re-circulated within the BSC and 30% is exhausted to the room	BSL-2 & BSL-3
Class II A2	100 lfm	70% of HEPA filtered supply is re-circulated within the BSC and 30% is exhausted through building exhaust	BSL-2 & BSL-3
Class II B1	100 lfm	30% of internal air is re-circulated within the BSC and 70% is exhausted through a hard-connected duct	BSL-2 & BSL-3
Class II B2	100 lfm	100% of internal air is exhausted from the cabinet through a hard-connected duct	BSL-2 & BSL-3
Class II B3	100 lfm	70% of HEPA filtered supply is re-circulated within the BSC and 30% is exhausted via an internal plenum that is negatively pressured in relation to the room and exits via connection to building exhaust	BSL-2 & BSL-3
Class III	N/A	Supply air inlets and exhaust through 2 HEPA Filters	BSL-3 & BSL-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 2002, 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.

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 the test report should be forwarded to EH&S Biosafety.

### **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.

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 a 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. EH&S recommends each laboratory develop a chemical decontamination procedure and require that the procedure be followed by each user 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).

## Prior to using Biological Safety Cabinets

Turn on the blower motor and let the unit run for at least 10 minutes to allow the cabinet to purge ambient air within the unit and establish an internal equilibrium.

1. After the unit has equilibrated, wipe down the interior (work surface, side and back walls, and interior of the view screen) with 10% v/v sodium hypochlorite solution (household bleach) or other appropriate compound (for example, Wescodyne®, a bound iodine solution) prior to placing equipment or supplies into the BSC.
2. Decontaminating solutions intended for use inside a BSC should be made up 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. Carefully consider the tasks to be performed and place only necessary equipment and supplies in an appropriate quantity 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.

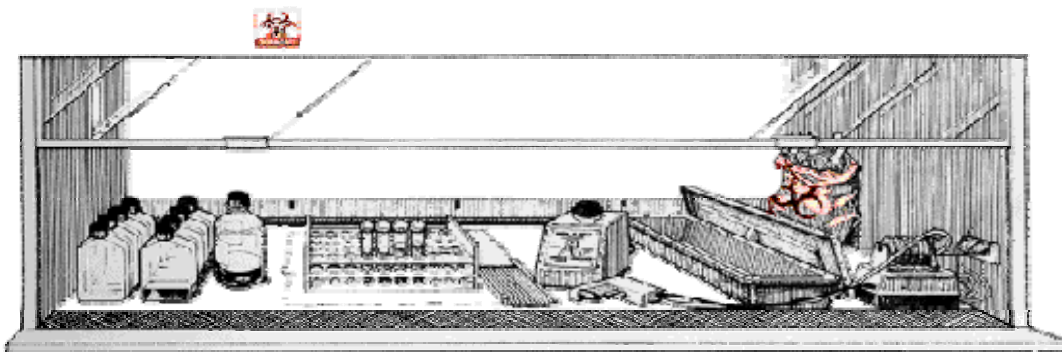


Figure 1. Proper Arrangement of Materials in a BSC

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. The two most common flammables used in BSCs are the alcohol burner and the Bunsen burner. Since standard Class II BSC electrical systems are not spark proof and 70% of Class II BSC air is re-circulated within the cabinet and that HEPA filters remove only particulates, flammable and volatile vapors may build up inside the cabinet creating a fire or explosion hazard. Use of chemicals in a BSC that is vented to the laboratory may create an inhalation hazard for all persons in the laboratory.

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. Bacticerator®



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 an “authorized” person is likely to invalidate the warranty.

### Improper Use of Biological Safety Cabinets

Avoid keeping excess supplies like pipette tips, paper towels, and other lab ware in the BSC. 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. 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.

Consider the following examples of *improper use* of a BSC.

1. Drilling, grinding or cutting into an internal or external surface of the cabinet for any reason is prohibited. As mentioned previously, BSC design and performance is vigorously tested to meet NSF International “listing” criteria. Any change to the physical structure of a Biosafety cabinet 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.
2. Using a biosafety cabinet as a chemical fume exhaust hood when manipulating large quantities (above 250ml) of acid or base 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 are not spark-proof, and Class II BSCs are not constructed to be gas-tight. Also consider that most biosafety cabinets at NMSU are exhausted back into the laboratory. The presence of flammable or combustible vapor and gas in this environment can be problematic.

## **BIOHAZARD SPILL CLEAN UP**

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 decontaminating a spill of viable organisms. 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 every other day.

Typically cell and tissue culture procedures involve anywhere between 10 ml and 500 ml of liquid (higher volumes for pilot plant or scale up projects) containing an unknown number of cells or organisms. 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**

The three components of the risk assessment for spill clean up in research and teaching laboratories are listed below and followed by an explanation of its respective impact on the spill clean up procedure. Major spills must be reported to EH&S.

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. Note that organisms classified for work at BSL-1 are usually NOT infectious and organisms classified for work at BSL-2 may be pathogenic, but they are usually NOT infectious via aerosol exposure. The decision to vacate the area should be made on a case-by-case basis for the particular organisms and agents involved. EH&S & the IBC may be consulted.
2. What is the largest volume of liquid culture manipulated and what is the highest titer of the organism attained per vessel (or colony population per plate carried at one time) attained in your protocol? 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.
3. Also, a high titer of organisms in a culture or an overgrown plate(s) may enhance the risk of exposure if the vessel or plate(s) hits the floor and break open. 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.
4. For spills inside of biological safety 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. As mentioned previously such rapid motions will compromise containment and permit aerosols to escape containment 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 out of the BSC and the underside inspected and if necessary cleaned.

5. 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.

### **Liquid Spill Clean Up Procedure**

PPE Requirements: The operator wears gloves, a lab coat and eye protection at a minimum.

1. Alert other persons in the vicinity that a spill has occurred. (Especially for large volume spills).
2. Based on the result of the risk assessment and prior planning, the area may or may not be evacuated.
3. Cover the spill with paper towels to contain the spill to as small an area as possible and absorb the liquid.
4. Apply decontaminating solution (usually 10% sodium hypochlorite bleach) to paper towels. Let stand for 10 minutes.
5. Use a wastebasket to discard the used paper towels, and other soiled materials.
6. Add a sufficient number of paper towels to absorb the entire volume of liquid (culture and decontaminating agent) involved in the spill.
7. Carefully transfer the paper towels to the wastebasket.
8. Repeat the application of decontaminating agent to the work surface.
9. Let stand for 10 minutes.
10. Wipe up and discard the soiled paper towels into the wastebasket.

