UNIVERSITY OF
NOTRE DAME

Biosafety Manual

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UNIVERSITY OF NOTRE DAME BIOSAFETY MANUAL
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1.0 PURPOSE

To ensure a safe working environment for all University activities and for compliance with all applicable federal, state, and local regulations concerning the use of biological agents, biological toxins, select agents, and recombinant DNA in the laboratory The precautions and guidelines in this Biosafety Plan are compatible with current knowledge and regulations.

2.0 SCOPE

This Biosafety Manual applies to all laboratory employees working in laboratories that use biological agents, biological toxins and recombinant DNA.

3.0 ACRONYMS AND DEFINITIONS

ABSL -Animal Biological Safety Level
BL - Biological Safety Level
CDC - Center for Disease Control
ESPM - Equipment Surplus Property Management
HEPA - High-Efficiency Particulate Air
IACUC - Institutional Animal Care and Use Committee
IBC - Institutional Biosafety Committee
MSDS - Material Safety Data Sheet
NIH - National Institutes of Health
NSF - National Sanitation Foundation
NRC - National Research Council
ORDA - Office of Recombinant DNA Activities (NIH)
OSHA - Occupational Safety and Health Administration
PI - Principal Investigator
RAC - Recombinant DNA Advisory Committee (NIH)
rDNA - Recombinant DNA
RM&S - Risk Management and Safety
OR - Office of Research

Biological Toxin - A colloidal proteinaceous poisonous substance that is a specific product of the metabolic activities of a living organism and is usually very unstable, notably toxic when introduced into the tissue, and typically capable of inducing antibody formation.

Biosafety Level 3 Facility - A facility specifically designed for the use of Class 3 organisms. Formerly referred to as a P3 facility.

Blood-Borne Pathogens - Pathogenic microorganisms that are present in human blood that can cause disease in humans. These pathogens include, but are not limited to HBV and HIV.

Baseline Serum - A blood sample drawn from a human for archiving for future reference by a physician.

Class I Biosafety Cabinet - An enclosure with an inward airflow through the front opening. Provides protection for the worker and the laboratory environment but not to product being utilized in the cabinet.

Class II Biosafety Cabinet An enclosure with an inward airflow through the front opening. Provides protection to the worker, the environment, and the product being utilized in the cabinet.

Class 1 Organisms - Oganisms not known to cause disease in healthy adults.

Class 2 Organisms - Organisms associated with human disease, infectious through auto-inoculation ingestion, mucous membrane exposure.

Class 3 Organisms - Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences.

Containment - Used to describe safe methods for managing infectious agents in the laboratory environment where they are being handled and maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.

Host - Organism in which the rDNA replicates.

Negative Airflow - Directional airflow from areas exterior to a laboratory into the laboratory.

Primary (p) Containment - methods to protect the internal laboratory environment.

rDNA - DNA prepared by breaking up and splicing together DNA from several different species of organisms.

rDNA Insert - That (those) strand(s) of foreign DNA being inserted into the host/vector.

Secondary (s) Containment - methods to protect the environment external to the laboratory.

Select Agent - is defined by CDC and USDA as biological agents or toxins deemed as a threat to the public, animal or plant health, or to animal or plant products.

Sharps - Any object that can penetrate the skin, e.g., needle, scalpel, knife, etc.

Vector - Carrier used to introduce rDNA into the host system and that facilitates replication.

4.0 RESPONSIBILITIES

4.1 Institutional Biosafety Committee - The Institutional Biosafety Committee (IBC), a University Committee appointed by the President, has the ultimate responsibility for biosafety and shall establish regulations and guidelines, as defined in this Biosafety Manual, for the safe use of biohazardous materials at the University of Notre Dame. The duties of the IBC are:

1. Oversee all research and teaching activities involving biohazardous agents including review and approval prior to initiation, annual reviews and updates, reviews of laboratory safety equipment and procedures, and certification of compliance with all applicable rules and regulations governing the use of biohazardous materials.
2. Advise University faculty, staff and students in matters related to biohazards and biosafety with their respective areas of responsibility.
3. Develop, recommend, and implement policies and procedures for biological risk assessment and biological risk reduction throughout the University.
4. Develop emergency plans for the containment and resolution of accidental spills and other related emergencies with an emphasis on risk reduction, personnel protection, and environmental protection.
5. As an agent of the Institution, ensure that all principal investigators are sufficiently trained in appropriate containment practices, secondary containment procedures, accidental spill containment, and their responsibilities as principal investigators.
6. Conduct investigation of serious violations or problems and to make recommendations to the Faculty member, Department Chair, Dean and/or Provost.

