UNLV
UNIVERSITY OF NEVADA LAS VEGAS

Institutional Biosafety Manual

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Executive Summary

The mission of the Division of Research and Graduate Studies is to create an environment which is conducive to performing superior research and initiating innovative scholarly pursuits. Our entire team is dedicated to providing university faculty, staff, and students with the support necessary to fulfill UNLV's goal of becoming a nationally recognized research institution. It is our intent to help "open the doors" to research for all who wish to recognize their full creative and intellectual potential.

In support of this mission, it is the goal and philosophy of the Institutional Biosafety Committee (IBC) to facilitate legitimate scientific investigations using recombinant DNA, biohazardous and infectious materials. This manual is intended to enable such investigations by summarizing the best practices, precautions and facilities that are necessary to safely handle and use these materials.

Additionally, investigators are encouraged to have open discussions with the UNLV campus Biosafety Officer and the IBC. This will serve the dual purpose of assuring regulatory compliance and ensuring appropriate specific precautions are utilized.

Finally, it is the intent of the UNLV IBC that this manual be viewed as a living document that can be amended as appropriate to continually improve the procedures needed for the safe use of biological material at UNLV.

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Date

Ron Smith, Ph.D.
Vice President for Research and Graduate Studies

_____ _____
Date

Ronald Gary, Ph.D.
Chair, Institutional Biosafety Committee

_____ _____
Date

Gary Snodgrass
Biosafety Officer

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I. Introduction

The University of Nevada Las Vegas (UNLV) is actively committed to preserving the health and safety of our faculty, staff and students. As part of this commitment, this biosafety manual was developed to serve as a guide and resource for faculty, staff and students to understand internal policies and procedures related to biological safety. The content of this manual includes UNLV policies and procedures, current regulatory guidance and best management safety practices for work with potentially hazardous biological materials including infectious agents, recombinant DNA, human derived materials and biologically derived toxins.

When followed, the procedures in this manual will provide an increased level of protection for laboratory workers as well as allow compliance with regulatory entities concerned with biological safety.

Most biological laboratories contain chemicals and may contain radiological materials. This manual was designed with a focus on biological agents; for specific guidance on chemicals or radiological hazards consult the UNLV Chemical Hygiene Plan and UNLV Radiation Safety Manual, respectively.

This manual is applicable to all UNLV faculty, staff and students as well as research performed at any of the UNLV facilities by non-affiliated persons. It shall serve as policy for all work involving potentially hazardous biological materials.

A. Definitions

Biosafety:
Development and implementation of administrative policy, work practices, facility design, and safety equipment to prevent transmission of biological agents to workers, other persons, and the environment.

Biosafety Level (BSL):
A description of the degree of physical containment being employed to confine microorganisms and to reduce the potential for exposure of laboratory workers, persons outside of the laboratory, and the environment. In Appendix G of the NIH Guidelines, these are graded from BSL-1 (the least stringent) to BSL-4 (the most stringent). These levels are also detailed extensively in the BMBL.

Biological Safety Officer (BSO):
An individual appointed by an institution to oversee management of biosafety risks. The NIH Guidelines require that a BSO be appointed when the institution is engaged in large-scale research or production activities, or in research requiring containment at BSL-3 or BSL-4. The duties of the UNLV BSO are described in this Manual.

Biosafety in Microbiological and Biomedical Laboratories (BMBL):
A document published by the CDC and NIH detailing recommendations for work with a variety of infectious agents in various laboratory settings. It describes combinations of standard and special microbiological practices, safety equipment, and facilities constituting BSL1-4, as well as giving recommendations for various specific infectious agents.

Biosecurity:
Protection of high-consequence microbial agents and toxins (or critical information pertaining to those agents or toxins) against theft or diversion by those who intend to misuse them.

Bloodborne Pathogens:
Pathogenic microorganisms that are present in human blood and can cause disease in humans. These pathogens include, but are not limited to, hepatitis B virus (HBV) and human immunodeficiency virus (HIV).

Contaminated:
The presence or the reasonably anticipated presence of blood or other potentially infectious materials on an item or surface.

Decontamination:
The use of physical or chemical means to remove, inactivate, or destroy pathogens (or other microorganisms) on a surface or item to the point where they are no longer capable of transmitting infectious particles. This process renders the surface or item safe for handling, use, or disposal.

Institution:
In the context of the NIH Guidelines, an institution is any public or private entity, including federal, state, and local governments.

Institutional Biosafety Committee (IBC):
An institutional committee created according to NIH Guidelines to review research involving recombinant DNA. The role of IBCs has evolved over time, and many committees also review other forms of research that entail biohazardous risks as part of their institutionally assigned responsibilities.

NIH Guidelines for Research Involving Recombinant DNA Molecules (National Institutes of Health Guidelines):
A document created in 1976 that outlines principles for the safe conduct of research employing recombinant DNA technology. The NIH Guidelines detail practices and procedures for the containment of various forms of recombinant DNA research, for the proper conduct of research involving genetically modified plants and animals, and for the safe conduct of human gene transfer research. As a "living" document, it is periodically revised to keep pace with the changing state of science.

Office of Biotechnology Activities (OBA):
The NIH office responsible for developing, implementing, and monitoring NIH policies and procedures for the safe conduct of recombinant DNA activities, including human gene transfer.

Personal Protective Equipment (PPE):

Specialized clothing or equipment worn by an employee for protection against a hazard. General work clothes (e.g., uniforms, pants, shirts, or blouses) that are not intended to function as protection against a hazard are not considered to be personal protective equipment.

Recombinant DNA Advisory Committee (RAC):
An NIH advisory committee whose principal role is to provide advice and recommendations to the NIH Director on 1) the conduct and oversight of research involving recombinant DNA, including the content and implementation of the NIH Guidelines, and 2) other NIH activities pertinent to recombinant DNA technology. A major element of the committee's role is to examine the science, safety, and ethics of clinical trials that involve the transfer of recombinant DNA to humans. More details about RAC membership and responsibilities can be found on the RAC page of the OBA Website, as well as in its Charter.

Recombinant DNA molecules:
According to current NIH Guidelines, molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or molecules that result from their replication.

Responsible Official:
A facility official who has been designated the responsibility and authority to ensure that the requirements of Title 42, Code of Federal Regulation, Part 73, are met.

Select agent:
Specifically regulated pathogens and toxins as defined in Title 42, Code of Federal Regulation, Part 73, including pathogens and toxins regulated by both Department of Health and Human Services and U.S. Department of Agriculture (i.e., overlapping agents or toxins).

Sterilize:
The use of a physical or chemical procedure to destroy all microbial life, including highly resistant bacterial endospores.

Universal Precautions:
An approach to infection control. According to the concept of Universal Precautions, all human blood and certain human body fluids are treated as if known to be infectious for HIV, HBV, and other bloodborne pathogens.

B. Abbreviations

ATCC – American Type Culture Collection

BI – Biological Indicators

BMBL – *Biosafety in Microbiological and Biomedical Laboratories*

BSC – Biological Safety Cabinet

BSL – Biological Safety Level

BSO – UNLV Biological Safety Officer

CDC – United States Centers for Disease Control and Prevention

CFR – Code of Federal Regulations

DNA – Deoxyribonucleic Acid

DOT – United States Department of Transportation

EBV – Epstein-Barr Virus

HBV – Hepatitis B Virus

HCV – Hepatitis C Virus

HEPA – High Efficiency Particulate Air (filter)

HHS – United States Department of Health and Human Services

HIV – Human Immunodeficiency Virus

HPV – Human Papillomavirus

IACUC – UNLV Institutional Animal Care and Use Committee

IATA - International Air Transport Association

ICAO - International Civil Aviation Organization

IBC – UNLV Institutional Biosafety Committee

IRB – UNLV Institutional Review Board

MSDS – Material Safety Data Sheet

NIH – National Institutes of Health

OPIM – Other Potentially Infectious Materials

OSHA - Occupational Safety and Health Administration

PHS – United States Public Health Service

PI – Principal Investigator

PPE – Personal Protective Equipment

SOPs – Standard Operating Procedures

UNLV – University of Nevada Las Vegas

USDA – United States Department of Agriculture

USPS - United States Postal Service

II. Responsibilities

Biosafety at UNLV is a critical element to advancing our University and protecting our personnel and therefore is a coordinated effort between the Division of Research and Graduate Studies, the IBC, the BSO, Principal Investigators and staff. To be most effective, all members involved in this critical program must understand their roles and responsibilities. UNLV biosafety responsibilities are delineated in this section.

A. Division of Research and Graduate Studies

The Vice President for Research and Graduate Studies is ultimately responsible for the oversight of biological research activities. The Vice President for Research and Graduate Studies has responsibility for ensuring that research is conducted in full conformity with the provisions of the Biosafety manual and all other University policies and procedures. Federal, state, and local statutes, codes and regulations. The Vice President for Research and Graduate Studies is ultimately responsible for:

- Promoting the importance of safety in all research activities;
- Supporting the laboratory safety programs that protect all UNLV faculty, staff, students and our laboratory visitors;
- Appointing an Institutional Biosafety Committee which works closely with the Associate Vice President for Research and Graduate Studies to develop and effectively implement biosafety at UNLV;
- Supporting the provisions of this document for facilities working with biologically hazardous materials.

B. Institutional Biosafety Committee

The Institutional Biosafety Committee (IBC) is responsible for overseeing the effective operation, policies, and compliance of the conduct of biological research at UNLV. The IBC also:

- Has specific duties as outlined in the UNLV IBC Policies and Procedures, in addition to the general oversight responsibilities;
- Has responsibility for investigation of activities or accidents that violate UNLV programs and/or are a health threat to faculty, staff, students or the community.
- Reports unresolved or significant findings to the Vice President for Research and Graduate Studies.

C. Biosafety Officer

The Biosafety Officer (BSO) serves as the campus expert for biological safety and is responsible for providing guidance for all aspects of the biosafety program at UNLV. Specific duties include:

- Providing regular biosafety training to all faculty, staff, students and members of the IBC;
- Reviewing all research protocols submitted to the IBC and providing technical guidance;
- Preparing the Biosafety Manual and revising as necessary;
- Distributing the Biosafety Manual to any faculty member working with biological materials;

- Conducting laboratory visits to provide biosafety guidance and monitor compliance with the UNLV Biosafety Manual;
- Investigating accidents involving infectious agents;
- Informing the IBC of pertinent biosafety information and program administration issues;
- Providing guidance on purchase of biological safety equipment including biological safety cabinets;
- Reviewing plans for new laboratories and renovations, and providing recommendations for laboratory ventilation and laboratory design;
- Providing consultation for shipping infectious agents;
- Providing support for clean-up and decontamination of biological materials.

D. Deans/Department Chairs

Deans/Department Chairs are responsible for the implementation of safe practices and procedures in their schools or departments. They are responsible for:

- Promoting a positive safety culture in their school/department;
- Ensuring that their school/department's activities are compliant with relevant research safety policies, regulations, laws and guidelines;
- Ensuring that all faculty, staff and students in their purview have had appropriate biological safety instruction.

E. Principal Investigators

Principal Investigators are directly and primarily responsible for the safe conduct of research in their laboratory. They provide operational oversight for all activities and staff in the laboratory and assure compliance. Specific duties include, but are not limited to:

- Creating a safety culture in the laboratory that is positive and encourages open discussion of biosafety concerns, problems or violations of procedure;
- Completing laboratory specific SOPs (Appendixes A-C) for their level of research and ensuring that all laboratory staff are knowledgeable in the biosafety SOPs;
- Maintaining and making available to their staff a copy of the UNLV Biosafety Manual;
- Ensuring that all laboratory staff, maintenance personnel, and laboratory visitors have been appraised of the biological risks present in the laboratory;
- Registering all necessary projects (including recombinant DNA; human infectious agent research; research involving human blood, cells, tissues and other potentially infectious human materials (OPIM), research involving biological toxins; and research involving select agents) with the UNLV IBC;
- Not initiating or modifying the above listed projects without prior approval of the IBC;
- Assuring that personnel working with hazardous biological materials are adequately experienced and trained for the safe handling of such materials. Training must be documented and retained with this manual for at least 3 years after the employee leaves that laboratory;

- Immediately reporting to the BSO any significant violations of the biosafety policies and procedures or any potential exposure to hazardous biological materials.

F. Staff

Staff is defined for the purposes of this manual as any person who works in the laboratory in a technical (rather than purely administrative) capacity. This includes, but is not limited to, faculty, professional and classified staff members, graduate assistants, students, interns, visiting scholars and volunteers. Specific duties under this policy include, but are not limited to:

- Following all laboratory and UNLV biosafety and security practices and procedures;
- Reading the entire laboratory customized UNLV Biosafety Manual and asking for assistance understanding any portions that are not comprehended;
- Reviewing and familiarizing themselves with all protocols and organisms used in the laboratory regardless of whether they are working directly with the organism;
- Completing all required safety training;
- Knowing all emergency and spill response procedures established by the Principal Investigator or supervisor;
- Reporting to the Principal Investigator or supervisor all problems, violations in procedure, exposure events or spills as soon as they occur.

III. Adopted Reference

Since its initial publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) published by NIH/CDC, it has been recognized by most in the Biosafety field as the text that contains the most appropriate, accurate and detailed information on biological safety in the United States. Therefore, UNLV has adopted it for the main body of our biosafety manual. The BMBL contains information about:

- The history of biosafety and detail about the need for a biosafety program;
- Biological risk assessment;
- Principles of biosafety;
- Laboratory biosafety level criteria;
- Recommended biosafety levels for infectious agents;
- Vertebrate animal biosafety level criteria, for vivarium research facilities;
- Recommended biosafety levels for activities in which experimentally or naturally infected vertebrate animals are used;
- Principle of laboratory biosecurity;
- Occupational health and immunoprophylaxis;
- Agent summary statements;
- Bacterial agents;
- Fungal agents;
- Parasitic agents;
- Rickettsial agents;
- Viral agents;
- Arboviruses and related zoonotic viruses;
- Alphabetic listing of 597 arboviruses and hemorrhagic fever;
- Toxin agents;
- Prion diseases;
- Primary containment for biohazards: selection, installation and use of biological safety cabinets;
- Decontamination and disinfection;
- Transportation of infectious substances;
- Agriculture pathogen biosafety;
- Arthropod containment guidelines;
- Select agents and toxins;
- Integrated Pest Management;
- Working with human, non-human primate and other mammalian cells and tissues;
- Guidelines for work with toxins of biological origin;
- NIH oversight of research involving recombinant biosafety issues.

The latest version of the BMBL is available in hyperlinked format on the CDC Office of Health and Safety Website at http://www.cdc.gov/od/ohs/. All employees working with potentially hazardous biological agents are expected to familiarize themselves with the BMBL. It is highly recommended for BSL-2 and above laboratories that you print the latest version of the BMBL and insert it into this section for reference for your laboratory staff. Should you need assistance on interpretation of the BMBL please contact the UNLV Biosafety Officer.