The resulting waste (paper towels, other materials) does not require autoclaving. Autoclaving bleach or paper towels containing bleach will vaporize the bleach resulting in the release of  $\text{Cl}^-$  ions into the atmosphere. Under no circumstances should bleach or materials containing bleach be autoclaved.

**Note:** For information on chemical spill clean up see the NMSU Hazard Communication Plan, Section 4.1.7 at [http://www.nmsu.edu/safety/programs/lab\\_safety/app01a\\_model\\_chp.htm](http://www.nmsu.edu/safety/programs/lab_safety/app01a_model_chp.htm).

## **BLENDING, MIXING, SONICATING AND CELL DISRUPTION**

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, eye protection, and hearing protection for some sonicating procedures.

### **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

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 and 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 the centrifuge, 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. Be sure to 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. The centrifuge lid may be left open until the decontamination procedure is completed.

## 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. Laboratory exposures to potentially infectious agents, recombinant DNA, and toxins are reportable to NMSU EH&S, Employee Health Center and the laboratory director under NMSU policy and 29 CFR 1910.1030, the Bloodborne Pathogens Standard. The classical path to a disease state is composed of three distinct steps; (1) an exposure event that leads to (2) an infection and if the infection persists, the condition progresses to (3) a disease. The following describes the risks for the five routes of exposure to potentially infectious agents, recombinant DNA molecules, and toxins along with risk reduction measures.

### Ingestion

- Risk: Occupational exposure by ingestion occurs when food or drink that is contaminated with any research material is consumed or when food or drink is touched by contaminated hands or utensils and subsequently consumed.
- Risk reduction: Exposure by ingestion is substantially reduced by keeping food and drink out of the laboratory, frequent hand washing - 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 potentially infectious agent or toxin without wearing protective gloves, a lab coat, 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. Evaluate Band-Aid or other protective coverings of cuts and abrasions prior to entering the laboratory.

### Mucous Membrane Contact (eyes and mouth)

- Risk: Exposure of eyes or mouth 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, and use full-face shield when handling liquid nitrogen, viable cultures, acids or bases in quantity outside of containment. Avoid chewing gum and applying cosmetics in the laboratory especially when handling hazardous materials.

### Sharps Injury

- Risk: Exposure via a sharp injury (laceration, needle-stick, or scrape) with a contaminated needle, razor blade or other tool represents the most serious threat of infection because 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, feeding cells and 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: Manipulation of potentially infectious bacterial or viral cultures (flasks, vials, etc.) is restricted to a biological safety cabinet that is certified at least annually. Use engineering controls (BSCs, ventilated animal cage racks and change stations, dedicated exhaust of animal holding rooms) to reduce inhalation hazards associated with animal dander, excreta, and spent cage bedding.

## DISPOSAL PROCEDURE FOR LABORATORY MICROBIOLOGICAL WASTES

### **Biohazard (Infectious) Waste**

In accordance with the provisions of the New Mexico Environment Department Solid Waste Management Regulations (EID/SWMR-s), materials identified, as biohazard must be handled with special consideration. Primary investigators are responsible for preparation of a written procedure for steam sterilization and implementation of personnel training and documentation.

1. Segregate ordinarily autoclaved trash from biohazard (infectious) trash.
2. Maintain written log for each sterilization unit used to autoclave infectious waste.
3. Certify in writing (TRAINING RECORD on reverse side) that personnel understand the written operating procedures for each steam sterilizer including time, temperature, pressure, type of waste, type of container, closure on container and steam sterilization integrator. A copy of each TRAINING RECORD must be sent to the Environmental Health & Safety, Dept 3578.
4. Place the steam sterilization integrator (Castle/ Getinge 800-950-9912, catalogue number 61301603658) at the innermost area of the biohazard waste bag to demonstrate that an effective sterilization temperature and pressure has been reached throughout the package.
5. Place 12 inches of temperature sensitive tape diagonally across the biohazard symbol.
6. Autoclave at sufficient temperature, pressure and time to render all contents non-viable. Waste shall not be considered sterilized if either the indicator or tape fails to demonstrate that a temperature of at least 250 degree Fahrenheit or 121 degrees Celsius was reached during the process. Attach exposed indicator to the outside of the biohazard bag.
7. All material certified as non-infectious by steam sterilization and not otherwise regulated as hazardous, special or radioactive can be disposed by landfilling.

### **Ordinary Autoclaved Waste (Not Infectious)**

All activities which utilize culture plates or other biological growth media regardless of use must be autoclaved.

1. All personnel must be trained on the operating procedures for each autoclave and sign a written log which shall be maintained for each sterilization unit.
2. A 12 inch strip of temperature sensitive tape must be on each autoclave load. The exposed words "autoclaved" or "sterile" must be displayed to demonstrate that the sterilization procedure was performed before releasing as ordinary trash.

### **Sharps**

All sharps must be placed in a rigid, puncture proof container manufactured for use in sharps disposal and autoclaved as described above. Call Environmental Health & Safety for pick-up and disposal of autoclaved sharps-never put sharps in ordinary trash.



**PRESERVED BIOLOGICAL WASTES**

User:	Phone No:	Date:
Dept:	Building:	Room No:

**DRY SOLID WASTE<sup>1</sup>**

Description <sup>2</sup> :
----------------------------

<sup>1</sup>NO FREE STANDING LIQUIDS ACCEPTED

<sup>2</sup>Describe type of biological material

**BULK LIQUID WASTE**

Percentage of Total Container Volume	Chemical Identification

**Procedure for Preserved Biological Waste Disposal**

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.
  - Dry Solid Waste  
Use plastic liner inside bio-waste container supplied by EH&S.  
Place dry carcasses into lined container. No free standing liquids are acceptable.
  - Liquid Waste  
Use sturdy transport boxes with cardboard separators for bulk liquid waste bottles.
3. Complete label (above) and secure to outside of container and/or box.
4. Call Environmental Health and Safety, 646-3327, to schedule pick-up or request bio-waste containers.
5. Fresh biological material (must not contain preservative or regulated material) shall be taken to the City Landfill. A limited amount of carcass disposal can be arranged through the Animal Care Facility, 646-3241.

## SHIPPING RESEARCH MATERIALS

This section is intended to inform the NMSU community on U.S. and International shipping regulations and that commercial carriers are likely to have additional requirements for transporting packages containing research materials. The information provided is introductory, and is not intended to substitute for the comprehensive training requirements specified in the regulations. Not all shipping entities accept all types of packages, for example United Parcel Service and the U.S. Postal Service have very restrictive requirements for packages containing hazardous materials.