4.2 Risk Management and Safety Department (RM&S)

1. Provide industrial hygiene and safety support for all laboratory operations.
2. Transport and dispose of all infectious waste in compliance with all applicable federal, state, and local ordinances.
3. Assist, as necessary, in the emergency response, cleanup, and decontamination of biological spills and accidents.
4. Shut down research or teaching activities in laboratories that do not meet the necessary standards for the biosafety level required for the agents being used.
5. Maintain compliance with all shipping requirements for biological agents and toxins including certifying individuals to ship specific Hazardous Materials or Dangerous Goods.
6. Provide assistance in determining shipping requirements for all biological samples and be responsible for shipment if the sample falls under the dangerous goods classification.

7. Provide initial and annual retraining on biosafety level criteria, biosafety practices, and biohazardous waste.
8. Conduct annual laboratory inspections to ensure compliance with appropriate regulations and biosafety guidelines.

4.3 Office of Research (OR)

1. Provide the necessary liaison between Principal Investigators, the Institutional Biosafety Committee, granting agencies, and regulatory agencies.
2. Serve as the Office of Record for documentation involving the Institutional Biosafety Committee.
3. Provide all necessary documentation, forms, regulatory guidelines and regulations, etc. for Principal Investigators.

4.4 Institutional Animal Care and Use Committee (IACUC)

1. Provide appropriate animal care that meets or exceeds federal, state, and local requirements and specifications.
2. Ensure that animal housing systems are designed and utilized in a manner that will minimize the potential exposure of other animals or personnel to potentially biohazardous agents.
3. In cooperation with the investigator and the Institutional Biosafety Committee, develop and implement specific standard operational procedures, in adherence to the ABSL classification of the agent being used addressing animal care, research procedures, and procedures in case of accident or equipment failure.
4. Ensure that all animal care personnel are adequately trained and aware of the potential risk associated with each agent.
5. Develop emergency plans for handling accidental spills, personnel exposures, unintentional animal exposure, equipment failure, etc.

4.5 Principal Investigator (PI)

1. Ensure compliance with appropriate National Institute of Health guidelines and all conditions stated in the protocol approved by the Institutional Biosafety Committee.
2. Submit protocol applications for all activities or modifications of activities involving biohazardous materials and obtain approval by the Institutional Biosafety Committee prior to initiation of the activities or modifications.
3. Ensure that all laboratory staff, including students, are trained in the accepted procedures in laboratory practices, containment methods, disinfectant and disposal practices, and required actions in the event of an accidental spill.
4. Develop a Laboratory Safety Plan, including an emergency action plan for accidents and spills, as an addendum to this manual, when required.

5. Ensure proper handling and disposal of all infectious wastes as outlined in the *Infectious Waste Guide* (see Appendix C).
6. Request immunizations for laboratory personnel when working with biological agents for which there is an effective vaccine available.
7. Maintain all biosafety equipment in appropriate operating condition. Decontaminate laboratory equipment prior to maintenance or disposal.
8. Maintain records of microorganisms and toxins used in the laboratory and biosafety cabinets.

4.6 Laboratory Worker

1. Conduct no activities under the research protocol until the protocol is approved by the IBC and appropriate training is completed.
2. Follow all procedures and containment methods established for activities conducted.
3. Properly utilize all laboratory protective equipment including proper clothing, personal protective equipment, and containment devices.
4. Report all accidents and spills to the Principal Investigator or RM&S as soon as possible.
5. Report unsafe conditions to the Principal Investigator, RM&S, or the Institutional Biosafety Committee.
6. Notify RM&S of any biological sample needing to be shipped (whether in the United States or internationally) at least 48 business hours prior to the desired shipping date.

5.0 REGULATORY COMPLIANCE

Recombinant DNA activities - The NIH *Guidelines for Research Involving Recombinant DNA Molecules* governs all rDNA activities including those exempt by the guidelines.