IV. Institutional Biosafety Committee

The Institutional Biosafety Committee ensures that all potentially hazardous biological materials utilized at UNLV facilities or by UNLV faculty and staff are given appropriate review and oversight. The IBC also encourages safe biological research for the protection of employees and students at UNLV. To fulfill these aims the UNLV IBC Policies and Procedures (Appendix D) have been developed.
These Policies and Procedures are applicable if you work with:

- Recombinant DNA;
- Human infectious agent research;
- Research involving human blood, cells, tissues and other potentially infectious human materials (OPIM),
- Research involving biological toxins; or
- Research involving select agents.

It is the Principal Investigators' responsibility to register all of these materials with the IBC as described in Appendix D.

V. Biohazardous Waste

Biohazardous waste includes laboratory and clinical materials that are known or suspected of containing biological agents that could pose a risk to human health. Also included as biohazardous waste are materials of human origin including blood, cells, tissues and all sharps used in medical or dental procedures.

A. Types of Biohazardous Waste

Biohazardous waste includes, but is not limited to:

- Human blood, blood products, body fluids, cell and tissue culture;
- Organisms with recombinant DNA;
- Cultures and stocks of infectious agents;
- Specimens from medical, pathological and research laboratories;
- Disposable culture/Petri dishes;
- Devices used to transfer, inoculate and mix cultures;
- Wastes from the production of biological material;
- Discarded live and attenuated vaccines;
- Toxins;
- Potentially infectious bacteria, viruses, fungi and spores;
- Animal carcasses, body parts and bedding from animals exposed to pathogens in research;
- All sharps (contaminated and uncontaminated) including needles and syringes, scalpels, razors, microtome blades, glass pipettes, slides and cover slips, plastic pipettes, small plastic pipette tips, and broken glass; and
- Non-sharps that have come into contact with the aforementioned waste stream.

B. Proper Waste Management

Waste must be segregated at the point of origin by the generator. Culture plates and vials containing pathogenic organisms must be autoclaved with a biological indicator prior to disposal (see section E below) using autoclave safe bags (orange, red or clear). Clear bags have been approved to reduce the public concern if a sterilized bag is found in a dumpster or landfill.

Biohazard waste containers must be rigid and leak-proof (no cardboard boxes) with a tight fitting lid. The containers may be any color, but they must be labeled with either the words "Biohazardous Waste" or a biohazard symbol and the word "Biohazard". The labels must be placed on both the lid and the sides of the container. The labels must be visible from all sides of the container.

All sharps, including all listed above, and any other items that may puncture human skin must be placed in a red sharps container. This container should only be filled 2/3 full before sealing and disposal.

Biohazardous wastes that are contaminated with radioactive and/or chemical materials are not regulated as medical waste. They are regulated as radioactive, mixed, or chemical waste depending on contamination. This waste must not be put into the biohazard containers.

C. Biohazard Waste Labeling

Biohazardous Waste must be contained in an approved biohazard waste bag at all times. Biohazardous waste bags must be labeled with either the words "Biohazardous Waste" or a biohazard symbol and the word "Biohazard". These bags must be disposable and impervious to moisture and have strength sufficient to preclude ripping, tearing, or bursting under normal conditions of usage and handling. A figure illustrating the biohazard symbol follows.

D. Uncontaminated waste that physically resembles Biohazardous Waste

Uncontaminated sharps must be placed in a sharps container. Cultures of non-pathogenic microorganisms and unused Petri dishes with agar should be autoclaved and disposed of either in the biohazardous waste stream or normal waste stream. Plastic bottles, jars and centrifuge tubes that have not contacted biohazardous materials may be disposed of through the normal waste stream. Glass bottles or jars should be contained in a "broken glass" container, then disposed of through the normal waste stream.

E. Decontamination by Autoclaving

Autoclaving, using saturated steam under high pressure, is one of the most effective methods for decontaminating biohazardous materials. When using laboratory or departmental autoclaves to decontaminate biohazardous materials, the Principal Investigator/Supervisor is responsible in meeting the following requirements:

- Maintaining a log book where the operator records the date, name, cycle time, and biological indicator results.
- Autoclaving for the appropriate time.
- Documenting proper validation techniques when decontaminating material.

Biological Indicators (BI) are the most accepted means of monitoring the sterilization process as they directly determine whether the most resistant microorganisms are present. BI ampoules are autoclaved along with a load of waste. Upon completion of the cycle and following manufacturer's instructions, the ampoules should be incubated for at least 24-48 hours and observed for any sign of positive growth that would indicate that the sterilizing process did not work. Results should be recorded and kept in the autoclave log book. Indicators such as autoclave tape or strips do not ensure sterilization, but can be used to show that autoclaving has been performed.

Autoclaves should be maintained on a regular service contract to ensure the system is working properly. In addition, calibration should be performed annually by the manufacturer or a qualified autoclave technician as part of the annual maintenance contract.

F. Spills of Biohazardous Waste

If a spill should occur involving a biohazardous waste:

Response:

- Do not panic.
- Avoid inhaling airborne material, quickly notify others in the area to prevent contamination of additional personnel and environment.
- Close the area, post a warning sign, and allow agents to settle (~30 minutes).
- Inform the Principal Investigator/Supervisor, and, if assistance is needed, contact the Risk Management & Safety department.

Clean-up:

- Assemble a spill response clean-up team.
- Have a complete biological spill kit ready to go before you start the clean-up.
- Before re-entering the area to proceed with the clean-up: don gowns, gloves, shoe covers, full covered face shield, and an appropriate tight fitted respirator, if applicable.
- Start at the edge and work toward the center of the spill covering it with paper towels or absorbent material.
- Pour effective disinfectant over the paper towels and spill starting around the edges and working toward the center. Saturate the area with the disinfectant.
- Allow sufficient contact time (~30 minutes).
- Pick up any pieces of broken glass or sharp objects with forceps/tongs and put in a sharps container. *Sharps containers do not have to be included in the spill kit. Responders should attempt to use the sharps container in the laboratory area.*
- Use fresh paper towels soaked in effective disinfectant to wipe up spill, working from the edges to the center.
- Discard all disposable materials in the biohazard waste bag, then place the bag in a second biohazard bag. Secure outer bag and disinfect by autoclaving.
- Dispose of materials through biological waste stream.

VI. Biological Spill Response and Clean-up

Work with inherently hazardous materials such as infectious agents presents some degree of risk including risk of spills. Therefore, emergency procedures should be detailed in the laboratory specific SOPs.
Laboratories that have biological material should have the necessary clean-up material in their laboratory in case a spill happens. A Biological Spill Kit should be assembled prior to working with biological material and readily available in the event there is a potential biological spill in the laboratory.

A. Biological Spill Kit

Biological Spill Kits should contain basic items such as:

- Effective disinfectant agent.
- Small disposable broom with dustpan.
- Forceps/Tongs: for handling sharps.
- Paper towels or other suitable absorbent.
- Biohazard bags: for the collection of contaminated spill clean-up items.
- Gloves: for hand protection.
- Face protection (eye wear and mask)
- A waterproof copy of the UNLV spill response and clean-up procedure.

B. Response for Biosafety Level 1 Spill and Clean-up

Biosafety Level 1 work involves working with agents not known to consistently cause disease in healthy adult humans and is a minimal potential hazard to laboratory personnel and the environment. Laboratory work is not separated from the general traffic areas and special containment equipment of facility design is not required. Work is normally conducted on open bench tops. Laboratory personnel should have specific training in the procedures conducted in the laboratory and are supervised by the Principal Investigator/Supervisor that has general training in microbiology or a related science.
Each laboratory that is conducting Biosafety Level 1 work should provide a biological spill kit for laboratory personnel. A Biological Spill Kit should be assembled prior to working with biological material and readily available in the event there is a potential biological spill in the laboratory.
Additional Personal Protective Equipment (PPE) that is not included in the kit that the individual cleaning up the spill would need is a laboratory jacket/disposable gown.

Procedure:

Response:

- Do not panic.
- Notify others in the area to prevent contamination of additional personnel and environment.
- Remove any contaminated clothing and wash exposed skin with disinfectant.

Clean-up:

- Assemble a spill response clean-up team.
- Have a complete biological spill kit ready to go before you start the clean-up.
- Wearing a laboratory coat, gloves, and face protection start at the edges and working toward the center of the spill, cover the spill with paper towels or other absorbent material.
- Pour disinfectant over the paper towels and spill starting around the edges and working toward the center. Saturate the area with the effective disinfectant.
- Allow sufficient contact time (~30 minutes).
- Pick up any pieces of broken glass or sharp objects with forceps/tongs and put in a sharps container. *Sharps containers do not have to be included in the spill kit. Responders should attempt to use the sharps container in the laboratory area.*
- Use fresh paper towels soaked in the effective disinfectant to wipe up spill, working from the edges to the center.
- Discard all disposable materials in the biohazard waste bag as you clean-up the spill.
- Close and secure the biohazard waste bag, then place the bag in a second biohazard bag. Secure outer bag and disinfect by autoclaving.
- Dispose of materials through biological waste stream.

NOTE: Individuals involved in a spill that results in a biological exposure should inform the Principal Investigator/Supervisor in order to initiate a Notice of Injury or Occupational Disease – Incident Report. Additionally, the Principal Investigator/Supervisor must notify the Risk Management & Safety department by submitting a Supervisor's Incident and Analysis Report immediately.

C. Response for Biosafety Level 2 Spill and Clean-up

Biosafety Level 2 (BSL-2) work involves working with agents that have moderate potential hazard to personnel and the environment. BSL-2 work is performed by laboratory personnel that have specific training in handling pathogenic agents and are directed by Principal Investigators/Supervisors that are competent scientists. BSL-2 work must be performed in a laboratory that has limited access. Additionally, BSL-2 procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

Each laboratory that is conducting BSL-2 work should provide a biological spill kit for laboratory personnel. A Biological Spill Kit should be assembled prior to working with biological material and be readily available in the event there is a potential biological spill in the laboratory.

Additional PPE that is not included in the kit that the individual cleaning up the spill would need includes a disposable gown (water-proof that closes in the back), an appropriate tight fitting respirator (individuals that need respirators must enroll in UNLV's Respiratory Program provided by Risk Management & Safety), and disposable shoe covers.

Procedure:

Response:

- Do not panic.

- Avoid inhaling airborne material; quickly notify others in the area to prevent contamination of additional personnel and environment.
- Remove any contaminated clothing, turn contaminated clothing areas inward, and place in a biohazard bag (evacuate).
- Wash exposed skin with disinfectant.
- Close the area, post a warning sign, and allow agents to settle (~30 minutes).
- Inform Principal Investigator/Supervisor, and, if assistance is needed, consult the Risk Management & Safety department.

Clean-up:

- Assemble a spill response clean-up team.
- Have a complete biological spill kit ready to go before you start the clean-up.
- Before re-entering the room to proceed with the clean-up: don gowns, gloves, shoe covers, full covered face shield, and tight fitted respirator with a N95 cartridge, if applicable.
- Start at the edge and work toward the center of the spill covering it with paper towels or absorbent material.
- Pour effective disinfectant over the paper towels and spill starting around the edges and working toward the center. Saturate the area with the disinfectant.
- Allow sufficient contact time (~30 minutes).
- Pick up any pieces of broken glass or sharp objects with forceps/tongs and put in a sharps container. *Sharps containers do not have to be included in the spill kit. Responders should attempt to use the sharps container in the laboratory area.*
- Use fresh paper towels soaked in effective disinfectant to wipe up spill, working from the edges to the center.
- Discard all disposable materials in the biohazard waste bag as you clean-up the spill.
- Close and secure the biohazard waste bag, then place the bag in a second biohazard bag. Secure outer bag and disinfect by autoclaving.
- Dispose of materials through biological waste stream.

NOTE: Individuals involved in a spill that results in a biological exposure should inform the Principal Investigator/Supervisor in order to initiate a Notice of Injury or Occupational Disease – Incident Report. Additionally, the Principal Investigator/Supervisor must notify the Risk Management & Safety department by submitting a Supervisor's Incident and Analysis Report immediately.

D. Response for a Spill in a Biological Safety Cabinet and Clean-up

A Biological Spill Kit should be assembled prior to working with biological material and readily available in the event there is a potential biological spill in the biological safety cabinet.

Procedure:

Response:

- Do not panic.
- Allow cabinet's blower to run during clean-up.

- Quickly notify others in the area to prevent contamination of additional personnel and environment.
- Inform Principal Investigator/Supervisor, and, if assistance is needed, consult Risk Management & Safety department.

Clean-up:

- Assemble a spill response clean-up team.
- Have a complete biological spill kit ready to go before you start the clean-up.
- Before re-entering the biological safety cabinet to proceed with the clean-up: don gowns, gloves, full covered face shield. If your face will cross the air barrier while cleaning the cabinet also don a tight fitted respirator with a N95 cartridge if applicable.
- Since the method of protection is an air barrier, movements in and out of the hood can cause significant disruption of the protective barrier in Class II Biological Safety Cabinets. Any movement within the cabinet should be done slowly and perpendicular to the face opening to reduce disruptions.
- Start at the edge and work toward the center of the spill covering it with paper towels or absorbent material.
- Pour effective disinfectant over the paper towels and spill starting around the edges and working toward the center. Saturate the area with the disinfectant.
- Allow sufficient contact time (~30 minutes).
- Pick up any pieces of broken glass or sharp objects with forceps/tongs and put in a sharps container. *Sharps containers do not have to be included in the spill kit. Responders should attempt to use the sharps container in the laboratory area.*
- Use fresh paper towels soaked in effective disinfectant to wipe up spill, working from the edges to the center. When sodium hypochlorite is used, a final wipe with 70% ethanol will be needed to remove the residual chlorine that can corrode stainless steel surfaces if not cleaned off.
- Discard all disposable materials in the biohazard waste bag as you clean-up the spill. Potentially contaminated materials should be surface decontaminated before removal from the cabinet.
- Close and secure the biohazard waste bag, then place the bag in a second biohazard bag. Secure outer bag and disinfect by autoclaving.
- Dispose of materials through biological waste stream.

NOTE: Individuals involved in a spill that results in a biological exposure should inform the Principal Investigator/Supervisor in order to initiate a Notice of Injury or Occupational Disease – Incident Report. Additionally, the Principal Investigator/Supervisor must notify the Risk Management & Safety department by submitting a Supervisor's Incident and Analysis Report immediately.

E. Response for a Spill in a Centrifuge and Clean-up

There are various types of centrifuges utilized in the laboratory. Individuals using centrifuges should be familiar on how to operate them. A Biological Spill Kit should be assembled prior to working with biological material and readily available in the event there is a potential biological spill in the centrifuge.

Procedure:

Response:

- Do not panic.
- Avoid inhaling airborne material; quickly notify others in the area to prevent contamination of additional personnel and environment.
- Close the area, post a warning sign, and allow agents to settle (~30 minutes).
- Inform Principal Investigator/Supervisor, and, if assistance is needed, consult Risk Management & Safety department.