Shipping is a highly regulated process that assigns civil and criminal liability to the organization and the shipper for any violation. *The discovery of an improperly packaged material in commerce can result in a civil penalty of not less than \$275 per day up to a maximum of \$32,500 per day. Criminal charges may result in penalties of a fine up to \$500,000 and/or 60 months in prison.* For packaging and shipping purposes, research materials considered in this section are defined as biologicals, diagnostic specimens, or infectious agents. There are differences in the packaging, paperwork, and container requirements for each respective material to ensure the package arrives at its destination in good order (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). The following briefly describes the components that make up a “package” as defined in the regulations.

1. A leak-proof primary container wrapped in a absorbent material sufficient for the quantity contained, AND
2. An outer container (usually a box) manufactured according to regulatory specification AND
3. 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
4. Is marked with the correct shipping name for the material AND
5. Is marked with the UN identification number of the materials AND
6. Is labeled with appropriate decals AND
7. Is free of extraneous markings, labels, stains or discoloration AND
8. Clearly identifies the addressee AND
9. Clearly identifies the consignee AND
10. The correct paperwork accompanies the package.

Within the continental United States shipping materials via ground transport is regulated by the U.S. Department of Transportation under the Title 49 Code of Federal Regulations Parts 172, 173, & 176, the Hazardous Materials Regulations (HMRs). Shipping research materials via air is guided by the International Air Transport Association (IATA) Dangerous Goods Regulations (DGRs). No less than 4 agencies (DOT, BIS, FAA, FMCSA) are charged with enforcing federal shipping regulations. Note that shipping companies are required to maintain files of “discrepant” shipping events involving *any* violation of the regulations. Regulatory review of shipping company files that reveal multiple or repeat discrepant shipping events at a given entity will result in a regulatory inspection of the entity. Regulatory inspections often result in the entity being fined.

## 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 CO<sub>2</sub> 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.).

## The Shipping Process

Preparation for shipping research materials should be planned well in advance. Be sure to allow sufficient time to obtain packaging materials, and if necessary dry ice, and EH&S review of shipping documents. There are several preliminary determinations that must be made prior to shipping research materials. First is determining whether 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 MTA explicitly prohibits subsequent transfer of cell cultures and other materials purchased without ATCC's written permission. For a fee (currently \$150.00) you may submit a form requesting permission from ATCC to transfer materials to a secondary location. Contact ATCC Intellectual Property & Asset Management Office 1-703-365-2773 for further 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. Also determine if there is a NM or U.S.-issued permit required 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.

The second consideration is that 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 or working directly with the BSO and Physical Science Laboratory (PSL) shipping staff to ensure the package conforms to carrier-specific requirements for the material being shipped.

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. Persons planning to ship hazardous materials and research materials must contact EH&S at 646.3227 for assistance with classifying the materials and selection of the proper packaging and labels. The Physical Science Laboratory mailroom (522-9411 or 522-9407) is the designated NMSU shipping point for these materials.



**NMSU Institutional Biosafety Committee Application**  
 c/o Environmental Health & Safety  
 Academic Research Ctr. Unit C MSC 3578  
 505-646-3327

FOR OFFICE USE ONLY
Application No:
Receipt Date:
Status:
Approval Date:

**APPENDIX A, The IBC Application**

Instructions for completing this form can be found at [IBC Application Form Instructions](#). Form is to be filled out completely and submitted electronically to [John Balog](#).

**SECTION I: ADMINISTRATIVE INFORMATION**

PRINCIPAL INVESTIGATOR INFORMATION		
Name:	Date:	
Title:	Department:	
Phone:	Fax:	Lab Location:
Campus Mail Stop Code:	Email address:	
PROJECT INFORMATION	Proposed Biosafety Level:	← As entered in Section IV Part C
Title of Research Project:		
CATEGORY OF APPLICATION	This application is: <input type="checkbox"/> Initial (New) <input type="checkbox"/> Updated, the existing IBC approval will expire.	
FUNDING SOURCE(S) INFORMATION		
Name of PI on Grant:		
Funding Agency/Source:		
Grant Title:		
<input type="checkbox"/> Yes <input type="checkbox"/> No	Approval of this protocol is needed for grant application deadline?	Grant deadline date:

**SECTION II. INSTITUTIONAL & REGULATORY APPROVALS/ REGISTRATIONS**

OTHER INSTITUTIONAL REVIEWS/APPROVALS/PERMITS		
<b>A. USE OF VERTEBRATE ANIMALS</b> Does this biosafety activity involve the use of animals?	<input type="checkbox"/> Yes <input type="checkbox"/> No Registration with the NMSU IAUCU is required for all work with live animals	If Yes, have you registered with the Institutional Animal Care and Use Committee (IAUCU)? <input type="checkbox"/> Yes <input type="checkbox"/> No If answer is Yes, attach a copy of the registration form to this application If answer to above question is "No", proceed to <a href="#">IACUC</a>
<b>B. USE OF RADIATION</b> Does this biosafety activity involve the use of radioactive materials?	<input type="checkbox"/> Yes <input type="checkbox"/> No A permit from the URSC is required for all work with radioactive materials	If Yes, have you registered with the University Radiation Safety Committee (URSC)? <input type="checkbox"/> Yes <input type="checkbox"/> No If answer is "Yes", attach a copy of the permit to this application If answer to above question is "No", proceed to: <a href="#">Radiation Safety Manual Section IV Permitting and Registration</a>
<b>C. USE OF HUMAN SUBJECTS</b> Does this biosafety activity involve the use of human subjects?	<input type="checkbox"/> Yes <input type="checkbox"/> No Permission to use human subjects in research must be granted by the NMSU IRB	If yes, have you applied for permission with the Institutional Review Board (IRB)? <input type="checkbox"/> Yes <input type="checkbox"/> No If answer is "Yes", attach a copy of the permit to this application If answer to above question is "No", proceed to: <a href="#">NMSU Human Subjects Policy and Procedures IRB</a>
<b>D. FEDERAL PERMITS</b> Does this biosafety activity require any Federal permits that are not included in A, B, or C above?	<input type="checkbox"/> Yes <input type="checkbox"/> No Permit (s) from Federal agencies (e.g. USDA/APHIS) are required for handling of certain biological materials, including <a href="#">Select Biological Agents and Toxins</a> (list)	If yes, have you acquired the necessary permits? <input type="checkbox"/> Yes ( <input type="checkbox"/> application submitted) <input type="checkbox"/> No If answer is "Yes", attach a copy of permit(s) If answer is "No", please explain. For USDA permits see: <a href="http://www.aphis.usda.gov/ppg/permits/index.html">http://www.aphis.usda.gov/ppg/permits/index.html</a> For Select Agent application see: <a href="#">CDC: Select Agent Program: Forms</a>