Non-rDNA activities involving microorganism and exempt rDNA microorganism - Activities involving these agents are not federally regulated but it is the position of the IBC and RM&S that the procedures and containment levels outlined in CDC publication *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) will govern such activities at the University.

Biological Toxins - These agents are not governed by NIH or CDC regulations or guidelines. Although Material Safety Data Sheets (MSDS) are available for most of these agents, specific exposure levels, to our knowledge, have not been established. RM&S will work with the PI to interpret the MSDS and to establish work and disposal procedures which will protect the users of the materials and the environment outside the laboratory.

Blood and Other Body Fluids - OSHA's standard on bloodborne pathogens will govern any activity involving human blood or other potentially infected body fluids. Compliance with this standard is administered by RM&S. Information on the university's blood-borne pathogen program can be found in the Blood-Borne Pathogens Policy.

Tuberculosis - OSHA has published guidelines for activities which potentially expose people to tuberculosis pending publication of an OSHA Standard regulating such exposures in the clinical setting. Compliance with the current guideline and the future standard is administered by RM&S. Research activities involving *Mycobacterium tuberculosis* will be governed accordingly by NIH or CDC guidelines. Should the OSHA standard apply to the research environment in the future, those requirements will be made available to the PI's.

Chemicals - Chemical usage in educational and research laboratories are governed by OSHA Standard 1910.1450, *Occupational Exposures to Hazardous Chemicals in Laboratorie*s, and is administered by RM&S.

Radioactive Materials and Radiation-Producing Devices - Regulated by the Nuclear Regulatory Commission and the Indiana State Department of Health-Radiological Health Section. The radiation safety program is administered by the Radiation Control Committee and enforced by the Radiation Safety Officer. The Radiation Safety Manual contains university procedures for using radioactive materials and radiation-producing devices.

6.0 SUMMARY OF BIOSAFETY LEVELS

Assignment of Biosafety Levels (BL) - It is the responsibility of the PI to initially assign the BL to his/her protocol. This level may be changed either upward or downward during review by the IBC. All parties involved in assigning BLs will follow the standard levels of 1, 2 or 3 as outlined in either NIH's *Guidelines for Research Involving Recombinant DNA Molecules* or CDC's *Biosafety in Microbiological and Biomedical Laboratories.* The BLs correspond directly to the class of organism to be used in a protocol. The classes are defined as follows:

Class 1	Organisms not known to cause disease in healthy adults.
Class 2	Associated with human disease, infection transmitted through autoinoculation, ingestion, and mucous membrane exposure.
Class 3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences.
Class 4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission.

7.0 PROTOCOL SUBMISSION AND REVIEW

Mandatory Submission of Protocol Application - PI's proposing research/academic activities involving microorganisms (including exempt and non-exempt rDNA activities) and biological toxins must complete the university's Biosafety Protocol Application form and submit it to Office of Research, for review and approval action prior to initiation of the activity.

Voluntary Submission of Protocol Application - Researchers working with blood or other potentially infectious body fluids, carcinogens, mutagens or teratogens in the absence of microorganisms are requested to complete the university's Biosafety Protocol Application form and forward to IBC/RM&S to assistant in the administration of the OSHA programs governing

occupational exposures to these agents. The review of these protocol applications will be undertaken by IBC in cooperation with RM&S. There will be no assignment of a biosafety level for these protocols.

7.1 Protocol Review and Approval

1. rDNA Activities - All protocols involving rDNA activities must follow the requirement of the National Institutes of Health as presented in the latest edition of theNIH *Guidelines for Research Involving Recombinant DNA Molecules* and all supplements published thereafter in the Federal Register. In the case of gene therapy protocols, transferring drug resistence to organisms not known to acquire the trait naturally, and the cloning of highly potent toxins, it is the responsibility of the PI and the IBC to ensure review by the NIH as required under current government guidelines. Non-exempt BL 1 and 2 and exempt rDNA protocols not requiring review by federal agencies can be approved by the IBC. Areas to address when rDNA protocols are being prepared for submittal to OR are:
   a. rDNA Insert
      (1) Synthetic and associated sequence(s)
      (2) Potential protein product
   b. Vector
      (1) Carrier used to introduce rDNA into the host system that facilitates replication
      (2) Plasmids, organelles, viruses
   c. Host
      (1) Organism in which the rDNA replicates
      (2) Bacteria, yeast, plant, animal cells
   d. Containment - Several containment methods are described below:
      (1) Biological Containment:
         (a) Limit infectivity of vector or vehicle for specific hosts
         (b) Limit dissemination and survivability of host and/or vector in the environment
      (2) Physical Containment:
         (a) Specifically designed equipment and facilities used to physically contain microbes
         (a) Limit access to facilities
   e. Good Laboratory Practices:
         (a) Specifically designed practices and procedures used to physically contain microbes
         (b) Mechanisms for inactivation and disposal of microbes