Clean-up:

- Assemble a spill response clean-up team.
- Have a complete biological spill kit ready to go before you start the clean-up.
- Before re-entering the room to proceed with the clean-up: don gowns, gloves, full covered face shield, and an appropriate tight fitted respirator, if applicable.
- Remove rotors and swinging buckets, and transfer to the closest biological safety cabinet. Cleanout the inside of the centrifuge with the appropriate disinfectant.
- Cleanout the rotors and buckets with the appropriate disinfectant.
- Discard all disposable materials in the biohazard waste bag as you clean-up the spill.
- Close and secure the biohazard waste bag, then place the bag in a second biohazard bag. Secure outer bag and disinfect by autoclaving.
- Dispose of materials through biological waste stream.

NOTE: Individuals involved in a spill that results in a biological exposure should inform the Principal Investigator/Supervisor in order to initiate a Notice of Injury or Occupational Disease – Incident Report. Additionally, the Principal Investigator/Supervisor must notify the Risk Management & Safety department by submitting a Supervisor's Incident and Analysis Report immediately.

F. Response for a Spill that Happens During Transport in a Public Area:

Response:

- Do not panic.
- Avoid inhaling airborne material; quickly notify others in the area to prevent contamination of additional personnel and environment.
- Close the area, post a warning sign, and allow agents to settle (~30 minutes).
- Inform Principal Investigator/Supervisor and Risk Management & Safety department for assistance.
- Do not attempt to clean-up the spill if the proper supplies are not available.

VII. Emergency Response in the Event of a Disaster

In the case of a fire, earthquake, explosion or other disaster, exit the laboratory immediately and follow approved procedures detailed in the UNLV Emergency Response Manual depending on the scenario. If you are contaminated with biological materials during the emergency, seek the nearest drench shower for decontamination, then seek medical attention from your preferred medical provider.

VIII. Transportation of Biological Materials

Regulations related to biological agents have become increasing stringent over time and especially so during recent years. Transportation of biological materials is an area that has very specialized and specific regulations and should not be attempted without consulting the UNLV Biosafety Officer. Severe penalties for non-compliance with shipping rules can result in personal fines of up to $250,000 and up to a year jail sentence with up to a $500,000 fine per incident for the associated organization. This section will cover regulatory information on shipping infectious substances, information for individuals that must follow shipping regulations, training requirements, permits, and transporting within UNLV.

A. Shipping Infectious Substances

Shipping of infectious agents and other biological materials are regulated by governmental and consensus organizations (non-governmental).

Shipping regulations categorize biological materials in the following manner:

- Unregulated biological material;
- Infectious substances (Category A infectious substances);
- Diagnostic specimens (Category B infectious substances);
- Biological products; or
- Genetically modified organisms and microorganisms.

Different requirements apply to each of the above listed categories of biological materials. Shipping terminology differs from that utilized in research situations; therefore you should request assistance from Risk Management & Safety.

The list of agencies that regulate the shipment of biological materials includes:

- US Department of Transportation (DOT)
- US Public Health Service (PHS)
- Occupational Safety and Health Administration (OSHA)
- United States Postal Service (USPS)
- International Air Transport Association (IATA)
- International Civil Aviation Organization (ICAO)

It is very important to comply with all regulations for appropriate shipping of infectious agents. It is not allowed to carry infectious agents on an airplane with you, in carry on luggage or checked luggage. It is also not appropriate to drive with infectious substances over public roads without proper training, manifests, and placards.

B. Training Requirements IATA, ICAO, 49CFR

Dangerous Goods training is required under all national and international regulations (IATA 1.5, ICAO 1.4.1, 49CFR 172.702) for anyone shipping hazardous materials. Within 2 years of initial training, shippers should receive recurrent training as per IATA 1.5 and ICAO 1.4.2.2.

The employer must provide training to all shippers. However, the shipper is under obligation to receive further training if shipping hazardous materials of a class or division they have insufficient training in.

The department of Risk Management & Safety offers training to UNLV faculty, students, and staff at no charge to become certified to ship using the IATA DGR and 49 CFR. Unless trained, an individual may not be involved in any part of the shipping process, other than to provide information and supplies to a trained person.

1. How is a Person Trained?

A person is trained when they have gone through the proper shipping training course offered by the department of Risk Management & Safety. Individuals must contact Risk Management & Safety to enroll in a class. A record of current training is maintained by the individual in his or her department and the department of Risk Management & Safety.

2. Record Requirements

According to 49 CFR 172.704, an employer must test employees to ensure that they have been trained on the material(s) that they ship. Additionally, the employer must retain a copy of the training record for each individual. CFR 172.704 states, that training records are valid for **three** years. Training records are valid for **two** years under ICAO/IATA. If there is a change in the regulations, then individuals must be retrained and the new training records become valid. Training records must be kept on file for the duration of employment and 90 days after an employee has left the organization.

IATA 1.5.4.1 requires the employer to keep a record of training which must include:

- The individual's name.
- The most recent training completion date.
- A description, copy or reference to training materials used to meet the training requirement.
- The name and address of the organization providing the training; and
- Evidence, which shows that a test has been completed satisfactorily.

Records of training must be made available upon request to the appropriate requesting authority.

IATA: *Training valid for**: 2 years**; records must be kept for: **2 years***
ICAO: *Training valid for: **2 years**; records must be kept for: **duration not specified***
49CFR: *Training valid for: **3 years**; records must be kept for: **duration of employment plus 90 days***

C. Permits

U.S. Customs officials require import permits to accompany all shipments of infectious substances, animals, animal-derived materials, insects, etiologic agents, biological toxins, or genetically modified organisms that cross international borders. Regulations that govern the transfer of biological materials help to minimize or eliminate the possible threats to public health and agriculture. The receiver is often best suited to obtain import permits upon request by the shipper. The shipper is responsible for obtaining export permits. International shipments must not be made until these permits have been granted by the proper national authority and approved by the department of Risk Management & Safety.

1. Importing into the United States

All shipments that come into the United States are reviewed by the U.S. Customs and Border Protection agency. Individuals should check the appropriate governmental organization prior to shipping the material.

- CDC Permits: www.cdc.gov/od/aipp/
- USDA Permits: www.aphis.usda.gov/vs/import_export.htm
- U.S. Fish and Wildlife Service Permits: www.fws.gov/

Some U.S. governmental agencies require individuals/companies to obtain an import permit. Packaging and labeling according to IATA guidelines are required to ship. U.S. Importation permits are issued only to the importer, located in the United States. The importer is legally responsible for assuring that the package that is being shipped follows the PHS (Federal) and IATA (International) regulations. The importer has the responsibility to send the labels and one or more copies of the permit to the individual shipping. Shipping labels and permit information include a universal biohazard symbol, address of the importer, permit number, and the expiration date of the permit.

Importing permits must be approved by the department of Risk Management & Safety.

2. Exporting from the United States

Export permits may be required depending on the nature of the shipment. An import permit might be required in addition to the export permit in the country the package is being exported to. Any package that requires an export permit must complete the permit and have it approved by the appropriate government agency before shipping the package.

Export permits must be approved by the department of Risk Management & Safety.

D. Transporting within UNLV

For transporting biological material between laboratories within a building, the biological material is placed in an enclosed, labeled, primary container. The primary container is then placed in a durable, leak proof secondary container (e.g. a sealable biohazard plastic bag) that has a biohazard symbol on it.

For transporting outside the building, the biological material is placed in an enclosed, labeled, primary container. The primary container is then placed in a durable, leak proof secondary container (e.g. a sealable biohazard plastic bag) that has a biohazard symbol on it. The secondary container is lastly placed into a rigid sturdy outer container that has a lid that snaps that prevents it from opening.

Following transport of biological materials between laboratories in a building or outside a building, the transporter must decontaminate the outside of the secondary container and remove gloves for transportation. Once at the destination, the transporter should re-glove prior to opening the secondary container.

For questions about appropriate secondary containers, rigid sturdy outer containers, or safety practices, contact the UNLV Biosafety Officer.

IX. Decontamination

Decontamination is a process in which a laboratory worker cleans an area, equipment, device, or material so it is safe to work in/on or handle. The main purpose of decontaminating is to reduce the level of microbial contamination so that infection transmission is eliminated. There are various processes that are utilized to decontaminate. At UNLV, one decontamination process for individuals working in the laboratory is washing their hands with soap and water. Another process is autoclaving biohazardous waste.

For laboratory surfaces such as bench tops, liquid chemical germicides formulated as disinfectants may be used for decontamination. The most common disinfectants that are used for decontamination are sodium hypochlorite solutions, oxidative solutions, peracetic acid, phenols, and iodophors. Disinfectants chosen must be effective against agents that are being used in the laboratory. Individuals should refer to the agent's Material Safety Data Sheet (MSDS), if available, to determine the type of disinfectant to use. A source of microbial MSDS is "Material Safety Data Sheets (MSDS) for Infectious Substances" from the Public Health Agency of Canada, found at http://www.phac-aspc.gc.ca/msds-ftss/index-eng.php.

MSDSs detail what type of disinfectant should be used, the concentration level, and the contact time needed to disinfect. Concentration and contact time can vary depending on the formulation and the manufacturer's instructions for use.

UNLV requires laboratories to have a decontamination procedure in place and available in the laboratory prior to work. A decontamination procedure should describe what disinfectant will be used, the concentration level, and the contact time needed to disinfect. It should also include the rationale for the disinfecting agent, the approach to its application, and other parameters.

X. Biological Safety Cabinets

Biological Safety Cabinets (BSCs) are among the most effective and the most commonly used primary containment devices in laboratories working with infectious agents. They also provide sterility for experiments that require clean spaces.

A. General Information

There are three general types of biological safety cabinets (Class I, II, and III) that provide different levels of sterility, personal and environmental protection. Appendix A of the BMBL (http://www.cdc.gov/od/ohs/biosfty/biosfty.htm) gives an in-depth discussion of the types of cabinets and their uses.

Prior to purchase of any HEPA filtered laminar flow equipment consult with the UNLV Biosafety Officer. Certain types of Laminar Flow Hoods or Clean Air Benches appear very similar to Biological Safety Cabinets but do not provide personnel protection.

All staff members should be trained on proper methods for working in a biological safety cabinet. Since the method of protection is an air barrier movements in and out of the hood can cause significant disruption of the protective barrier in Class II Biological Safety Cabinets. Contact the department of Risk Management & Safety for in-depth training on Biological Safety Cabinets.

B. Certification

NSF International Standard 49 entitled "Class II Laminar Flow Biohazard Cabinetry" and the BMBL require testing and certification of biological safety cabinets upon initial installation, whenever relocated or repaired, and annually thereafter. UNLV requires adherence to the above listed standards whenever working at BSL-2 or above.

An accredited Biosafety Cabinet field certifier must be hired for biological safety cabinet certification. At the time of certification a notice must be posted on the cabinet that includes the name and contact information for the certifier, the date of certification and date of expiration. Contact the UNLV Biosafety Officer for a list of accredited certifiers that service the UNLV campus.

C. Preparation

Prepare a written checklist of materials needed for the procedure. All materials should be placed in the cabinet beforehand to reduce the number of air disruptions. Only the material and equipment needed for the immediate work should be placed in the cabinet. Any additional supplies that will be needed should be left outside the cabinet. Any movement within the cabinet should be done slowly and perpendicular to the face opening to reduce disruptions. Placing cabinets away from high-traffic areas may also reduce air disruptions inside the cabinet.

Laboratory coats should be worn fully buttoned over street clothes and protective gloves worn during any procedures. Before beginning work, adjust the stool height so that the individual's face is above the front opening and their arms can easily enter and exit the cabinet. Ensure that the cabinet sash is at the appropriate level. The front grille must be kept free from paper, wrappers, slides, etc. All operations inside the cabinet must be performed at least four inches from the inside of the grille.

If the cabinet has been shut down, the blowers should be operated at least three minutes before beginning work to allow the cabinet to "purge." In addition, the cabinet should be wiped with 70% ethanol or other disinfectant as determined by the Principal Investigator/Supervisor. If sodium hypochlorite is used, a second wipe with 70% ethanol is needed to remove the residual chlorine that can corrode stainless steel surfaces. Materials and equipment that will be placed in the cabinet should also be wiped down with 70% ethanol to reduce contaminants within the Biological Safety Cabinet.

D. Object Placement

Absorbent toweling can be placed on the work surface but not near the front grille opening. All materials should be placed as far back as possible, toward the rear edge of the work surface and away from the front grille. Aerosol-generating equipment, such as vortex mixers and tabletop centrifuges, should similarly be placed near the rear of the cabinet. Active work should flow from the clean to the contaminated area across the work surface. Items that take up a large amount of space, such as a biohazard bag, should be placed to one side of the cabinet. Potentially contaminated materials should be surface decontaminated before removal from the cabinet.

E. Decontamination

All equipment and supplies should be surface decontaminated and removed from the cabinet once work has been completed. At the end of the day, final surface decontamination of the cabinet should include wiping down the work area, the sides and back of the cabinet, and the interior glass. If working with radioactive materials, the cabinet needs to be monitored for radioactivity and properly decontaminated before removal of items. Laboratory personnel should remove gloves and coats in an appropriate manner to avoid contamination. Once PPE has been removed, wash hands with soap and water.

XI. Standard Biosafety Level 1 Practices and Procedures

BSL-1 laboratory work involves working with well characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. These laboratories are not normally separated from the general traffic patterns in the building. Standard microbiological practices are used while working on open bench tops. Laboratory personnel should have specific training in the laboratory and are supervised by the Principal Investigator (PI) or Supervisor who have general training in microbiology or a related science.

A. Standard Microbiological Practices

1. Principal Investigator/Supervisor must enforce the institutional policies that control access to the laboratory.
2. Individuals must wash their hands after working with hazardous materials and when exiting the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is 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. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of splashes or aerosols.
7. Work surfaces are decontaminated on completion of work or at the 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 an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak proof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the facility are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.
9. The universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Additional information that needs to be posted includes: the laboratory's biosafety level, the Principal Investigator/Supervisor's name that is responsible, telephone number, and required procedures for entering and exiting the laboratory.
10. An insect and rodent control program is in effect.

B. Special Practices

Not required.

C. Safety Equipment (Primary Barriers) and Personal Protective Equipment

1. Containment devices such as biological safety cabinets are generally not required.
2. Wearing laboratory coats is recommended to prevent contamination of personal clothing.
3. When there is a potential to create splashes of microorganisms or other hazardous material protective eyewear should be worn. Individuals that wear contact lens should also wear eye protection.

4. Individuals are required to wear gloves to protect their hands from exposure to hazardous materials.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratories should have doors that close for access control.
2. Laboratories must have sinks to wash hands.
3. Laboratory floor and furniture should be designed to be cleaned easily. Space between the counters and cabinets should be easily accessible for cleaning.
4. Laboratories that have windows on them should have screens on the windows.