SECTION III: LOCATION OF ACTIVITIES

LOCATION Approval of the proposed activity is given only for the locations listed below.			
<b>NMSU</b> Indicate which NMSU locations are used for this activity?	For each Yes, Complete the table below.		Room number(s)/site identifier
	Building/Site, Greenhouse or Field Location		
<input type="checkbox"/> Yes NMSU main campus	1. 2.		Name and phone number of contact person at this site
<input type="checkbox"/> Yes NMSU off-campus Research Center	1. 2.		
<input type="checkbox"/> Yes NMSU field site	1. 2.		
<input type="checkbox"/> Yes NMSU greenhouses	1. 2.		
<input type="checkbox"/> Yes Other			
<b>NON-NMSU</b> Does any part of this activity occur at a non-NMSU facility or site?	<input type="checkbox"/> Yes <input type="checkbox"/> No	If Yes, complete all of the following information for each non NMSU facility	
Do any NMSU personnel associated with this activity physically participate in the activity at this non-NMSU facility?	<input type="checkbox"/> Yes <input type="checkbox"/> 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?	<input type="checkbox"/> Yes <input type="checkbox"/> No	If Yes, write the materials transferred in this space.  Do you have the necessary permits required for transfer of these materials? <input type="checkbox"/> Yes <input type="checkbox"/> No	
Does this facility/or specific area where the work is conducted have an IBC approval for work at the appropriate biosafety level?	<input type="checkbox"/> Yes <input type="checkbox"/> No	If Yes, what is the approved BL? <input type="checkbox"/> BL1 <input type="checkbox"/> BL2 or <input type="checkbox"/> BL3 Have you attached a copy of the non NMSU facility IBC approval to this form <input type="checkbox"/> Yes <input type="checkbox"/> 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	
Name of non-NMSU Facility		Contact Name	
Address		Contact Title	
City, State, Zip, Country		Contact Phone Number	

SECTION IV. TYPE OF BIOLOGICALS AND BIOSAFETY ACTIVITY

<b>A. BIOHAZARDOUS AGENTS:</b> Check all boxes that apply to this project. Include any biological material that should be brought to the attention of the IBC.		
1. <input type="checkbox"/> Arthropod (e.g., mosquitoes, ticks) 2. <input type="checkbox"/> Bacteria 3. <input type="checkbox"/> Cells or tissues (Animal source) 4. <input type="checkbox"/> Cells or tissues (Human or non-human primate source) blood, or body fluids, unfixed tissue including immortalized cell lines. 5. <input type="checkbox"/> Fungi 6. <input type="checkbox"/> Mold 7. <input type="checkbox"/> Parasite (e.g., Plasmodium spp.)	8. <input type="checkbox"/> Recombinant DNA (rDNA), Attach completed <a href="#">WORKSHEET 1</a> 9. <input type="checkbox"/> Use of expression vectors <input type="checkbox"/> Yes viral vector <input type="checkbox"/> Yes cosmid, phagemid, plasmid vector 10. <input type="checkbox"/> Toxin ( <input type="checkbox"/> chemical or <input type="checkbox"/> biological product) 11. <input type="checkbox"/> Virus - Animal ( <input type="checkbox"/> exotic or <input type="checkbox"/> endemic to NM) 12. <input type="checkbox"/> Virus - Plant ( <input type="checkbox"/> exotic or <input type="checkbox"/> endemic to NM) 13. <input type="checkbox"/> Yeast  <input type="checkbox"/> Other, list material below (e.g. <i>dura mater</i> from human, non-human primate, livestock, rickettsia etc.) _____	
Check the appropriate box that indicates the source of each material listed above. <input type="checkbox"/> A commercial vendor (ATCC or as part of a kit, i.e., Stratagene, Promega, etc.) <input type="checkbox"/> Hospital or clinic If using a "kit", provide the Manufacturer and product number _____ <input type="checkbox"/> Isolation from environmental samples (water, soil, etc.) <input type="checkbox"/> Isolated from a plant or animal <input type="checkbox"/> Colleagues and collaborators in <input type="checkbox"/> Academia or <input type="checkbox"/> Industry <input type="checkbox"/> Other (specify) _____		
For each box checked above provide the COMMON NAME, SCIENTIFIC NAME (genus & species, strain and Manufacturer's kit name and product number). Check each box that applies in the "SPECIFICS" column.		
COMMON NAME	SCIENTIFIC NAME (Genus & species, strain)	Yes No SPECIFICS
		<input type="checkbox"/> <input type="checkbox"/> Certified BSC will be used for propagation / manipulation of agent. <input type="checkbox"/> <input type="checkbox"/> Vaccine is available ( <input type="checkbox"/> licensed or <input type="checkbox"/> IND). <input type="checkbox"/> <input type="checkbox"/> Vaccination recommended. <input type="checkbox"/> <input type="checkbox"/> Special precautions to be used are listed below. _____
		<input type="checkbox"/> <input type="checkbox"/> Certified BSC will be used for propagation / manipulation of agent. <input type="checkbox"/> <input type="checkbox"/> Vaccine is available ( <input type="checkbox"/> licensed or <input type="checkbox"/> IND). <input type="checkbox"/> <input type="checkbox"/> Vaccination recommended. <input type="checkbox"/> <input type="checkbox"/> Special precautions to be used? (If yes, list below). _____
		<input type="checkbox"/> <input type="checkbox"/> Certified BSC will be used for propagation / manipulation of agent. <input type="checkbox"/> <input type="checkbox"/> Vaccine is available ( <input type="checkbox"/> licensed or <input type="checkbox"/> IND). <input type="checkbox"/> <input type="checkbox"/> Vaccination recommended. <input type="checkbox"/> <input type="checkbox"/> Special precautions to be used are listed below _____

B. RECOMBINANT DNA		
<b>USE OF RECOMBINANT DNA MOLECULES.</b> Does this research involve the use of recombinant DNA molecules?	<input type="checkbox"/> Yes <input type="checkbox"/> No	Specific source (e.g. tomato – scientific name, for <i>E. coli</i> and other cells supplied as part of commercial prep kit, list the origin of the cell line.) Reference (author, year, journal, vol./issue, page numbers)
Materials	Genus, species and strain of organism & source of DNA	
Source of DNA <input type="checkbox"/> plant <input type="checkbox"/> animal <input type="checkbox"/> other		
Use of expression vectors? <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Yes viral vector (identify promoters) ⇒ <input type="checkbox"/> Yes plasmid vector (describe insert) ⇒		