2. Non-rDNA Protocols Involving Microorganisms or Toxins - IBC approval is required for all protocols involving microorganisms. Protocols involving biological toxins in the absence of microorganisms will be under the review authority of the IBC, but will not be assigned a biosafety level.

8.0 TRAINING

8.1. All IBC members, PI's, and laboratory staff members conducting activities involving microorganisms or biotoxins, are required to receive training in biosafety, regardless of the level of activity they propose to use (BL1, BL2, or BL3). This commitment can be met by attending the training sessions given by the IBC and/or RM&S or viewing the video training film approved by the IBC. Record of attendance will be maintained by RM&S. Additionally, the PI is responsible for the development and administering of training to their Laboratory staff members and students.

This training shall address biosafety and laboratory safety relative to the activities on-going in the laboratory. Training should include, but not necessarily be limited to, procedures and techniques, laboratory safety rules, emergency response, spill containment and cleanup, and instructions on the operating parameters and procedures for use of laboratory equipment (chemical fume hood, biosafety cabinets, autoclaves, centrifuge, etc.). **Some equipment, when used improperly, can give less than desired results and may even result in accidents with severe injury to the user.**

PIs must provide training to lab personnel regarding lab specific SOPs. Records of training must be maintained by PI and a copy sent to RM&S.

8.2. Training must be given to all new laboratory employees or students and all members of the laboratory staff should receive annual refresher training provided by Risk Management. Record of attendance and the training provided should be maintained by the RM&S. Records are to be kept for a minimum of three (3) years. Biosafety references are available through RM&S which can assist the PI in meeting training requirements. PI's will also ensure that everyone involved in their laboratory operations also participate in other training requirements relative to health and safety as administered by RM&S. Examples of this training would include, but not necessarily be limited to, Blood-Borne Pathogens, Hazards Communications, Chemical Hygiene Plan, Care and Usage of Laboratory Animals, etc.

9.0 CONTAINMENT

Definition - Containment is the use of safe methods for managing infectious agents in the laboratory environment where they are to be handled or maintained.

Purpose - to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.

9.1 Types of Containment

1. Primary (p):methods to protect the internal laboratory environment, e.g., microbiological techniques and appropriate safety equipment.
2. Secondary (s):methods to protect the environment external to the laboratory, e.g., facility design and operational practices.

9.2 Required Levels of Containment - In general, the requirements listed below are considered minimum methods for containment at the appropriate BL. The PI is responsible for referring to NIH's *Guidelines for Research Involving Recombinant DNA Molecules* or CDC's *Biosafety in Microbiological and Biomedical Laboratories* for specific containment requirements. The PI is also responsible for ensuring that secondary methods, e.g., facility design features or special practices are incorporated into the protocol when primary or standard practices are not sufficient for containment. The IBC, in its review of protocols, will give serious consideration for the need of additional containment.

1. BL1: Laboratory work involves the use of organisms not known to cause disease in healthy adults and present a minimal potential hazard to laboratory personnel and the environment. Special containment equipment is not required and work is generally conducted on the open bench top.
   Laboratory Practices include:
   - Standard Microbiological Practices (p)
   - Required Personal Protective Equipment (p)
   - Open bench top sink required (s)
2. BL2: Laboratory work involves the use of agents that present a moderate potential hazard to laboratory personnel and the environment. With the added hazards, access to the laboratory is limited when work is being conducted and training is given to the laboratory personnel on the proper handling and the hazards of the organisms being used. A procedural guide for handling of agents may be needed. A biological safety cabinet is used for material manipulations where there is a potential for aerosols being generated.
   Laboratory Practices include:
   - BL1 practices (p) plus:
   - Limited access