* Adopted from *Biosafety in Microbiological and Biomedical Laboratories*, 5th Ed. U.S. Department of Health and Human Services, 2007.

XII. Standard Biosafety Level 2 Practices and Procedures

BSL-2 laboratory work involves agents that pose moderate hazards to personnel and the environment. BSL-2 builds upon BSL-1; however, it differs from BSL-1 in that laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures, access to the laboratory is restricted when work is being conducted, and all procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

A. Standard Microbiological Practices

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments 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, and applying cosmetics are not permitted in the work areas. Food is 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. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of splashes or aerosols.
7. Work surfaces are decontaminated on completion of work or at the 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 an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak proof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the facility are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.
9. The universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Additional information that needs to be posted includes: the laboratory's biosafety level, the name of the responsible Principal Investigator/Supervisor, telephone number, and required procedures for entering and exiting the laboratory.
10. An insect and rodent control program is in effect.

B. Special Practices

1. Access to the laboratory is limited or restricted by the laboratory director 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 policies and 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. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).
4. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.
5. Biosafety procedures are incorporated into standard operating procedures or in a Biosafety Manual adopted or prepared specifically for the laboratory by the laboratory director. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
6. 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 for procedural or policy changes. The laboratory Supervisor ensures that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
7. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
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. Plasticware 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 which re-sheathe the needle, needleless 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 before disposal, according to any local, state, or federal regulations.
8. Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.
9. Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

10. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
11. Animals not involved in the work being performed are not permitted in the laboratory.

C. Safety Equipment (Primary Barriers) and Personal Protective Equipment
1. Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:
a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include 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 embryonate eggs.
b. 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, smocks, or uniforms designated for laboratory use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.
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 they should not be worn outside the laboratory. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

D. Laboratory Facilities (Secondary Barriers)
1. Provide lockable doors for facilities that house restricted agents (as defined in 42 CFR 72.6).
2. Consider locating new laboratories away from public areas.
3. Each laboratory contains a sink for hand washing.
4. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.
5. 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.
6. 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.

7. 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.
8. An eyewash station is readily available.
9. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
10. 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.
11. HEPA filtered exhaust air from a Class II biological safety cabinet can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. Biological safety cabinets can also be connected to the laboratory exhaust system by either a thimble connection or a direct connection. The air system operation must be verified to assure proper safety cabinet performance.
12. All laboratory waste should be decontaminated (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

* Adopted from *Biosafety in Microbiological and Biomedical Laboratories*, 5th Ed. U.S. Department of Health and Human Services, 2007.

XIII. Standard Biosafety Level 3 Practices and Procedures

BSL-3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents.

All procedures involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment. The laboratory has special engineering and design features.

It is recognized, however, that some existing facilities may not have all the facility features recommended for Biosafety Level 3 (i.e., double-door access zone and sealed penetrations). In this circumstance, an acceptable level of safety for the conduct of routine procedures, (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, susceptibility testing, etc.), may be achieved in a Biosafety Level 2 facility, **providing** 1) the exhaust air from the laboratory room is discharged to the outdoors, 2) the ventilation to the laboratory is balanced to provide directional airflow into the room, 3) access to the laboratory is restricted when work is in progress, and 4) the recommended Standard Microbiological Practices, Special Practices, and Safety Equipment for Biosafety Level 3 are rigorously followed. The decision to implement this modification of Biosafety Level 3 recommendations should be made only by the laboratory director.

The following standard and special safety practices, equipment and facilities apply to agents assigned to Biosafety Level 3:

A. Standard Microbiological Practices

1. The laboratory director is responsible for making access to the laboratory limited and restricted.

2. Persons wash their hands after handling infectious materials, after removing gloves, and before they leave the laboratory.

3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the laboratory. 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 for this purpose only.

4. Mouth pipetting is prohibited; mechanical pipetting devices are used.

5. Policies for the safe handling of sharps are instituted.

6. All procedures are performed carefully to minimize the creation of aerosols.

7. Work surfaces are decontaminated after completion of work and after any spill of viable material.

8. The universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Additional information that needs to be posted includes: the laboratory's biosafety level, the name of the responsible Principal Investigator/Supervisor, telephone number, and required procedures for entering and exiting the laboratory.

9. All items that leave a BSL-3 laboratory must be decontaminated before exiting the laboratory by an approved decontamination method, such as autoclaving.

10. An insect and rodent control program is in effect.

11. The laboratory supervisor is required to have laboratory personnel receive the appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Annually, laboratory personnel must receive updates or additional training when procedural or policy changes occur. All laboratory personnel should be provided with information regarding immune competence and conditions. Staff should be encouraged to present themselves to the institution's healthcare provider for appropriate counseling and guidance, should they become ill and suspect exposure to the BSL-3 agent(s) being worked with.

B. Special Practices

1. Laboratory doors are kept closed when experiments are in progress.

2. The laboratory director controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. 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 risk of acquiring infections. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. No minors are allowed in the laboratory.

3. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures, enter the laboratory or animal rooms.

4. When infectious materials or infected animals are present in the laboratory or containment module, a hazard warning sign, incorporating the universal biohazard symbol, is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for immunizations, respirators, or other personal protective measures.

5. Laboratory personnel receive the appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing), and periodic testing as recommended for the agent being handled.

6. Baseline serum samples are collected as appropriate and stored for all laboratory and other at-risk personnel. Additional serum specimens may be periodically collected, depending on the agents handled or the function of the laboratory.

7. A biosafety manual specific to the laboratory is prepared or adopted by the laboratory director and biosafety precautions are incorporated into standard operating procedures. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

8. 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 for procedural changes.

9. The laboratory director is responsible for ensuring that, before working with organisms at Biosafety Level 3, all personnel demonstrate proficiency in standard microbiological practices and techniques and in the practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the laboratory director or other competent scientist proficient in safe microbiological practices and techniques.

10. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.

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. Plasticware 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 which re-sheathe the needle, needleless systems, and other safe 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 should be decontaminated before disposal, and disposed of according to any local, state, or federal regulations.

11. All open manipulations involving infectious materials are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench. Clean-up is facilitated by using plastic-backed paper toweling on non-perforated work surfaces within biological safety cabinets.

12. Laboratory equipment and work surfaces should be decontaminated routinely with an effective disinfectant, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials.

a. Spills of infectious materials are decontaminated, contained and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious material. Spill procedures are developed and posted.

b. Contaminated equipment must be decontaminated before removal from the facility for repair or maintenance or packaging for transport, in accordance with applicable local, state, or federal regulations.

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

14. All potentially contaminated waste materials (e.g., gloves, laboratory coats, etc.) from laboratories are decontaminated before disposal or reuse.

15. Spills and accidents that result in overt or potential exposures to infectious materials are immediately reported to the laboratory director. Then the laboratory director immediately notifies the Biosafety Officer. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.

16. Animals and plants not related to the work being conducted are not permitted in the laboratory.

C. Safety Equipment (Primary Barriers) and Personal Protective Equipment

1. Protective laboratory clothing such as solid-front or wrap-around gowns, scrub suits, or coveralls are worn by workers when in the laboratory. Protective clothing is not worn outside the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when overtly contaminated.

2. Gloves must be worn when handling infectious materials, infected animals, and when handling contaminated equipment.

3. Frequent changing of gloves accompanied by hand washing is recommended. Disposable gloves are not reused.

4. All manipulations of infectious materials, necropsy of infected animals, harvesting of tissues or fluids from infected animals or embryonate eggs , etc., are conducted in a Class II or Class III biological safety cabinet.

5. When a procedure or process cannot be conducted within a biological safety cabinet, then appropriate combinations of personal protective equipment (e.g., respirators, face shields) and physical containment devices (e.g., centrifuge safety cups or sealed rotors) are used.

6. Respiratory and face protection are used when in rooms containing infected animals.

D. Laboratory Facilities (Secondary Barriers)

1. The laboratory is separated from areas that are open to unrestricted traffic flow within the building, and access to the laboratory is restricted. Passage through a series of two self-closing doors is the basic requirement for entry into the laboratory from access corridors. Doors are lockable. A clothes change room may be included in the passageway.

2. Each laboratory room contains a sink for hand washing. The sink is hands-free or automatically operated and is located near the room exit door.

3. The interior surfaces of walls, floors, and ceilings of areas where BSL-3 agents are handled are constructed for easy cleaning and decontamination. Seams, if present, must be sealed. Walls, ceilings, and floors should be smooth, impermeable to liquids and resistant to the chemicals and disinfectants normally used in the laboratory. Floors should be monolithic and slip-resistant. Consideration should be given to the use of coved floor coverings. Penetrations in floors, walls, and ceiling surfaces are sealed or capable of being sealed to facilitate decontamination. Openings such as around ducts and the spaces between doors and frames are capable of being sealed to facilitate decontamination.

4. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and those 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. All windows in the laboratory are closed and sealed.

7. A method for decontaminating all laboratory wastes is available in the facility and utilized, preferably within the laboratory (i.e., autoclave, chemical disinfection, incineration, or other approved decontamination method). Consideration should be given to means of

decontaminating equipment. If waste is transported out of the laboratory, it should be properly sealed and not transported in public corridors.

8. Biological safety cabinets are required and are located away from doors, from room supply louvers, and from heavily-traveled laboratory areas.

9. A ducted exhaust air ventilation system is provided. This system creates directional airflow which draws air into the laboratory from "clean" areas and toward "contaminated" areas. The exhaust air is not recirculated to any other area of the building. Filtration and other treatments of the exhaust air are not required, but may be considered based on site requirements, and specific agent manipulations and use conditions. The outside exhaust must be dispersed away from occupied areas and air intakes, or the exhaust must be HEPA-filtered. Laboratory personnel must verify that the direction of the airflow (into the laboratory) is proper. It is recommended that a visual monitoring device that indicates and confirms directional inward airflow be provided at the laboratory entry. Consideration should be given to installing an HVAC control system to prevent sustained positive pressurization of the laboratory. Audible alarms should be considered to notify personnel of HVAC system failure.

10. HEPA-filtered exhaust air from a Class II biological safety cabinet can be recirculated into the laboratory if the cabinet is tested and certified at least annually. When exhaust air from Class II safety cabinets is to be discharged to the outside through the building exhaust air system, the cabinets must be connected in a manner that avoids any interference with the air balance of the cabinets or the building exhaust system (e.g., an air gap between the cabinet exhaust and the exhaust duct). When Class III biological safety cabinets are used they should be directly connected to the exhaust system. If the Class III cabinets are connected to the supply system, it is done in a manner that prevents positive pressurization of the cabinets (see Appendix A).

11. Continuous flow centrifuges or other equipment that may produce aerosols are contained in devices that exhaust air through HEPA filters before discharge into the laboratory. These HEPA systems are tested at least annually. Alternatively, the exhaust from such equipment may be vented to the outside if it is dispersed away from occupied areas and air intakes.

12. Vacuum lines are protected with liquid disinfectant traps and HEPA filters, or their equivalent. Filters must be replaced as needed. An alternative is to use portable vacuum pumps (also properly protected with traps and filters).

13. An eyewash station is readily available inside the laboratory.

14. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

15. The Biosafety Level 3 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have

been met prior to operation. Facilities should be re-verified, at least annually, against these procedures as modified by operational experience.

16. Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment, the site conditions, or other applicable federal, state, or local regulations.

* Adopted from *Biosafety in Microbiological and Biomedical Laboratories*, 5th Ed. U.S. Department of Health and Human Services, 2007.

XIV. Toxin Work

A. General

The laboratory facilities, equipment, and procedures appropriate for work with toxins of biological origin must reflect the intrinsic level of hazard posed by a particular toxin as well as the potential risks inherent in the operations performed. If both toxins and infectious agents are used, both must be considered when containment equipment is selected and policies and procedures are written. If animals are used, animal safety practices must also be considered.

B. Standard and Special Practices

At a minimum, BSL-2 safety practices shall be used for all work with toxins. Work shall only be done in BSL-2 laboratories, unless the IBC deems that the work does not require BSL-2 facilities. Some toxins will require BSL-3 practices and facilities. The investigator shall review the requirements of the toxin, and include justification for the BSL chosen for handling the material, with the registration of the toxin to the IBC. This should address, at a minimum, any infectious material that will be related to research with this toxin, the diluents used with this toxin, and the electrostatic properties of the toxin. This justification should also be incorporated into the laboratory specific toxin SOPs that the investigator develops.

C. Training

Training specific to the toxin(s) is required and shall be documented by the Biosafety Officer for all laboratory personnel working with toxins, before starting work with the toxin. All laboratory personnel shall, at regular intervals, undergo refresher training for the specific hazards of the laboratory they are working in. Refresher trainings shall also be documented. Refresher trainings and documentation are the responsibility of the laboratory director.

D. Inventory Control and Security Measures

An inventory control system shall be in place.

When not in use, toxins shall be stored in locked storage rooms, cabinets, or freezers. This is in addition to locked outer doors on the laboratory. If the laboratory cannot be reasonably locked (i.e. open access from adjacent laboratories, the laboratory can be accessed by those not given adequate training for the toxin), the toxins should be stored behind two different locks. When the toxin is not being used, the storage unit should be locked at all times. Any stock solutions, working solutions, broths, etc. should be stored in a locked storage unit.

Access to areas containing toxins shall be restricted to those whose work assignments require access.

As part of the inventory control system, all toxins shall be inventoried as part of normal use to maintain an exact count of the quantity of the toxin present in the laboratory. The stocks of the toxin(s) shall be inventoried, at regular intervals, independently of normal use to verify the running inventory. An up-to-date and accurate inventory list must be maintained. The information the Principal Investigator/Supervisor must include in the log are:

- Date the material was taken and used

- The name of person using the material
- Vial number used
- Start amount of material
- Amount used for the procedure
- End amount of material
- Procedure the material was used for

Only approved individuals and the UNLV Biosafety Officer may have access to toxins. The approved users are listed on the UNLV Toxin Laboratory SOPs form that is submitted to and approved by the Institutional Biosafety Committee. The PI will also specify on the SOPs form the working and storage locations of the toxin(s). Prior to obtaining toxin(s) the PI must notify the Biosafety Officer at 702-895-4226 and describe their plans. Once an individual is no longer working at UNLV, the Principal Investigator/Supervisor must immediately notify the Biosafety Officer.

E. Standard Operating Procedures

Preparation of primary containers of toxin stock solutions and manipulations of primary containers of dry forms of toxins must be conducted in a chemical fume hood, a glove box, or a biological safety cabinet or equivalent containment system approved by the biosafety officer. HEPA and/or charcoal filtration of the exhaust air may be required, depending on the toxin.

The laboratory worker must verify inward airflow of the hood or biological safety cabinet before initiating work. All work must be done within the operationally effective zone of the hood or biological safety cabinet.

When toxins are in use, the room shall be posted to indicate "Toxins in Use, Authorized Personnel Only." Any special entry requirements shall be posted on the entrance(s) to the room. Only personnel whose presence is required shall be permitted in the room while toxins are in use.