C. PROPOSED BIOSAFETY LEVEL	
What is the proposed Biosafety Level for this activity? <input type="checkbox"/> BSL-1 <input type="checkbox"/> BSL-2 <input type="checkbox"/> BSL-3 <a href="#">CDC BMBL Section III</a> <a href="#">CDC BMBL Section VI</a> <a href="#">NIH Guidelines Section II-B</a> <a href="#">NIH Guidelines Appendix G</a> <a href="#">NIH Guidelines Appendix I</a>	<ul style="list-style-type: none"> <li>• 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.</li> <li>• Biosafety Level 2 involves work with agents of moderate potential hazard to personnel and the environment.</li> <li>• Biosafety Level 3 involves clinical, diagnostic, teaching, research, or production facilities in which works done with indigenous or exotic agents that may cause serious or potentially lethal disease as a result of exposures by the inhalation route.</li> <li>• 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.</li> </ul> <p>Source: <a href="#">NIH Guidelines April 2002</a> Section II-B, Appendices G&amp; I  <a href="#">CDC BMBL 4th Edition</a> Sections III, VI, VII</p>

**V DESCRIPTION OF ACTIVITY**

A. LAY SUMMARY
<i>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.</i>

B. PROCEDURES
<b>Research Methods/ Procedures</b> 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.
<ol style="list-style-type: none"> <li>1. <i>Include any activities which may produce aerosols, or which may increase the hazard of working with the biohazardous agent(s).</i></li> <li>2. <i>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)</i></li> </ol>

**Option 1:** Review the template language below describing the method of disposal of biohazardous substances; recombinant DNA transformed organisms (e.g., incineration, autoclaving, chemical disinfections).

**Option 2:** If chemical disinfectant other than a 10% dilution of 6.0% sodium hypochlorite is used, state chemical and concentration in the space provided..

1. 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 EH&S 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.

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 EH&S (6-3327) for pick up of chemical waste and full sharp containers.

*2. 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.)*

**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.

	ROOM	BUILDING	MANUFACTURER	MODEL	SERIAL NO.	CERT / TEST DATE
<input type="checkbox"/> BSC <input type="checkbox"/> CAB <input type="checkbox"/> Autoclave						
<input type="checkbox"/> BSC <input type="checkbox"/> CAB <input type="checkbox"/> Autoclave						

SECTION VI: PERSONNEL

Complete for each of your personnel listed below.

**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) Relevant experience  
 (2) Where obtained (NMSU or other), and  
 (3) Number of years experience.

Name	Title	Description of Experience
1.		(1) (2) (3)
2.		(1) (2) (3)
3.		(1) (2) (3)
4.		(1) (2) (3)
5.		(1) (2) (3)

The BSO will review records of EH&S 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.

B. 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

SECTION VII. SAFETY PLANS

Safety Plans (laboratory safety plan and emergency response plan) will be reviewed during lab survey.

Established Exposure Response Procedures described below:

<p><b>Accidental Exposure:</b> Indicate that you agree with the text supplied below by checking this box <input type="checkbox"/> OR delete the text below and describe your alternative exposure response.</p>	
<p>Material (rDNA, Bacteria, Virus etc.)</p>	<p>Response Procedure</p>
	<p>Available evidence suggests that materials used in this research <input type="checkbox"/>are / <input type="checkbox"/> 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 <i>E. coli</i> K-12 derivatives 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 EH&amp;S. Injured persons who need or want medical treatment will report to the Employee Health Center (646-6600) or Student Health Center (646-1512).</p>
<p>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 BSO.</p>	

SECTION VIII: PRINCIPAL INVESTIGATOR STATEMENT

Each PI must forward a signed hard copy "[Principal Investigator Statement](#)" via campus mail to EH&S MSC 3578.

Has a signed hard copy Principal Investigator statement been submitted to EH&S? Yes  No

SECTION IX: IBC APPROVAL

*The content of this signed form represents the final application reviewed and approved by the NMSU IBC.*

---

John D. Kemp, Ph.D., IBC Chair 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

NMSU INSTITUTIONAL BIOSAFETY COMMITTEE  
 c/o Environmental Health & Safety  
 MSC-3578, Academic Research Ctr. Unit C 4200 Research Drive  
 Phone: 505.646.3327 Fax: 505.646.7898



### NMSU IBC ACTIVITY MODIFICATION REPORT Must be filed within 60 days of change.

NAME OF PRINCIPAL INVESTIGATOR: \_\_\_\_\_

APPLICATION NO: \_\_\_\_\_

APPLICATION TITLE: \_\_\_\_\_

RESEARCH TYPE:(check all that apply)  Cell Culture  Molecular Biology (DNA)  rDNA (animal or plant)  
 Environmental samples  Animal husbandry  Wildlife/Arthropods  Plant (Greenhouse)  
 Plant (Field)  Other: \_\_\_\_\_

**Minor Modifications:**

- Personnel added \_\_\_\_\_  
(List new personnel & attach training and experience record form )
- Personnel terminated. (List personnel no longer involved) \_\_\_\_\_
- Laboratory is being renovated and the project is suspended until completion of renovation. BSO must inspect renovated BSL-2 and BSL-3 laboratories. Post-renovation inspection by BSO required.
- Laboratory is moving to another floor or building. BSO inspection of new lab space required.
- Please identify new location. \_\_\_\_\_
- Project is voluntarily suspended (due to PI sabbatical, or is awaiting funding, or minor lab renovation).
- Project no longer uses live animals.
- Project no longer involves use of potentially infectious agents or pathogens.
- Project no longer involves use of recombinant DNA.
- Project is no longer active or funded.
- Other: \_\_\_\_\_

**Major Modifications**

- 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
- Substantial changes in the IBC-approved procedures (new technology or novel rDNA construct) or initial acquisition of new organisms or toxins. Submit description of changes to BSO. IBC Chair will review on a case-by-case basis. A new IBC application may or may not be required.
- Other: \_\_\_\_\_

\_\_\_\_\_  
 PI Signature

\_\_\_\_\_  
 Date

## APPENDIX C



EH&S Biosafety  
Annual Laboratory  
Survey Form  
646-3327

Survey Date: \_\_\_\_\_  
 Name of Surveyor: \_\_\_\_\_  
 Laboratory Contact: \_\_\_\_\_  
 PI  Tech  Bldg. Monitor  Other

PI Name:	Phone:
Office Bldg & Room:	Fax:
Dept:	PI Email:
Lab Bldg.:	Application No.:
Lab Room(s):	Expiration Date:
Project Title:	Facility: Practices:

### STANDARD OPERATING PROCEDURES

1. Laboratory Safety Plan describing routine safety practices and an Emergency Response/Action Plan describing what to do in the event of a natural disaster (flood, earthquake) and unnatural disasters (fire, building system failure) are available for review.  Y  N  N/A

Comment: \_\_\_\_\_

2. Access to the laboratory is controlled (for experiments & other times).  Y  N  N/A

Comment: \_\_\_\_\_

3. Eating, drinking, handling contact lenses, and storing food items in the lab is prohibited.  Y  N  N/A

Comment: \_\_\_\_\_

4. Personnel wash their hands after handling viable materials, after removing gloves, and before leaving the lab.  Y  N  N/A

Comment: \_\_\_\_\_

5. Mechanical pipetting devices are used; mouth pipetting is prohibited.  Y  N  N/A

Comment: \_\_\_\_\_

6. Safe sharps handling and disposal procedures are practiced.  Y  N  N/A

Comment: \_\_\_\_\_

7. Work surfaces are decontaminated at least daily & after spills.  Y  N  N/A

Comment: \_\_\_\_\_

8. Cultures, stocks and regulated waste are decontaminated prior to disposal.  Y  N  N/A

Comment: \_\_\_\_\_

9. Materials that are to be decontaminated outside the immediate laboratory are placed in durable, leak proof containers for transport.  Y  N  N/A

Comment: \_\_\_\_\_

10. Decontaminated materials are packaged for disposal off-site (red bag biohazard box or similar).  Y  N  N/A

Comment: \_\_\_\_\_

11. A biohazard sign is posted at the entrance to the laboratory, and includes emergency contact information for PI & alternate.  Y  N  N/A  
 Comment: \_\_\_\_\_
12. Lab coats, gowns or other coverings are available to prevent contamination of street clothes.  Y  N  N/A  
 Comment: \_\_\_\_\_
13. Gloves are removed prior to exiting the laboratory, when opening doors, using telephone, and other "common" non-lab items.  Y  N  N/A  
 Comment: \_\_\_\_\_
14. Sink, hand soap, and paper towels are available for hand washing.  Y  N  N/A  
 Comment: \_\_\_\_\_
15. Eyewash stations are available and operating properly.  Y  N  N/A  
 Comment: \_\_\_\_\_
16. Only persons who have been advised of the hazards and meet lab-specific training requirements may enter the laboratory.  Y  N  N/A  
 Comment: \_\_\_\_\_
17. Standard Operating Procedures incorporate biosafety procedures & staff *read* and *follow* these procedures.  Y  N  N/A  
 Comment: \_\_\_\_\_
18. Laboratory Director ensures that all laboratory and support personnel have been trained with respect to the potential hazards associated with the work, with annual updates or more frequently as needed.  Y  N  N/A  
 Comment: \_\_\_\_\_
19. Cultures, tissues, & specimens are placed in leak-proof containers for transport.  Y  N  N/A  
 Comment: \_\_\_\_\_
20. Decontamination of contaminated equipment that must be shipped, repaired or disposed of off-site is documented.  Y  N  N/A  
 Comment: \_\_\_\_\_
21. Biological Safety Cabinets are properly located, & certified annually.  Y  N  N/A  
 Comment: \_\_\_\_\_
22. Face protection (thermal gloves, face shields, splash guards) are available & used when working with liquid nitrogen and other splash hazards.  Y  N  N/A  
 Comment: \_\_\_\_\_

23. Proper PPE (lab coats, gowns, smocks, gloves, coveralls) used is removed when experiment is completed.  Y  N  N/A

Comment: \_\_\_\_\_

24. All propagation of viable potentially infectious organisms is conducted in certified BSC & viable potentially infectious organisms are not manipulated on the open bench. (required for IBC-approved BSL-2)  Y  N  N/A

Comment: \_\_\_\_\_

25. All waste, unwanted cultures, specimens, tissues blood specimens are decontaminated *within* the lab using an autoclave that is routinely tested and certified for effectiveness.  Y  N  N/A

Comment: \_\_\_\_\_

26. Vacuum lines are protected with liquid traps and/or HEPA filters.  Y  N  N/A

Comment: \_\_\_\_\_

27. Each incident of injury, spill, or exposure is immediately reported to the Principal Investigator and to the Biosafety Officer.  Y  N  N/A

Comment: \_\_\_\_\_

28. Were there any work-related injuries in your lab during the past year?  Y  N  N/A A

Comment: \_\_\_\_\_

### Facility & Equipment

The laboratory is negatively pressured with respect to the surrounding corridor.  Yes  No

Autoclave:  Yes  No Mfr & Model: \_\_\_\_\_ Room #: \_\_\_\_\_

Biological Safety Cabinet (BSC):  Yes  No Clean Air Bench  Yes  No

Test Report On File:  Yes  No

Mfr: \_\_\_\_\_ Model: \_\_\_\_\_ Serial No.: \_\_\_\_\_ Room No: \_\_\_\_\_

Certification Date: \_\_\_\_\_ Certification Company:  ENV Services,  Other \_\_\_\_\_

Mfr: \_\_\_\_\_ Model: \_\_\_\_\_ Serial No.: \_\_\_\_\_ Room No: \_\_\_\_\_

Certification Date: \_\_\_\_\_ Certification Company:  ENV Services,  Other \_\_\_\_\_

Centrifuge:  Yes  No

[Microfuge:  Tabletop:  Floor Super speed:  Ultra: ]

Chemical Fume Exhaust Hood:.....  Yes  No Tested w/in the year:  Yes  No

Eye wash station located w/in 50 ft.:  Yes  No Testing frequency: weekly , monthly , semi-annually

Fire Extinguisher: .....  Yes  No Tested w/in the year:  Yes  No

Liquid Nitrogen Storage:.....  Yes  No Shared tank.....  Yes  No

Refrigerator:.....  Yes  No Freezer:.....  Yes  No

## APPENDIX D

### PROTECTIVE GLOVE USE POLICY

Memorandum

March 24, 2004

To: NMSU Faculty, Staff & Students

From: John T. Balog, Program Manager, Biosafety Officer  
Environmental Health & Safety

c: John Kemp, Ph.D., Chair, NMSU Institutional Biosafety Committee

Subject: Glove Use Policy

It is the policy of the NMSU Institutional Biosafety Committee and EH&S 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, 4<sup>th</sup> 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 EH&S at 505.646.3327 with questions.