All high risk operations shall be conducted with two knowledgeable individuals present. Each must be familiar with the applicable procedures, maintain visual contact with the other, and be ready to assist in the event of an accident.

Before containers are removed from the hood, cabinet, or glove box, the exterior of the closed primary container shall be decontaminated and placed in a clean secondary container. Toxins shall be transported only in leak/spill-proof secondary containers.

Contaminated and potentially contaminated protective clothing and equipment shall be decontaminated using methods known to be effective against the toxin before removal from the laboratory for disposal, cleaning or repair. If decontamination is not possible/practical, materials (e.g. used gloves) shall be disposed of as toxic waste. Materials contaminated with infectious agents as well as toxins shall also be autoclaved or otherwise rendered non-infectious before leaving the laboratory.

The interior of the hood, glove box, or cabinet shall be decontaminated periodically, for example, at the end of a series of related experiments. Until decontaminated, the hood, box, or cabinet shall be posted to indicate that toxins are in use, and access to the equipment and apparatus restricted to necessary, authorized personnel.

F. Practice Procedure

Before the use of the toxin(s), it is highly recommended that the PI and all laboratory workers undergo a practice run of the procedure to be used with the toxin(s) using (a) similar, but inert, stimulant(s). It is advised that outside observers be used to help spot troubled spots within the procedure. This practice may be done using an agent that fluoresces under black light to further illuminate possible sources of exposure. This may be used as part of the initial required training for all laboratory personnel.

G. Safety Equipment

The safety equipment guidelines listed under BSL-2 and BSL-3 should be reviewed and incorporated as appropriate into protocols for work with toxins.

1. When using an open-fronted fume hood or biological safety cabinet, protective clothing, including gloves and a disposable long-sleeved body covering (gown, laboratory coat, smock, coverall, or similar garment), shall be worn so that hands and arms are completely covered. Street clothes shall never be exposed to toxins. In the event that street clothes become contaminated or potentially contaminated, they shall be properly decontaminated before they may be removed from the laboratory or disposed of as toxic waste.

2. Standard eye protection (e.g. safety glasses) must be worn in the laboratory at all times. Additional eye protection shall be worn if an open-fronted containment system is used.

3. Other protective equipment may be required, depending on the characteristics of the toxin and the containment system. For example, use additional respiratory protection if aerosols may be generated and it is not possible to use containment equipment or other engineering controls.

4. When handling dry forms of toxins that are electrostatic:

    a. Do not wear gloves (such as latex) that help generate static electricity
    b. Use glove bag within a hood or biological safety cabinet, a glove box, or a class III biological safety cabinet.

5. When handling toxins that are percutaneous hazards (irritants, necrotic to tissue, or extremely toxic from dermal exposure), select gloves that are known to be impervious to the toxin.

6. Consider both toxin and diluent when selecting gloves and other protective clothing.

7. If infectious agents and toxins are used together in an experimental system, consider both when selecting protective clothing and equipment.

H. Laboratory Facilities
Laboratory facility recommendations listed under BSL-2 and BSL-3 and OSHA standards should be reviewed and incorporated as appropriate into protocols for work with toxins.

When vacuum lines are used with systems containing toxins, they shall be protected with a HEPA filter to prevent entry of toxins into the lines. Sink drains shall be similarly protected when water aspirators are used.

I. Risk Assessment
Prior to work beginning with the toxin(s) a risk assessment must be performed by the investigator. This should address the need for and quantity of the toxin(s), including the possibility that this process could be performed with less quantity or another type or form of toxin(s). This assessment should be an ongoing process that should evolve and adapt as situations change within the laboratory. This is an important tool that should be used to prevent exposure. Other laboratory workers should be included in risk assessment within the laboratory to help ensure that unwanted exposures and loss or theft of toxins does not occur.

J. Registration
All toxins shall be registered with the IBC before they arrive on campus. Unregistered toxins shall not be used. Registration must be done using the Research Protocol Proposal Form and the UNLV Toxin Laboratory SOPs form, found at:
http://www.unlv.edu/Research/IBC/forms.htm.

Registration of toxins with the IBC is not only a safety measure in the best interest of the researcher, the laboratory workers and the larger UNLV community, as well as a university policy, it is also a legal protection for the researcher with regards to 18 USC § 175 (Prohibitions with respect to biological weapons).

Any toxins that were received before the ratification of this section of the Institutional Biosafety Program shall be registered in accordance with these guidelines as soon as possible.

XV. Select Agent and Toxin Work

The U.S. Departments of Health and Human Services (HHS) and Agriculture (USDA) published final rules, which implement the provisions of the USA PATRIOT Act and Public Health Security and Bioterrorism Preparedness and Response Act of 2002 setting forth the requirements for possession, use, and transfer of select agents and toxins. The select agents and toxins identified in the final rules have the potential to pose a severe threat to public health and safety, to animal and plant health, or to animal and plant products.

This rule covers all select agents, even if you had them in your possession prior to the effective date of the final rules (April 18, 2005). You must notify the UNLV Biosafety Officer immediately if you discover select agents and toxins or posses select agents and toxins.

UNLV currently does not participate in the Select Agent Program and is not registered to obtain select agents and toxins. Individuals who possess select agents must register with the CDC and/or APHIS through the designated institutional Responsible Official (RO). Currently at UNLV, there is no appointed RO. If you are planning to obtain a select agent in the future please contact the Biosafety Officer immediately. Registration with the CDC could take months to years and will require adequate notification.

Visit **http://www.cdc.gov/od/sap/final_rule.htm** for complete details on the regulations. The list of select agents and toxins are continuously being updated and at the time of this publication they include:

HHS SELECT AGENTS AND TOXINS

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
Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of eight gene segments (Reconstructed 1918 Influenza 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
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 melitensis
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

USDA SELECT AGENTS AND TOXINS
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 QUARANTINE (PPQ) 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 (citrus variegated chlorosis strain)

XVI. Exempt Strains of Select Agent Work

A. General

The Security, Department of Health and Human Services (HHS) and the Security, United States Department of Agriculture (USDA) has established a procedure by which attenuated strains of select agents or toxins that do not pose a severe threat to plant or animal health, plant or animal products, or public health and safety may be excluded from the list of select agents and toxins (7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73).

Individuals using attenuated strains must use them for specific purposes stated in their IBC protocol. PIs must delineate risks of attenuated strains becoming virulent on their protocol. If the attenuated strains are manipulated in such a way that virulence is restored or enhanced, then the individual(s) and research will be subject to the select agent and toxin regulations. If this is planned to occur, the PI should immediately contact and inform the Biosafety Officer. Since there is a chance for these exempt strains to be potentially manipulated into biological weapons, UNLV requires laboratories with these agents to use biosafety level 2 precautions, to follow biosafety level 2 practices, and to have specific security measures in place.

Attenuated strains of select agents and toxins excluded:

Coccidioides posadasii Δchs5 strain
Coccidioides posadasii Δcts2/Δard1/Δcts3 strain
Conotoxins specifically excluded are: the class of sodium channel antagonist μ-conotoxins, including GIIIA; the class of calcium channel antagonist ω-conotoxins, including GVIA, GVII, MVIIA, MVIIC, and their analogs or synthetic derivatives; the class of NMDA-antagonist conantokins, including con-G, con-R, con-T and their analogs or synthetic derivatives; and the putative neurotensin agonist, contulakin-G and its synthetic derivatives
Junin virus vaccine strain Candid 1
Yersinia pestis strains which are Pgm^- due to a 102-kb region of the chromosome termed the pgm locus (i.e., Δpgm).
Yersinia pestis strains (e.g., Tjiwidej S and CDC A1122) devoid of the 75 kb low-calcium response (Lcr) virulence plasmid

Attenuated strains of Overlap select agents and toxins excluded:

Bacillus anthracis strains devoid of both plasmids pX01 and pX02
Bacillus anthracis strains devoid of the plasmid pX02
Brucella abortus Strain 19
Brucella abortus strain RB51
Coxiella burnetii Phase II, Nine Mile Strain, plaque purified clone 4
Francisella tularensis subspecies *novicida* (also referred to as *Francisella novicida*) strain, Utah 112 (ATCC 15482)
Francisella tularensis subspecies *holartica* LVS (live vaccine strain; includes NDBR 101 lots, TSI-GSD lots, and ATCC 29684)
Francisella tularensis ATCC 6223 (also known as strain B38)
Rift Valley Fever (RVF) virus vaccine strain MP-12
Venezuelan Equine Encephalitis (VEE) virus vaccine candidate strain V3526
Venezuelan Equine Encephalitis (VEE) virus vaccine strain TC-83

B. Standard and Special Practices

Work shall be done in BSL-2 laboratories, and BSL-2 safety practices shall be used for all work using attenuated strains of select agents or toxins. The investigator shall review the requirements of the toxin, and include justification for the BSL the material will be handled at in their IBC Protocol. This should address, at a minimum, any infectious material that will be related to research with this attenuated strain of select agent. This justification should also be incorporated into the laboratory specific SOPs that the investigator develops.

C. Training

Training specific to the attenuated strain is required for all laboratory personnel working with the attenuated strain and shall be documented and provided to the Biosafety Officer before starting work. All laboratory personnel shall at regular intervals undergo refresher training for their specific laboratory's hazards. Refresher trainings shall also be documented. Refresher trainings and documentation are the responsibility of the laboratory director.

D. Inventory Control and Security Measures

An inventory control system shall be in place.

Attenuated strains shall be stored in locked storage rooms, cabinets, or freezers when not in use. This is in addition to locked outer doors on the laboratory. If the laboratory cannot be reasonably locked (i.e. open access from adjacent laboratories, the laboratory can be accessed by those not given adequate training for the toxin), the attenuated strains should be stored behind two different locks. When the toxin is not being used, the storage unit should be locked at all times. Any stock solutions, working solutions, broths, etc. should be stored in a locked storage unit.

Access to areas containing attenuated strains shall be restricted to those whose work assignments require access.

As part of the inventory control system, all attenuated strains received shall be logged in a receiving log book. The log book must contain any paper work received with the attenuated strain, as well as, the ATCC number and where the strain came from.

All attenuated strains shall be inventoried as part of normal use to maintain an exact documentation of the quantity of the attenuated strain present in the laboratory. The stocks of the attenuated strain(s) shall be inventoried, at regular intervals, independently of normal use to verify the running inventory. An up-to-date and accurate inventory list must be maintained. The information the Principal Investigator/Supervisor must include in the inventory log are:

- Date the material was taken and used
- The name of person using the material
- Vial number used
- Start amount of material
- Amount used for the procedure

- End amount of material
- Procedure the material was used for

Only approved individuals may access the attenuated strains. These individuals are listed on the UNLV BSL-2 SOPs form that is submitted to the Institutional Biosafety Committee. Only approved users and the UNLV's Biosafety Officer may have access to the strains.

The Biosafety Officer must be notified of the working and storage locations of the attenuated strain. The PI shall notify the Biosafety Officer of all personnel working with the attenuated strain. Additionally, if individuals plan to possess attenuated strains, the Biosafety Officer must be notified at 702-895-4226. Once an individual leaves and is no longer working at UNLV the Principal Investigator/Supervisor must immediately notify the Biosafety Officer.

E. Standard Operating Procedures

Preparation of the primary containers of the attenuated strain must be performed in a biological safety cabinet or equivalent containment system approved by the Biosafety Officer. HEPA and/or charcoal filtration of the exhaust air may be required, depending on the attenuated strain.

The laboratory worker must verify inward airflow of the biological safety cabinet before initiating work. All work must be performed within the operationally effective zone of the biological safety cabinet.

When attenuated strains are in use, the room shall be posted to indicate "Attenuated Strains of Select Agents in Use, Authorized Personnel Only." Any special entry requirements shall be posted on the entrance(s) to the room. Only personnel whose presence is required shall be permitted in the room while attenuated strains are in use.

All high risk operations shall be conducted with two knowledgeable individuals present. Each must be familiar with the applicable procedures, maintain visual contact with the other, and be ready to assist in the event of an accident.

Before containers are removed from the cabinet, the exterior of the closed primary container shall be decontaminated and placed in a clean secondary container. Attenuated strains shall be transported only in leak/spill-proof secondary containers.

Contaminated and potentially contaminated protective clothing and equipment shall be decontaminated using methods known to be effective against the attenuated strain before removal from the laboratory for disposal, cleaning or repair. If decontamination is not possible/practical, materials (e.g. used gloves) shall be disposed of as biohazardous and/or hazardous waste as appropriate. Materials contaminated with infectious agents as well as toxins shall also be autoclaved or otherwise rendered non-infectious before leaving the laboratory.

The interior of the biological safety cabinet shall be decontaminated periodically, for example, at the end of a series of related experiments. Until decontaminated, the cabinet

shall be posted to indicate attenuated strains are in use, and access to the equipment and apparatus restricted to necessary, authorized personnel.

F. Practice Procedure

Before the use of the attenuated strain(s), it is highly recommended that the Principal Investigator/Supervisor and all laboratory workers undergo a practice run of the procedures using (a) similar, but inert, surrogate(s). It is advised that trained observers be used to help recognize potential problems with the procedure. This practice run may be completed using an agent that fluoresces under black light to further illuminate possible sources of exposure. This may be used as part of the initial required training for all laboratory personnel.

G. Safety Equipment

The safety equipment guidelines listed under BSL-2 should be reviewed and incorporated as appropriate into protocols for work with attenuated strains.

H. Laboratory Facilities

Laboratory facility recommendations listed under BSL-2 and OSHA standards should be reviewed and incorporated as appropriate into protocols for work with attenuated strains.

When vacuum lines are used with systems containing attenuated strains, they shall be protected with a HEPA filter to prevent entry of attenuated strains into the lines. Sink drains shall be similarly protected when water aspirators are used.

I. Risk Assessment

Prior to work beginning with the attenuated strain(s) a risk assessment must be performed by the investigator. This should address the need for and quantity of the attenuated strain(s), including the possibility that this process could be performed with less quantity or another type or form of bacterial strain(s). This assessment should be an ongoing process that should evolve and adapt as situations change within the laboratory. This is an important tool that should be used to prevent exposure. Other laboratory workers should be included in risk assessment within the laboratory to help ensure that unwanted exposures and loss or theft of attenuated strain(s) does not occur.

J. Registration

All attenuated strains shall be registered with the IBC before they arrive on campus. Unregistered attenuated strains shall not be used. Registration must be completed using the Research Protocol Proposal Form and the UNLV BSL-2 Laboratory SOPs form, found at: http://www.unlv.edu/Research/IBC/forms.htm.

Registration of attenuated strains with the IBC is not only a safety measure in the best interest of the researcher, the laboratory workers and the larger UNLV community, as well as a university policy, it is also a legal protection for the researcher with regards to 18 USC § 175 (Prohibitions with respect to biological weapons).

Any attenuated strains that were received before the ratification of this section of the Institutional Biosafety Program shall be registered in accordance with these guidelines as soon as possible.