## APPENDIX E ACRONYMS

APHIS	Animal & Plant Health Inspection Service, USDA
ARS	Agricultural Research Service, USDA
ATCC	American Type Culture Collection
BBP	Bloodborne Pathogen
BI	Biological Indicator
BIS	Bureau of Industry Security, U. S. Dept. of Commerce
BSL	Biosafety Level
BSC	Biological Safety Cabinet
BSO	Biosafety Officer
CAB	Clean Air Bench
CAR	Customer Acceptance of Responsibility, ATCC Form 62
CI	Chemical Indicator
CFM	Cubic Feet per Minute
CFU	Colony Forming Unit
CSREES	Cooperative State Research, Education, and Extension Service, USDA
DGR	Dangerous Goods Regulations, IATA
DOT	U.S. Department of Transportation
EH&S	Environmental Health & Safety, NMSU
EPA	Environmental Protection Agency
FAA	Federal Aviation Administration, U. S. Dept. of Transportation
FMCSA	Federal Motor Carrier Safety Administration, U. S. Dept. of Transportation
GMO	Genetically Modified Organism
HEPA	High Efficiency Particulate Air
HHS	U.S. Department of Health & Human Services
HMR	Hazardous Materials Regulations, U. S. Dept of Transportation
IACUC	Institutional Animal Care & Use Committee, NMSU
IATA	International Air Transport Association, an airline industry group.
IBC	Institutional Biosafety Committee, NMSU
IRB	Institutional Review Board, NMSU
LAF	Laminar Air Flow
lfm	Linear Feet per Minute
NIH	National Institutes of Health
NSF	National Sanitation Foundation International
MTA	Material Transfer Agreement, Vendors & other sources of research materials
OBA	Office of Biotechnology Activities, NIH
OFS	Office of Facilities and Services (formerly PPD), NMSU
OLAW	Office of Laboratory Animal Welfare, NIH
OSHA	Occupational Safety & Health Administration, U. S. Dept. of Labor
PPQ	Plant Protection & Quarantine, USDA
RSC	Radiation Safety Committee, NMSU
RSPA	Research and Special Programs Administration, U. S. Dept. of Labor
USDA	United States Department of Agriculture
μl	Micro liter
VS	Veterinary Services, USDA

## **APPENDIX F INTERNET SHIPPING RESOURCES**

1. Airborne Express Hazardous Materials Shipments:  
<http://www.dhl-usa.com/USSvcs/detail/USSvcsDetai.asp?nav=USServices/USShipping/SpecialServices/Haz>
2. CDC Office of Health & Safety  
<http://www.cdc.gov/od/ohs/biosfty/shipdir.htm>
3. DOT Hazardous Materials Regulations:  
<http://www.myregs.com/dotrspa>
4. FedEx Hazardous Materials Shipments:  
<http://www.fedex.com/us/services/ground/addservopt/hazmat/index.html>
5. International Air Transport Association  
<http://www.iata.org/index>
6. United Parcel Service Hazardous Materials Guide:  
<http://www.ups.com/content/us/en/resources/prepare/hazardous/>
7. U.S. Postal Service Hazardous, Restricted, and Perishable Mail:  
<http://www.usps.com/cpim/ftp/pubs/pub52.pdf>
8. WHO Transport of Infectious Substances  
[http://www.who.int/csr/resources/publications/WHO\\_CDS\\_CSR\\_LYO\\_2004\\_9/en/](http://www.who.int/csr/resources/publications/WHO_CDS_CSR_LYO_2004_9/en/)

**APPENDIX G**  
**NEW MEXICO STATE UNIVERSITY (NMSU)**  
**INSTITUTIONAL BIOSAFETY COMMITTEE (IBC)**  
**OPERATING CHARTER 11-19-03**

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 regulatory agencies include but are not limited to:

- Health & Human Services (HHS) Center for Disease Control (CDC)  
<http://www.cdc.gov/od/ohs/biosfty/biosfty.htm> United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS)  
<http://www.aphis.usda.gov/>
- United States Department of Agriculture (USDA) 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).  
[http://www.nmenv.state.nm.us/OHSB\\_website/ohsb\\_home.htm](http://www.nmenv.state.nm.us/OHSB_website/ohsb_home.htm)
- National Institutes of Health (NIH) Recombinant DNA Guidelines (Guidelines) NIH Guidelines April 2002

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 Biosafety Officer 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 4th Edition), or **2.** NIH as risk group (RG) 2-4 agent (NIH Guidelines April 2002) (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). Also see the NACUA Agent and Toxin List as compiled by the National Association of College and University Attorneys (NACUA), as a summary of all of the lists

## **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 RESPONSIBILITIES 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) (NIH Guidelines April 2002)
  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). (NIH Guidelines April 2002)
  5. any possession, use, or transfer of HHS Select Agents and Toxins (42 CFR Part 73) (Select Agents Program), or USDA Biological Agents & Toxins (9 CFR Part 121) or listed Plant Pathogens (7 CFR Part 331) (Federal Register 9CFR 121 7CFR 331)
- The IBC will ensure that to the fullest extent practical, that 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 DNA 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 (EH&S) 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 Provost for Research.
- 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 Provost for Research 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 Provost for Research upon recommendation from but not limited to the Director of EH&S and the IBC Chair.
- The IBC Chair is appointed by the Vice Provost for Research and serves as the link between the Office of the Vice Provost 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:
    - o recombinant DNA technology
    - o molecular biology
    - o biological safety
    - o public health and epidemiology
    - o virology
    - o microbiology
    - o infectious diseases
    - o animal scientist
    - o plant pathogen or plant pest containment principles
    - o laboratory technician/non-doctoral
    - o 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 principals 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:
    - o officials of state or local public health or environmental protection agencies
    - o 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, Employee Health Services, Research Administration, University Council, Office of 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 Provost for Research 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 EH&S will make a recommendation for renewal of membership on the committee to the Vice Provost for Research.
- The IBC Chair is a continuous appointment by the Vice Provost for Research, with an annual confirmation from the committee to the Vice Provost for Research.
- The BSO is a continuous position appointment. The BSO is a professional position which reports to the EH&S Director.

## 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 not involve recombinant DNA, do not require review by the IBC.
    - o 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) (Select Agents Program), 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) (Federal Register 9CFR 121 7CFR 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 ([NIH Guidelines April 2002](#)) 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 Provost for Research 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 Director of EH&S and provides the necessary administrative support for the functions and business of the IBC.

### ADDITIONAL DEFINITIONS

- 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) 4th Edition. ([BMBL 4th 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 humans, (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. NIH recombinant DNA Guidelines Section II-I-A, and Appendix B. ([NIH Guidelines--Table of Contents](#)[NIH Guidelines April 2002](#))
- 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 Recombinant DNA Guidelines) when relevant.