***** Adopted from *Michigan State University, "Biological Safety Manual", Environmental Health and Safety Office of Radiation, Chemical and Biological Safety,* October 2007.

XVII. Human Cell and/or Tissue Work

A. General

Although risk of laboratory infection from working with cell cultures in general is low, risk increases when working with human and other primate cells, and primary cells from other mammalian species. Since individuals have an increased risk of laboratory infections working with human cells, tissue, body fluids, and primary cell lines, OSHA has developed a bloodborne pathogens standard that should be applied to all individuals working with human cells, tissue, body fluids, and primary cell lines.

B. Hazards

Some bloodborne pathogens that one can encounter while working with human cells and tissues are human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), etc. Other primate cells and tissues also present a risk to individuals working with them such as, cells immortalized with viral agents such as Simian virus 40 (SV-40), Epstein-Barr virus (EBV), adenovirus or human papillomavirus (HPV). NHP cells, blood, lymphoid and neural tissues should be treated as hazardous material.

C. Standard Practices

A risk assessment should be conducted before working with cells and/or tissues. The Centers for Disease Control and Prevention (CDC) and OSHA recommend that all cell lines of human origin be handled using BSL-2 practices and containment. All work should be performed in a biological safety cabinet, and all material decontaminated by autoclaving or disinfection before discarding. Laboratory coats, gloves and eye protection should be worn while performing procedures. Anyone working with human cells and/or tissues is included in UNLV's Bloodborne Pathogens Program as outlined in UNLV's Exposure Control Plan. The Principal Investigator/ Supervisor should make their employees/students familiar with the UNLV Exposure Control Plan, and a copy of the plan must be available in all laboratories.

All human cells and/or tissue work shall be approved by the IBC before work is performed on campus. Investigators must fill out the Research Protocol Proposal Form and the UNLV BSL-2 Laboratory SOPs form, found at: http://www.unlv.edu/Research/IBC/forms.htm.

XVIII. Recombinant Deoxyribonucleic Acid (DNA) Work

A. General

Recombinant DNA is used in various elements of experiments. Individuals working with shall follow the guidelines set by the National Institutes of Health (NIH). NIH established specific guidelines for constructing and handling of recombinant DNA. Any recombinant DNA experiment, which according to the *NIH Guidelines* requires approval by NIH, must be submitted to UNLV IBC and NIH or to another federal agency that has jurisdiction for review and approval.

B. Standard Practices

A risk assessment should be conducted before working with recombinant DNA. All work should be performed using standard microbiological practices. Individuals that will be working with HIV, HBV or other bloodborne pathogens are included in UNLV's Bloodborne Pathogens Program.

All recombinant DNA work shall be approved by the IBC before work is performed on campus. Investigators must fill out the Research Protocol Proposal Form and the UNLV BSL-2 SOPs form, found at: http://www.unlv.edu/Research/IBC/forms.htm.

C. Exempt Research

Under the *NIH Guidelines* some recombinant DNA research is exempt. Exempt research is listed as the following:

- Those that are not in organisms or viruses.
- Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.
- Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means.
- Those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).
- Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice of the Recombinant DNA Advisory Committee after appropriate notice and opportunity for public comment. See the following link for the NIH list: http://www4.od.nih.gov/oba/rac/guidelines_02/APPENDIX_A.htm.

Those that do not present a significant risk to health or the environment, as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment can be found at: http://www4.od.nih.gov/oba/rac/guidelines_02/APPENDIX_C.htm

The research listed above will need to be reviewed by the UNLV Biosafety Officer. He/she will make a recommendation to approve, disapprove or require modification of the proposal to the IBC Chair. The IBC Chair will then send an official response to the Principal Investigator.

XIX. Clinical Laboratory Work

A. General

Clinical laboratories examine human materials in order to provide information on diagnosis, prognosis, prevention, and treatment needed for disease. The materials found in clinical laboratories can be infectious and unknown to individuals working with the materials. General processing of clinical specimens is done safely at BSL-2.

B. Standard Practices

The standard guidelines listed under BSL-2 should be reviewed and incorporated as appropriate into protocols for clinical specimen work. When working with clinical specimens, protective clothing, including gloves and a disposable long-sleeved body covering (gown, laboratory coat, smock, coverall, or similar garment), shall be worn so that hands and arms are completely covered. Street clothes shall never be exposed to human specimens. In the event that street clothes become contaminated or potentially contaminated, they shall be properly decontaminated before they may be removed from the laboratory or disposed of as biohazardous waste. When handling clinical specimens, gloves should be worn.

Engineering controls, such as biological safety cabinets and splash shields, should be used for initial processing of clinical specimens and if splash of clinical material occurs.

Anyone working with clinical specimens is included in UNLV's Bloodborne Pathogens Program as outlined in UNLV's Exposure Control Plan. The Principal Investigator/ Supervisor should make their employees/students familiar with the UNLV Exposure Control Plan and a copy of the plan must be available in all laboratories.

* Adopted from *Michigan State University, "Biological Safety Manual", Environmental Health and Safety Office of Radiation, Chemical and Biological Safety,* October 2007.

XX. References

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XXI. Appendices

The following appendices contain forms (Biosafety Level 2 (BSL-2) SOPs, Biosafety Level 3 (BSL-3) SOPs, and Toxin SOPs) and the Institutional Biosafety Committee Policies and Procedures.

Appendix A (Available on-line at http://research.unlv.edu/IBC/forms.htm as a fill-in form format; a sample document is also available at the same URL)

UNIVERSITY OF NEVADA LAS VEGAS BSL-2 LABORATORY STANDARD OPERATING PROCEDURES (SOPS)

This SOPs document should include specific information for the laboratories and procedures being performed. It is meant to give detail in addition to UNLV's adopted* standard BSL-2 procedures (pages -) and Exposure Control Plan (available through rms.unlv.edu).

All faculty, staff and students should familiarize themselves with these procedures and sign page prior to starting work in this BSL-2 laboratory. Questions should be directed to the Principal Investigator. A copy of the SOPs must be forwarded to the UNLV Biosafety Officer and a copy must be retained in the laboratory's Biosafety Manual.

Principal Investigator:
BSL-2 Room Numbers:
Biohazards Being Used: (MSDS attach if available)
Description of Procedure(s):

Agent Description: The following agent (i.e. bacteria, virus, fungi, etc.), name of agent (genus and species) and strain of agent will be utilized in this procedure:

- Description of agent:
- Strain of agent (if applicable):

Disease: The agent utilized in this procedure has the ability to cause disease in humans and/or animals (signs and symptoms): (attach reference if available)

Hazards: The following materials and/or equipment associated with this procedure may present exposure hazards, health hazards, and/or physical hazards. Identify potential exposures that may occur during sample preparation, and/or experimental manipulations (i.e., use of sharps, aerosol generation during centrifugation, mixing or sonication, etc.):

Administrative Controls: The following administrative controls are in place to avoid exposures (i.e., training, signage, restricted entry, etc.):

Engineering Controls: The following safety equipment must be used when carrying out this procedure. (i.e., chemical fume hood, biological safety cabinet, sealed centrifuge rotors, etc.):

Protective Equipment: The following personal protective equipment must be worn when performing this procedure (type of glove, eye protection, laboratory coat, etc.):

Additional Special Handling Procedures: Include any transport between laboratories or buildings (i.e., secondary containment):

Decontamination/Clean-up Procedures: Specifics on products and procedures used to clean work areas. Include specifics on when these procedures will be performed and timing involved (i.e. contact time):

Labeling/Signage: Include any required labeling and signage (i.e. universal biohazard symbol label at the entrance doors and biohazard labels on all equipment being used to store BSL-2 agents):

Waste Disposal Procedures: Include specifics on collection, deactivation and transport for disposal:

Spill Response Procedures: Procedures to follow if a spill occurs. Procedures should include the materials that will be used to clean-up biological spills. The Biological Spill Response Procedure should include the type of disinfectant that will be used. A copy of the spill response procedure(s) should be available within the laboratory:

Injury/Exposure Response Procedures: Steps to be taken in the event of an exposure incident:

Unattended Operations: Portions of the experiment that may run unattended and steps taken to prevent accidental exposures:

Inventory Control Measures for Attenuated Strains - *Fill out section if applicable. If not applicable, then write not applicable (N/A)*:

- Include locations of locked storage rooms, cabinets, or freezers where attenuated strain(s) will be stored when not in use:
- The following individuals can only access the attenuated strain:
- Include how the inventory will be maintained and attach a copy of a blank inventory storage log sheet:

Training: Training courses will be taken by personnel performing this procedure (i.e. Biosafety Training, Chemical Hygiene Training, Bloodborne Pathogens Training) and additional training by PI/Supervisor:

Laboratory Facilities: Please check the items below that are available in the laboratory where the BSL-2 agent is used.

- Entrance/Exit door(s) have locks:
- Sink for hand washing:
- Furniture that is easily cleaned and decontaminated:
- Biological safety cabinet(s) with current certification sticker:
- Eyewash station:

Additional Laboratory Specific Safety Procedures:

I have read and understood all portions of these SOPs. I agree to contact the Principal Investigator should I have any questions or plan on making any modifications to the procedures detailed here.

NAME	SIGNATURE	DATE

Appendix B (Available on-line at http://research.unlv.edu/IBC/forms.htm as a fill-in form format)

UNIVERSITY OF NEVADA LAS VEGAS BSL-3 LABORATORY STANDARD OPERATING PROCEDURES (SOPS)

This SOPs document should include specific information for the laboratories and procedures being performed. It is meant to give detail in addition to UNLV's adopted* standard BSL-3 procedures (pages -).

All faculty, staff and students should familiarize themselves with these procedures and sign page prior to starting work in this BSL-3 laboratory. Questions should be directed to the Principal Investigator. A copy of the SOPs must be forwarded to the UNLV Biosafety Officer and a copy must be retained in the laboratory's Biosafety Manual. If applicable, please attach a copy of the completed National Select Agent Registry form(s) for the select agent or toxin you will be working with.

Principal Investigator:
BSL-3 Room Numbers:
Biohazards Being Used: (MSDS attach if available)
Description of Procedure(s):

APHIS-CDC Registration Number if applicable (or state if not applicable):

Agent or Toxin Description: The following agent or toxin (i.e. bacteria, virus, fungi, etc.), name of agent or toxin (genus and species, if applicable) and strain of agent or toxin that will be utilized in this procedure:

- Description of agent or toxin:
- Strain of agent or toxin:

Disease: The agent or toxin utilized in this procedure has the ability to cause disease in humans and/or animals (signs and symptoms): (attach reference if available)

Hazards: The following materials and/or equipment associated with this procedure may present exposure hazards, health hazards, and/or physical hazards. Identify potential exposures that may occur during sample preparation, and/or experimental manipulations (i.e., use of sharps, aerosol generation during centrifugation, mixing or sonication, etc.):

Administrative Controls: The following administrative controls are in place to avoid exposures (i.e., training, signage, restricted entry, etc.):

Engineering Controls: The following safety equipment must be used when carrying out this procedure. (i.e., chemical fume hood, biological safety cabinet, sealed centrifuge rotors, etc.):

Protective Equipment: The following personal protective equipment must be worn when performing this procedure (type of glove, eye protection, laboratory coat, etc.):

Additional Special Handling Procedures: Include any transport between laboratories or buildings (i.e., secondary containment):

Decontamination/Clean- Up Procedures: Specifics on products and procedures used to clean work areas. Include specifics on when these procedures will be performed and timing involved (i.e. contact time):

Labeling/Signage: Include any required labeling and signage (i.e. universal biohazard symbol label at the entrance doors and biohazard labels on all appliances being used to store BSL-3 agents or toxins):

Waste Disposal Procedures: Include specific methods for decontaminating all laboratory wastes (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method):

Spill Response Procedures: Procedures to follow if a spill occurs. Procedures should include the materials that will be used to clean-up biological spills. The Biological Spill Response Procedure should include the type of disinfectant that will be used. A copy of the spill response procedure(s) should be available within the laboratory:

Injury/Exposure Response Procedures: Steps to be taken in the event of an exposure incident:

Unattended Operations: Portions of the experiment that may run unattended and steps taken to prevent accidental exposures:

Inventory Control Measures for Agent or Toxin:

- Include locations of locked storage rooms, cabinets, or freezers where agent or toxin will be stored when not in use:
- The following individuals can only access the agent or toxin:
- Include how inventory will be maintained and attach a copy of a blank inventory storage log sheet:

Training: Training courses will be taken by personnel performing this procedure (i.e. Biosafety Training, Chemical Hygiene Training, Bloodborne Pathogens Training) and additional training provided by the PI/Supervisor:

Laboratory Facilities: Please check the items below that are available in the laboratory where the BSL-3 agent or toxin is used.

- Entrance/Exit door(s) have locks:
- Sink for hand washing:
- Furniture that is easily cleaned and decontaminated:
- Biological safety cabinet(s) with current certification sticker and located away from doors, heavily traveled laboratory areas:
- Eyewash station:
- Laboratory separated from areas that are open:
- Access to the laboratory is restricted to entry by a series of two self-closing doors(i.e. anti-room):
- Floors are slip resistant, impervious to liquids, and resistant to chemicals:
- Walls are constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated:
- Ceiling is sealed and finished in the same general manner as walls:

- Vacuum lines protected with HEPA filters, or their equivalent:
- Laboratory duct air ventilation system sustains directional airflow by drawing air into the laboratory from "clean" areas toward "potentially contaminated" areas:

HEPA Filtration System Procedures: Include information on HEPA filter housing and schedule for certifying the system annually:

I have read and understood all portions of these SOPs. I agree to contact the Principal Investigator should I have any questions or plan on making any modifications to the procedures detailed here.

NAME	SIGNATURE	DATE

Appendix C (Available on-line at http://research.unlv.edu/IBC/forms.htm as a fill-in form format)

UNIVERSITY OF NEVADA LAS VEGAS TOXIN LABORATORY STANDARD OPERATING PROCEDURES (SOPS)

This SOPs document should include specific information for the laboratories and procedures being performed. It is meant to give detail in addition to UNLV's adopted* standard Toxin Safety procedures (pages -).

All faculty, staff and students should familiarize themselves with these procedures and sign page prior to starting work in this laboratory. Questions should be directed to the Principal Investigator. A copy of the SOPs must be forwarded to the UNLV Biosafety Officer and a copy must be retained in the laboratory's Biosafety Manual.

Principal Investigator:

Toxin Use Room Numbers:

Toxins Being Used: (MSDS attached if available)

Description of Procedure(s):

Toxin Description: The following toxin (name of the toxin) will be utilized in this procedure:

- Description of toxin:

Disease: The toxin utilized in this procedure has the ability to cause disease in humans and/or animals (signs and symptoms):

Hazards: The following materials and/or equipment associated with this procedure may present exposure hazards, health hazards, and/or physical hazards. Identify potential exposures that may occur during sample preparation, and/or experimental manipulations (i.e., use of sharps, use of powdered toxins, aerosol generation during centrifugation, mixing or sonication, etc.):

Administrative Controls: The following administrative controls are in place to avoid exposures (i.e., training, signage, restricted entry, etc.):

Engineering Controls: The following safety equipment must be used when carrying out this procedure. (i.e., chemical fume hood, biological safety cabinet, sealed centrifuge rotors, etc.):

Protective Equipment: The following personal protective equipment must be worn when performing this procedure (type of glove, eye protection, laboratory coat, etc.):

Additional Special Handling Procedures: Include any transport between laboratories or buildings (i.e., secondary containment):

Decontamination/Clean-up Procedures: Specifics on products and procedures used to clean work areas. Include specifics on when these procedures will be performed and timing involved (i.e. contact time):

Labeling/Signage: Include any required labeling and signage (i.e. universal biohazard symbol label at the entrance doors and biohazard labels on all appliances being used to store toxins):

Waste Disposal Procedures: Include specifics on collection, deactivation and transport for disposal:

Spill Response Procedures: Procedures to follow if a spill occurs. Procedures should include the materials that will be used to clean-up biological spills. The Biological Spill Response Procedure should include the type of disinfectant that will be used. A copy of the spill response procedure(s) should be available within the laboratory:

Injury/Exposure Response Procedures: Steps to be taken in the event of an exposure incident:

Unattended Operations: Portions of the experiment that may run unattended and steps taken to prevent accidental exposures:

Inventory Control Measures for Toxin:

- Include locations of locked storage rooms, cabinets, or freezers where toxin will be stored when not in use:
- The following individuals can only access the toxin:
- Include how inventory will be maintained and attach a copy of a blank inventory storage log sheet:

Training: The following training courses will be taken by personnel performing this procedure (i.e. Biosafety Training, Chemical Hygiene Training, Bloodborne Pathogens Training) and additional training provided by the PI/Supervisor:

Laboratory Facilities: The items checked below are available in the laboratory where the toxin is used.

- Entrance/Exit door(s) have locks:
- Sink for hand washing:
- Furniture that is easily cleaned and decontaminated:
- Biological safety cabinet(s) with current certification sticker:
- Eyewash station:

Additional Laboratory Specific Safety Procedures:

I have read and understood all portions of these SOPs. I agree to contact the Principal Investigator should I have any questions or plan on making any modifications to the procedures detailed here.

NAME	SIGNATURE	DATE

Appendix D

Institutional Biosafety Committee

Policies and Procedures

November, 2008

CONTENTS

1. INTRODUCTION

The University of Nevada, Las Vegas (UNLV) is committed to protection of our faculty, staff, students and community through careful analysis of all biological research occurring at or affiliated with UNLV. The University recognizes and accepts responsibility, which it shares with its investigators and other researchers for determining that research involving biological materials meets or exceeds all federal, state, and local regulations.

These policies and procedures are intended to serve as a guide for registration for investigators and their staff who conduct research involving potentially hazardous biological materials. While these policies and procedures provide a general overview of the biological review process and the main regulatory requirements designed for human and environmental protection, the field of biosafety and biosecurity is continually evolving. Investigators should refer to the UNLV Institutional Biosafety Program for more in-depth information about biosafety. They should also ensure that they and their staff understand the information contained herein and follow any mandatory requirements, obtain additional information on any regulatory requirements or expectations relevant to their specific research, and contact the Institutional Biosafety Committee (IBC) with any questions they may have. As UNLV policy evolves, and rules change, the information will updated. Please make sure you have the latest information by checking the IBC Website: http://www.unlv.edu/Research/IBC/.

1.1 UNLV Institutional Biosafety Committee Roles and Responsibilities

Under *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* UNLV must establish an IBC that meets certain requirements and follows specific criteria for reviewing and approving Recombinant DNA Research. The IBC is required to

- conduct an independent assessment of the containment levels required by the *NIH Guidelines* for the proposed research;
- assess the facilities, procedures, practices, and training and expertise of personnel involved in recombinant DNA research;
- ensure that all aspects of Appendix M of the *NIH Guidelines* have been appropriately addressed by the Principal Investigator;
- ensure that no research participant is enrolled in a human gene transfer experiment until the RAC review process has been completed, Institutional Biosafety Committee approval (from the clinical trial site) has been obtained, Institutional Review Board approval has been obtained, and all applicable regulatory authorizations have been obtained;
- for human gene transfer protocols selected for public RAC review and discussion, consideration of the issues raised and recommendations made as a result of this review and consideration of the Principal Investigator's response to the RAC recommendations; ensure that final IBC approval is

granted only after the RAC review process has been completed;
- ensure compliance with all surveillance, data reporting, and adverse event reporting requirements set forth in the *NIH Guidelines*; and
- Notify the Principal Investigator of the results of the Institutional Biosafety Committee's review and approval.

The UNLV IBC also has the role of review and approval of all

- Human infectious agent research;
- Research involving human blood, cells, tissues and other potentially infectious human materials (OPIM);
- Research involving biological toxins; and
- Research involving select agents;
- Research involving exempt strains of select agents.

These activities cannot be carried out without prior IBC review and written approval.

1.2 The Foundation for IBC Review: *The NIH Guidelines*

The *National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* were developed to assure proper oversight of recombinant DNA research at institutions receiving funding from NIH for recombinant DNA research. Since 1976 these guidelines have been updated and published in the federal register. The latest version of the guidelines is available through NIH on their website. Compliance with these guidelines is required for any institution that wants to retain NIH funding.

2. OVERSIGHT OF BIOLOGICAL RESEARCH AT UNLV

2.1 Jurisdiction of the UNLV IBC

The IBC is responsible for reviewing all biological research falling within the following categories:

- Research sponsored by UNLV both funded and unfunded;
- Research conducted by or under the direction of any employee or agent of UNLV in connection with his or her responsibilities, even if conducted elsewhere;
- Research conducted by or under the direction of any employee or agent of UNLV using any property or facility of UNLV,

These categories cover <u>all</u> research in which UNLV and its faculty, staff, and students may be involved.

The IBC must review research under the direction of any employee or agent of UNLV even if it will be conducted at another institution. In such situations, the investigator and home institution remain legally responsible for the conduct of research at the other institution. Where another IBC also has jurisdiction over the research, the investigator should inform the UNLV IBC. The general policy of the UNLV IBC is to require submission of the project to the home IBC for review first, with submission to the other institution's IBC to follow. Final IBC approval at the home institution of the project as amended by the other IBC is required.

2.2 Reporting Relationships

The Vice President for Research and Graduate Studies is ultimately responsible for the oversight of biological research activities. The Institutional Biosafety Committee is responsible for overseeing the effective operation, policies, and compliance of the conduct of biological research at UNLV. No person or other committee--whether internal or external--can overturn an IBC decision to disapprove, terminate, or suspend a research protocol.

2.3 Scope of Authority of UNLV IBC

The UNLV Institutional Biosafety Committee has the following authority and responsibility over research at UNLV:

1. Review all research projects that will involve potentially hazardous biological materials prior to commencement of research;
2. Approve, disapprove, or require changes in all such research;
3. Notify federal government agencies and sponsors of approvals and disapprovals, or forward such notifications to investigators for submission as applicable;
4. Ensure prompt reporting by investigators to the RAC as well as any sponsoring agency of unanticipated problems involving risk to humans or environmental releases;
5. Ensure prompt reporting to the IBC by investigators of noncompliance with the IBC or federal policies or regulations, and report serious or continuing noncompliance to appropriate federal agencies;
6. Suspend or terminate a previously approved project and notify applicable agencies; and
7. Conduct continuing reviews of ongoing research as well as any other monitoring such research may require;

2.4 IBC Approval, Disapproval or Revision Decision

The IBC Chairperson notifies investigators by written document of approval, revisions, and disapprovals (or terminations), with enough detail to explain the decision to the investigator.

Should the IBC disapprove or terminate a research project, the principal investigator may request to present more information either in person or in writing to the IBC, explaining why he or she believes the project should be approved or continued. However, a final IBC decision to require modifications in, disapprove, suspend or terminate a project is incontrovertible. No other committee or official (University or Federal) can override these IBC decisions. Further, no committee or person can approve an investigator to conduct any research that the IBC has not approved.

2.5 Notification to RAC, NIH/OBA or Other Agencies of Approvals, Suspensions or Terminations, and Serious or Continuing Non-compliance

The IBC is required under the NIH Guidelines to ensure that the Principal Investigator submit certain protocols to the RAC for approval. It also must certify certain approvals and notify NIH/OBA regarding certain actions and activities. The IBC acts on behalf of the institution to certify the compliance of the project with the UNLV IBC Policies and Procedures to the relevant federal regulatory agencies and sponsors of the research, as applicable, and will provide such certifications to the principal investigators for forwarding to the applicable agency.

In the case of a suspension or termination, the IBC will consult with the Vice President for Research and Graduate Studies. The IBC will notify the funding agencies of the decision of the IBC.

Should the IBC receive a report of noncompliance with IBC policies or procedures or federal guidelines or regulations, the IBC will inform the Vice President for Research and Graduate Studies. If it appears that a project has been initiated without required IBC approval, or that other serious violations may have occurred, the IBC will require the investigator to suspend all activity at once. The IBC then implements procedures for investigating, remedying, and reporting noncompliance.

2.6 Serving as the IBC for an Unaffiliated Entity

Generally, the IBC reviews only research conducted at or involving UNLV employees, sponsorship, or information. However, on occasion, such as where another entity that does not have an IBC is the recipient of a grant under which UNLV faculty will be conducting the research (under a subcontract/award), the UNLV IBC may agree that it can serve as the IBC for the grantee. Where the UNLV IBC agrees to this arrangement, a memorandum of understanding (MOU) will be drafted and then signed by UNLV Vice President for Research and

Graduate Studies and the IBC Chair to permit the UNLV IBC to act as the review committee.

2.7 Cooperative Research

Cooperative research projects are those which involve more than one institution. In the conduct of such projects, each institution is responsible for biosafety review. Cooperative research being conducted by UNLV faculty/student at another university or facility will include UNLV IBC as the approval authority for such research. In such cases, UNLV IBC will review documents presented by the other university or facility and may seek consultation with its legal representative.

2.8 Research Conducted on UNLV Campus by Other Universities without UNLV Faculty as Co-Investigator

All research conducted by other universities without a UNLV faculty member as co-investigator will be required to have a memorandum of understanding (MOU) with appropriate signatures on file with the IBC. No research may begin until all approvals have been obtained. The protocol package must be submitted with approval from the requesting organization's IBC.

3. IBC MEMBERSHIP

The UNLV IBC is comprised of regular voting members and non-voting members. The IBC may utilize, as they deem necessary, non-voting members and consultant reviewers.

3.1 Regular Voting Members

3.1.1 Composition

The IBC shall have at least six regular voting members, including the Chair. The Chair may also serve as the animal or plant expert. At a minimum, the membership of the IBC shall include:

- A Biosafety Officer (Biological Safety Officer) with expertise in biohazard practices, procedures and containment;
- A Microbiologist, with knowledge of infectious disease research practices;
- An Animal Expert with knowledge of and experience with recombinant DNA research involving animals and containment protocols for animals;
- A Plant Expert with knowledge of and experience with recombinant DNA research involving plants and containment protocols for plants, plant pathogens and plant pests;
- Two (2) Community Members unaffiliated with UNLV.

The Chair shall select an alternate for an absent member and assure, in so far as possible, that the diversity of the membership in attendance reflects the overall IBC membership.

If human gene therapy protocol(s) are submitted, the committee must obtain expertise in this area either through membership or consultation.
The UNLV IBC generally will have more than the minimum number of members to ensure adequate and efficient reviews, as the Vice President for Research and Graduate Studies deems appropriate.

The Vice President for Research and Graduate Studies will appoint members to the UNLV IBC so that it will be sufficiently qualified through the experience and expertise of its members to review all biohazardous research at UNLV.

3.1.2 Terms

In general, UNLV IBC members are appointed for three-year terms. If a member is chosen to become the Chair, his or her term is extended as necessary. At the discretion of the Vice President for Research and Graduate Studies, memberships may be renewed.

3.1.3 Appointments

The Associate Vice President for Research is responsible for ensuring the appropriate composition of the UNLV IBC. To determine what expertise is needed, and who might be recommended to be appointed, he/she solicits recommendations for appointments from UNLV IBC members as well as the Chairs and Deans within UNLV schools and colleges. In addition, as he/she deems appropriate, the Associate Vice President for Research may solicit self-nominations from the faculty and full-time staff.

The Vice President for Research and Graduate Studies is the appointing authority for all IBC membership positions. Where a new community member is sought, the Vice President for Research and Graduate Studies will receive recommendations from the Associate Vice President for Research, knowledgeable UNLV faculty or may choose an alternative method of securing nominations. Solicitations for new members will highlight the desired qualifications based on gaps in the expertise of the UNLV IBC noted by IBC members or the Associate Vice President for Research. The Vice President for Research and Graduate Studies can appoint new members at his or her discretion.

3.2 Non-Voting Members

Members of UNLV staff or faculty may serve as non-voting members of the UNLV IBC should it be decided that such persons would be of assistance to the UNLV IBC in conducting their duties. A non-voting member cannot be counted in the quorum and cannot vote, but can participate in discussions and deliberations. The Vice President of Research and Graduate Studies may appoint a non-voting member who will serve for only as long as requested.

3.3 Consultants

The UNLV IBC may invite consultants to participate in discussions and deliberations on particular projects where they believe that additional expertise would assist in reviewing a particular protocol. The UNLV IBC Chair has the authority to invite such persons to participate. However, a consultant cannot be counted in a quorum, and cannot vote.

3.4 IBC Chair

The IBC will have a Chair chosen from the membership of the IBC, who is knowledgeable in biosafety, including the regulations, University and agency policies, and ethics relevant to such research. The Chair generally will serve for three-years. The Vice President for Research and Graduate Studies may in his/her discretion extend the term.

The Chair is responsible for conducting all meetings of the UNLV IBC. The chair is also responsible for reviews, delegating reviewers for protocols and initial reviews of adverse event reports. The Chair is responsible for ensuring that the IBC members are adequately informed about the requirements of the regulations for protocol review so that they conduct appropriate reviews.

The Chair will designate an appointee to serve in his/her absence. Whenever the Chair is not available, the designated appointee will assume the responsibilities of the Chair during the period of his or her absence.

3.6 Duties of IBC Members

Serving as an IBC member is considered to be an important role of faculty as well as an honor. It is recognized and appreciated that members serve in addition to their regular teaching, research and other service. Therefore, it is understood that on occasion a member may need to miss a scheduled IBC meeting. However, it is very important for continuity, scheduling, and well-rounded reviews that members attend IBC meetings. Membership is chosen based on the unique expertise that each member brings to an IBC. If a member cannot make a meeting,

he/she should notify the IBC Chair in advance (two weeks before meeting) so that an alternate can be secured. Because members serve at the pleasure of UNLV, failing to regularly attend meetings or the lack of diligence in performing duties will result in removal of a member from the IBC by the Vice President for Research and Graduate Studies.