## APPENDIX H REFERENCES

1. Environmental Health & Safety at NMSU, Effective Date November 1, 2000  
[http://www.nmsu.edu/safety/policies/policy\\_university\\_eh&s.htm#TABLE%20OF%20CONTENT](http://www.nmsu.edu/safety/policies/policy_university_eh&s.htm#TABLE%20OF%20CONTENT)
2. NMSU Administrative Policy and Procedure Manual  
<http://www.nmsu.edu/manual/chapter02/2.60.html>
3. NIH Guidelines for Research Involving Recombinant DNA Molecules in Research, April 2002 <http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html>
4. Laboratory Safety Monograph, A Supplement to the NIH Guidelines for Recombinant DNA Research January 1979, Out of print.
5. Information Systems for Biotechnology, Virginia Tech, 2001  
[http://www.isb.vt.edu/cfdocs/greenhouse\\_manual.cfm](http://www.isb.vt.edu/cfdocs/greenhouse_manual.cfm)
6. United States Environmental Protection Agency Terminology Reference system;  
<http://www.epa.gov/trs/>
7. Biosafety in Microbiological and Biomedical Laboratories, 4<sup>th</sup> Edition, May 1999  
<http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm>
8. National Sanitation Foundation International  
[http://www.nsf.org/business/biosafety\\_cabinetry/standards.asp?program=BiosafetyCab](http://www.nsf.org/business/biosafety_cabinetry/standards.asp?program=BiosafetyCab)
9. Title 20, Environmental Protection, Chapter 9 Solid Waste, Part 1, Solid Waste Management [http://www.nmenv.state.nm.us/swb/REG\\_REV6.F22word.doc](http://www.nmenv.state.nm.us/swb/REG_REV6.F22word.doc)
10. 49 Code of Federal Regulations Parts 172, 173 & 176  
[http://www.access.gpo.gov/nara/cfr/waisidx\\_03/49cfrv2\\_03.html](http://www.access.gpo.gov/nara/cfr/waisidx_03/49cfrv2_03.html)
11. Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets: <http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm>
12. NM Department of Game & Fish:  
[http://www.wildlife.state.nm.us/apps\\_permit/index.htm](http://www.wildlife.state.nm.us/apps_permit/index.htm)
13. USDA Veterinary Services Permits [http://www.aphis.usda.gov/vs/import\\_export.htm](http://www.aphis.usda.gov/vs/import_export.htm)
14. CDC Permit to Import or Transport Etiologic Agents, Hosts, or Vectors of Human Disease <http://www.cdc.gov/od/ohs/biosfty/0753.pdf>

**APPENDIX I**  
**COMBINED LIST OF HHS AND USDA SELECT AGENTS AND TOXINS**  
**7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73**

Abrin  
 Cercopithecine herpesvirus 1 (Herpes B virus)  
*Coccidioides posadasii*  
 Conotoxins  
 Crimean-Congo haemorrhagic fever virus  
 Diacetoxyscirpenol  
 Ebola viruses  
 Lassa fever virus  
 Marburg virus  
 Monkeypox virus  
 Ricin  
*Rickettsia prowazekii*  
*Rickettsia rickettsii*  
 Saxitoxin  
 Shiga-like ribosome inactivating proteins  
 South American Haemorrhagic Fever viruses  
 Flexal  
 Guanarito  
 Junin  
 Machupo  
 Sabia  
 Tetrodotoxin  
 Tick-borne encephalitis complex (flavi) viruses  
 Central European Tick-borne encephalitis  
 Far Eastern Tick-borne encephalitis  
 Kyasanur Forest Disease  
 Omsk Hemorrhagic Fever  
 Russian Spring and Summer encephalitis  
**QUARANTINE**  
 Variola major virus (Smallpox virus)  
 Variola minor virus (Alastrim)  
*Yersinia pestis*

**OVERLAP SELECT AGENTS AND TOXINS**

*Bacillus anthracis*  
 Botulinum neurotoxins  
 Botulinum neurotoxin producing species of *Clostridium*  
*Brucella abortus*  
*Brucella suis*  
*Burkholderia mallei* (formerly *Pseudomonas mallei*)  
*Burkholderia pseudomallei* (formerly *Pseudomonas pseudomallei*)  
*Clostridium perfringens* epsilon toxin  
*Coccidioides immitis*  
*Coxiella burnetii*  
 Eastern Equine Encephalitis virus  
*Francisella tularensis*  
 Hendra virus  
 Nipah virus  
 Rift Valley fever virus  
 Shigatoxin  
 Staphylococcal enterotoxins  
 T-2 toxin  
 Venezuelan Equine Encephalitis virus

African horse sickness virus  
 African swine fever virus  
 Akabane virus  
 Avian influenza virus (highly pathogenic)  
 Bluetongue virus (Exotic)  
 Bovine spongiform encephalopathy agent  
 Camel pox virus  
 Classical swine fever virus  
*Cowdria ruminantium* (Heartwater)  
 Foot-and-mouth disease virus  
 Goat pox virus  
 Japanese encephalitis virus  
 Lumpy skin disease virus  
 Malignant catarrhal fever virus  
 (Alcelaphine herpesvirus type 1)  
 Menangle virus  
*Mycoplasma capricolum*/M.F38/*M. mycoides capri*  
 (contagious caprine pleuropneumonia)  
*Mycoplasma mycoides mycoides*  
 (contagious bovine pleuropneumonia)  
 Newcastle disease virus (velogenic)  
 Peste des petits ruminants virus  
 Rinderpest virus  
 Sheep pox virus  
 Swine vesicular disease virus  
 Vesicular stomatitis virus (Exotic)

**USDA PLANT PROTECTION AND**

**SELECT AGENTS AND TOXINS**

*Candidatus Liberobacter africanus*  
*Candidatus Liberobacter asiaticus*  
*Peronosclerospora philippinensis*  
*Ralstonia solanacearum* race 3, biovar 2  
*Schlerophthora rayssiae* var *zeae*  
*Synchytrium endobioticum*  
*Xanthomonas oryzae* pv. *oryzicola*  
*Xylella fastidiosa* *Brucella melitensis*  
 (citrus variegated chlorosis strain)

Current as of 11-23-05

## **APPENDIX J**

### **OVERVIEW OF NMSU INTEGRATED PEST MANAGEMENT PROGRAM**

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. The Office of Facilities and Services (OF&S) maintains an IPM Program in the Landscape Management and Restoration Department that is compliant with EH&S Policy on Pesticide use. The use of pesticides at NMSU is subject to the OSHA Hazard Communication and Material Safety Data Sheets (MSDS) are readily available in the Landscape Management and Restoration Department (646-5957). 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.

Building occupants may report pest infestations to the Landscape Management and Restoration Department. Indoor applications are routinely scheduled when the building or room is unoccupied. Personnel are notified in advance and requested to vacate the building or room that is to be treated with pesticide. Only food-safe pesticides are used inside of buildings. Residues that may settle on work surfaces are inactivated and cleaned prior to re-occupancy.