3.7 Notification to NIH/OBA of Changes in IBC Membership

The Associate Vice President for Research will notify NIH/OBA in writing of changes in UNLV IBC membership.

4. IBC MEETINGS

The IBC must meet once during each semester and will be convened as needed during semester breaks.

4.1 Committee Meetings/Deadlines

IBC meetings are generally held during each quarter and as needed when research protocols require a vote. Protocols must be received two (2) weeks prior to the scheduled meeting of the full committee.

4.2 IBC Meeting Agenda

The Associate Vice President for Research prepares an agenda for each IBC meeting, listing all protocols that will be reviewed at the upcoming meeting (new and continuing), any adverse event reports to be reviewed by the committee, the projects that have been approved because they are exempt from the guidelines, and any other items for discussion.

4.3 IBC Meeting Procedures

Approvals of all protocols (all projects other than exempt) will be conducted only at convened meetings at which a majority of the members of the IBC are present. Any IBC member who is involved in any way in a research project being reviewed, or who has any other potential for conflict of interest, may not participate in the discussions or deliberations (other than to provide information as requested), nor vote on it. The IBC policy is to have such member leave the room during deliberations. When the Chair has a potential conflict of interest, he/she will designate someone to temporarily chair the meeting.

4.4 Actions that the IBC May Take at Meetings

UNLV IBC members will discuss each project and vote to approve or disapprove the project or proposed modification to an already approved project, or to defer a

decision until revisions are implemented, additional information is provided, or further expert review is obtained (including invitation of consultants). Under certain circumstances, if minor revisions in the submitted documents are required or a missing document of minor importance is to be obtained, the IBC may delegate the chair to subsequently issue an approval of the project on behalf of the IBC, upon completion of these tasks.

4.5 IBC Meeting Minutes

The office of the Associate Vice President for Research will prepare the IBC minutes of each meeting. The minutes will include the following information: 1) attendance; 2) actions taken by the IBC; 3) the number of members voting for, against, and abstaining in the decisions; 4) the basis for requiring changes in a project, or disapproving, suspending or terminating a project and 5) summary of the discussion of issues of concern and their resolution.

4.6 IBC Notification of Meeting Decisions

After each UNLV IBC meeting, the chair will notify the principal investigator in writing of the outcome of the review. The investigator will be informed, in writing, of whether the project was approved, whether it requires revisions before approval may be granted, whether additional information is needed from the investigator before approval can be voted upon, or whether it was disapproved (in sufficient detail for the investigator to understand). The investigator will also be informed at the time of approval, in writing, when an application for extension is due.

4.7 Time Sensitive Protocols

Normally, protocols must be received at least two weeks prior to the committee meeting at which they will be reviewed. This allows assigned reviewers enough time to conduct a thorough review prior to the scheduled meeting of the full committee.

On certain occasions, however, some protocols require a rapid response due to extenuating circumstance that fall beyond the control of the Principal Investigator. Protocols in this category may warrant a waiver of the required two-week submission period to allow a review by the IBC at the earliest regularly scheduled meeting or may require calling a meeting. In such cases, the protocol must be submitted with a memorandum explaining the circumstances giving rise to the request in enough detail that the request may be considered. The IBC Chair will make a determination whether the protocol qualifies for special handling and if it does, will advance the protocol to the next IBC meeting for early review or convene an IBC meeting. The investigator will be notified by the IBC Chair of the decision to grant or deny the request.

5. LEVELS OF IBC REVIEW: EXEMPT, FULL COMMITTEE REVIEW

Not all research requires review and approval by the IBC. Some research is "exempt" under federal regulations, but may still be reviewed according to UNLV policy. This category of research is reviewed by the UNLV biosafety officer. Investigators are required to submit all potentially biohazardous research to the UNLV IBC to formally determine the category of research:

5.1 Exempt Research

Under the *NIH Guidelines* some recombinant DNA research is exempt. Exempt research is listed as the following:

- Those that are not in organisms or viruses.
- Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.
- Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means.
- Those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).
- Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice of the RAC after appropriate notice and opportunity for public comment. See Appendix A: http://www4.od.nih.gov/oba/rac/guidelines_02/APPENDIX_A.htm.

Those that do not present a significant risk to health or the environment, as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See Appendix C: http://www4.od.nih.gov/oba/rac/guidelines_02/APPENDIX_C.htm

The research listed above and other categories detailed in section 5.3 of this document will be reviewed by the UNLV Biosafety Officer. He/she will make a recommendation approve, disapprove or require modification of the proposal to the IBC Chair. The IBC Chair will then send an official response to the Principal Investigator.

5.2 Full Committee Review

If the proposed research falls under the full-board review category detailed in section 5.3 of this document, all IBC members will be provided electronic copies of the protocol. All committee members will be expected to review the protocol

and submit questions to the IBC Chair or designee at least two business days prior to the next convened committee meeting. At that meeting, the Principal Investigator submitting the protocol must be available to answer questions (either in person or by telephone). The full committee will vote to approve, disapprove or require modification of the protocol. All experiments in the full-board review category require approval prior to initiation of the research.

5.3 Types of Research in Each Review Level

When protocols are submitted, they will be forwarded from the Associate Vice President for Research to the Biosafety Officer. The Biosafety Officer will determine which level the protocol should be reviewed at based on the following table:

Biosafety Officer Review	Full-Board Review
Exempt rDNA research as detailed by the *NIH Guidelines for Research Involving Recombinant DNA*	In vitro experiments using known BSL-2 agents
BSL-1 in vitro experiments where rDNA agents contain less than 2/3 of the genome of any animal virus	In vitro experiments using primary human material in combination with BSL-2 agents
Research involving established human cell lines not known to contain infectious agents	BSL-1 in vitro experiments where more than 2/3 of the genome of any animal virus is used
Research involving human sera or tissue from non-established or unknown sources	BSL-1 and BSL-2 in vivo experiments (IACUC approval required)
	Experiments using risk group 2 agents as host-vector systems
	Research involving human material likely to contain BSL-3 agents
	BSL-3 in vitro experiments
	BSL-3 in vivo experiments (IACUC approval required)
	Human gene transfer research (IRB approval required)
	Experiments using risk groups 2, 3, 4 or restricted agents as host-vector systems
	Experiments involving toxins or immune modulators
	Experiments involving use of infectious or defective DNA or RNA viruses in the presence of a helper virus in tissue culture systems
	Experiments involving viable rDNA-modified microorganisms tested in whole animals.
	Experiments involving plants infected with agents recognized to have potential for serious detrimental impact on managed or natural ecosystems
	Experiments involving greater than 10 liter volume cultures of rDNA containing organisms
	Biosafety Level change requests by the P.I. or any member of the IBC
	Any IBC research proposal requested to be brought before the Full-Board

6. CRITERIA FOR UNLV IBC REVIEW AND APPROVAL OF RESEARCH

6.1 General UNLV IBC Review

The *NIH Guidelines* detail the following specific items that must be reviewed for recombinant DNA research. These items must be reviewed on any submitted protocol including those for infectious agents, biological toxins and human materials:

- An independent assessment of the containment levels required by the *NIH Guidelines* for the proposed research;
- An assessment the facilities, procedures, practices, and training and expertise of personnel involved in the research;

In addition, the reviewer should assure that the protocol meets requirements specified in the *UNLV Institutional Biosafety Program*.

6.2 Additional Requirements for Select Agent Studies

The U.S. Departments of Health and Human Services (HHS) and Agriculture (USDA) published final rules for the possession, use, and transfer of select agents and toxins (42 C.F.R. Part 73, 7 C.F.R. Part 331, and 9 C.F.R. Part 121) in the Federal Register on March 18, 2005. All research conducted at UNLV must first satisfy these regulations (or subsequent amendments) then may be submitted for review to the UNLV IBC. As part of this submission, documentation of approval by the appropriate federal agency must be attached. IBC reviewers of select agent protocols must take into account requirements of the above listed federal regulations.

6.3 Additional Requirements for Biosafety Level 3 Studies

All research conducted at Biosafety Level 3 must comply with the UNLV Biosafety Manual (and latest edition of the CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories*). An assurance must be attached to all Biosafety Level 3 protocols detailing appropriate facilities and staff training for this type of research. All facilities shall be commissioned as BSL-3 laboratories. Appropriate protective devices (Biological Safety Cabinets, etc.) must be demonstrated as certified prior to approval of any such protocol.

6.4 Additional Requirements for Studies Involving Vertebrate Animals

All protocols involving biohazards and vertebrate animals must attach a copy of their UNLV Animal Care Protocol. For research involving vertebrate animals, coordination will be made during review of the protocol with the UNLV Animal Care and Use Committee to address any animal or biosafety issues involved with

the protocol. The supervisor of laboratory animal care will report any animal care concerns at the IBC meeting during review of the protocol.

Approvals for biohazard studies involving animals will be granted conditional approvals with the statement "This approval is for the biosafety portion of your protocol. You must maintain current animal care approvals during the entire project. If at any time your animal care protocol is discontinued or denied this IBC protocol is no longer approved".

6.5 Additional Requirements for Studies Involving Humans

For any research involving biohazards and human subjects UNLV IRB approval must be attached to the IBC protocol. Also, any involvement of recombinant DNA in human studies must be reviewed and approved by the RAC prior to initiation of the study as detailed in the *NIH Guidelines*. For research involving humans, coordination will be made during review of the protocol with the UNLV OPRS to address any human or biosafety issues involved with the protocol. The OPRS director will report any IRB concerns at the IBC meeting during review of the protocol.

Approvals for studies involving biohazards and humans will be granted conditional approvals with the statement "This approval is for the biosafety portion of your protocol. You must maintain current IRB approvals during the entire project. If at any time your IRB protocol is discontinued or denied this IBC protocol is no longer approved".

7. UNLV IBC MONITORING AND INVESTIGATOR REQUIREMENTS REGARDING RESEARCH IN PROGRESS

7.1 Amendments in Protocol

To obtain approval for proposed changes, investigators should submit an amendment form. The IBC will vote on major amendments to previously approved non-exempt projects during their meeting. Amendments to exempt projects and minor changes to non-exempt project will be reviewed by the UNLV Biosafety Officer. Minor changes include deletions of personnel, room changes (at BSL-2 or below), additions of qualified personnel and additional safety measures. Minor amendments may be approved by the IBC Chair based on the Biosafety Officer's recommendation in between meetings. Investigators <u>are not</u> permitted to implement any amendments without approval by the IBC except to eliminate apparent immediate hazards.

The date of approval of an amendment does not change the date by which the regularly scheduled continuing review of the project is to be completed.

7.2 Required Reporting of Adverse Events and Problems

A "serious adverse event" is any event occurring at any dose that results in any of the following outcomes: death, a life-threatening event, in-patient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization also may be considered a serious adverse event when, upon the basis of appropriate medical judgment, they may jeopardize the human gene transfer research subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

An adverse event is "associated with the use of a gene transfer product" when there is a reasonable possibility that the event may have been caused by the use of that product.

An "unexpected serious adverse event" is any serious adverse event for which the specificity or severity is not consistent with the risk information available in the current investigator's brochure.

Any of these events or any event involving exposure of any person to infectious materials, biological toxins or human blood or OPIM must be reported within 5 days of the event in writing to the Chair of the IBC. Even if an adverse effect was anticipated by the protocol (and disclosed to a subject), <u>if the effect has changed in nature, severity, or frequency in the study</u>, this must be reported to the IBC. Required reporting also includes, but is not limited to, any procedural errors during the research, a breach in confidentiality or privacy, emotional disturbances, noncompliance with the regulations or IBC policies, or any other problems occurring during the research. Investigators should err on the side of caution when determining whether an event is reportable to the IBC. Life-threatening adverse events must be reported to the IBC within 24 hours.

Adverse event reports, when involving recombinant DNA, must be reported under the *NIH Guidelines*. Any significant problems with or violations of the *NIH Guidelines* and any significant research-related accidents or illnesses must be reported to the appropriate institutional official and NIH/OBA within 30 days, unless the Institutional Biosafety Committee determines that a report has already been filed by the Principal Investigator. Reports to 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).

In the case of any adverse event involving hazardous biological materials, an IBC meeting will be called at the earliest date that a quorum will be possible. The IBC's review of adverse event reports will focus on reasons for exposures and any risks that may have changed. The IBC will determine if there is a need to revise

the protocol and whether approval should continue. Also, increased monitoring, training and safety measures may be required.

7.3 Additional Measures to Monitor Active Research Projects

In its discretion, and depending upon the perceived risk of the research, an IBC may require more active monitoring of a research project. The IBC can make this determination during their initial review of the research project.

To remain active, all non-exempt protocols must be reviewed no less than every three years. The IBC may require more frequent reviews if it considers that more oversight is necessary due to the nature of the study or degree of risk. The investigator will be informed in the original approval notice when the next review must be obtained, and may be reminded prior to expiration of the approval period (at least one month ahead of time). However, it is the explicit responsibility of the investigator to ensure that his/her project is approved for extension before expiration.

The principal investigator should submit a completed Protocol Renewal Form well in advance of the expiration date (minimum of 30 days). If approval for continuation has not been issued by the IBC prior to the expiration date, the investigator must terminate the research, and the project will need to be reviewed and approved as a new study.

The purpose of renewal review is to re-assess any safety risks based on the latest biosafety information. Modifications occurring during the three year period between reviews must be submitted via modification forms. In the renewal form Investigators should summarize the status of the study, and report any advancement or changes generally in the area under study that may impact the safety of continuing the study.

8. RECORD KEEPING

8.1 IBC Records

The Office of the Associate Vice President for Research maintains a file for each study, containing the following information: 1) research protocol(s) (all versions of the protocol are retained); 2) any approval documents from other committees or agencies; 3) notifications of IBC decisions, 4) records of protocol renewal activities, 5) reports on amendments and adverse events, and 6) correspondence between IBC and investigators of the project.

The files on a research project will be retained for at least three years after completion of the research.

8.2 IBC Meeting Records

Agendas and minutes of the IBC meetings are stored either on the computer or by hard copy and are kept indefinitely. Upon request, UNLV must make available to the public all IBC meeting minutes and any documents submitted or received from funding agencies which the latter are required to make available to the public.

8.3 IBC Member Records

Curricula vitae of active members of the IBC will be maintained in the files of the office of the Associate Vice President for Research, and will be updated in content as necessary. Each member's membership term status will be monitored and updated, as necessary.

9. EVIDENCE OF TRAINING

All Investigators are required to complete the Laboratory Specific SOPs included in the UNLV Biosafety Program prior to initiation of research for their respective projects. This document must be included in the IBC file. Additional documented training on the protocol may also be submitted for evidence of training.

Appendix E

Procedures for Amending this Program Manual

Amendments to this document may be proposed by a member of the IBC. The amendment may be adopted by a majority vote.