- Biohazard warning signs
- "Sharps" (needles, scalpel, knives, etc) precautions
- Lab Safety Plan as addendum to this manual when required.
- Class I or II biosafety cabinet or other physical containment device used for all manipulations of the agents that cause splashes or aerosols of infectious materials.
- Laboratory coats, gloves, face protection
- Autoclave (s)

3. BL3: Laboratory work involving indigenous or exotic agents that have the potential to cause serious illness or are lethal if inhaled. Access to the laboratory work area is restricted. The laboratory personnel are given specific training regarding proper handling and potential hazards of the agent that is being used. At this level of containment there is a specific need for administrative and procedural guidelines for operations.

   Laboratory Practices include:

   - BL2 practices plus
   - Controlled access
   - Decontamination of waste before removal from laboratory
   - Decontamination of lab clothing before laundering
   - Baseline serum (when appropriate, considering the agent(s) handled) will be taken and archived.
   - Class I or II biosafety cabinet or other physical containment devices used for all manipulations of agents
   - Protective lab clothing, gloves, eye, face protection, respiratory protection, as needed
   - Physical separation from access corridor
   - Self-closing, double door access
   - Exhausted air not recirculated
   - Negative airflow into laboratory

10.0 RECOMBINANT DNA

Recombinant DNA molecules are constructed outside living cells by joining foreign* natural or foreign* synthetic DNA segments to DNA molecules that can replicate in a living cell. Synthetic DNA segments likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) will be considered as equivalent to their natural DNA counterparts. If the synthetic DNA segment is not expressed "in vivo" as a biologically active polynucleotide or polypeptide product, it is exempt from the Guidelines.

*Rearrangements involving the introduction of DNA from different organisms or different strains of an organism will be considered recombinant DNA. Deletions, single-base changes and rearrangements within a single genome will not involve the introduction of foreign DNA and therefore would not be considered recombinant DNA.

10.1 GENERAL APPLICABILITY

The recombinant DNA guidelines are applicable to all recombinant DNA research within the United States or its territories which is conducted at or sponsored by an institution that receives any support for recombinant DNA research from the National Institutes of Health. Any individual receiving support for research involving recombinant DNA must be associated with or sponsored by an institution that can and does assume the responsibilities assigned in these guidelines.

10.2 CONTAINMENT

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information exists in the design of physical containment facilities and the selection of laboratory procedures applicable to organisms carrying recombinant DNAs. The existing programs can be divided into two categories: (i) A set of standard practices that are generally used in microbiological laboratories; and (ii) special procedures, equipment, and laboratory installations that provide physical barriers which are applied in varying degrees according to the estimated biohazard. Four biosafety levels consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and the hazards posed by agents and for the laboratory function and activity. Biosafety level 4 (BL4) provides the most stringent containment conditions, BL1 the least stringent.

Experiments on recombinant DNAs by their nature lend themselves to a third containment mechanism (iii), namely, the application of highly specific biological barriers. In fact, natural barriers do exist which limit either (1) the infectivity of a "vector" or "vehicle" (plasmid or virus) for specific hosts, or (2) its dissemination and survival in the environment. The vectors that provide the means for replication of the recombinant DNAs and/or the host cells in which they replicate can be genetically designed to decrease by many orders of magnitude the probability of dissemination for recombinant DNAs outside the laboratory.

10.3 GUIDELINES FOR COVERED EXPERIMENTS

Experiments involving recombinant DNA can be divided into four general classes as described below.

1. Experiments which require specific IBC review and NIH and IBC approval before initiation of the experiment.
2. Experiments which require IBC approval before initiation of the experiment.
3. Experiments which require IBC notification at the time of initiation of the experiment.

4. Experiments which are exempt from the procedures of the GUIDELINES.
IF AN EXPERIMENT FALLS INTO BOTH CLASS "A" AND ONE OF THE OTHER CLASSES, THE RULES PERTAINING TO CLASS "A" MUST BE FOLLOWED!

Class A Experiments - Require specific RAC (Recombinant DNA Advisory Committee) review and NIH and IBC approval before initiation of the experiment.

Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH, the publication of the Federal Register for thirty days of comment, review by the RAC, and specific approval by NIH. Included in this guideline (taken from the Federal Register, Vol. 51, No. 88, Wed. May 7, 1986, 16960) are the following criteria.

a. Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxic molecules lethal for vertebrates at an LD50 of less than 100 ng per kg body weight (e.g., microbial toxins such as botulinum toxins, tetanus toxin, diphtheria toxin, Shigella dysenteriae neurotoxin).
b. Deliberate release into the environment of any organism containing DNA.
c. Deliberate transfer of a drug resistant trait to microorganisms that are not known to acquire it naturally if such acquisition could compromise the use of the drug to control disease agents in human or veterinary medicine or agriculture.
a. Deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into human subjects.

Class B Experiments - Experiments which require IBC approval before initiation of the experiment.

Investigators performing experiments in this category must submit to their IBC, prior to initiation of the experiments, a registration document that contains a description of the source(s) of DNA, the nature of the inserted DNA sequences, the hosts and vectors to be used, whether a deliberate attempt will be made to obtain expression of a foreign gene, and if so what protein will be produced, and the containment conditions specified in this Guideline. Registration document must be dated and signed by the investigator and filed only with the local IBC. The IBC shall review all such proposals prior to initiation of the experiments. Included in this guideline (taken from the Federal Register, Vol. 51, No. 88, Wed. May 7, 1986, 16960) are the following criteria:

a. Experiments using human or animal pathogens (Class 2,3,4, or 5 agents) as host vectors.
b. Experiments in which DNA from human or animal pathogens (Class 2,3,4,or 5) agents is cloned into nonpathogenic prokaryotoic or lower eukaryotic host-vector systems.

c. Experiments involving the use of infectious animal or plant DNA or RNA viruses or defective animal or plant DNA or RNA viruses in the presence of helper virus in tissue culture systems.
d. Recombinant DNA experiments involving whole animals or plants.
e. Experiments involving more than 10 liters of culture. Physical containment for large-scale uses of organisms containing recombinant DNA molecules should be used.

Class C Experiments - Experiments which require IBC notification at the time of initiation of the experiment.
Experiments not included in "A or B" categories are to be considered as Class C experiments. All such experiments can be carried out at BL1 containment. For experiments in this category, a registration document as described in for Class B experiments must be dated and signed by the investigator and filed with the local IBC at the time of initiation of the experiment. The IBC shall review all such proposals, but IBC review prior to initiation of the experiment is not required. For example, experiments in which all components derive from non-pathogenic lower eukaryotes fall under Class C experiments and can be carried out at BL1 containment.

Class D Experiments - Exempt from Guidelines.
The following recombinant DNA molecules are exempt from the guidelines for research involving recombinant DNA molecules and no registration with the IBC is necessary.
a. Those that are not in organisms or viruses.
b. 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.
c. 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; also, 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).
d. Certain specified recombinant DNA molecules 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 Director, NIH, with the advice of the RAC (Recombinant DNA Advisory Committee) after appropriate notice and opportunity for public

comment.

e. Other classes of recombinant DNA molecules - if the Director, NIH, with advice of the RAC, after appropriate notice and opportunity for public comment, finds that they do not present a significant risk to health or the environment.

11.0 USE OF ANIMALS

11.1 The use of animals for teaching and research is controlled by the Institutional Animal Care and Use Committee (IACUC) which is administered through the Office of Research. The Animal Welfare Act of 1990, "Guide for the Care and Use of Laboratory Animals" (1986) and the Public Health Service Policy on "Humane Care and Use of Laboratory Animals" (1986) contain the federal requirements that are currently regulated and enforced by the IACUC.

11.2 All activities (teaching, research, and demonstrations) involving animals on the campus (or animal research involving any faculty/staff member conducted elsewhere) must be approved by the IACUC protocol procedure currently in operation.

11.3. The purchasing of all animals on campus can occur only with the written approval of the IACUC.

11.4 Animal protocols that involve the treatment of animals with pathogen, toxic substances or other biohazardous agents must be circulated for approval by the IBC. The IBC will determine the Biosafety Level for the project. Animal Biohazard Level 2 or higher must be used.

11.5 All research and teaching with rodents that do not involve biohazards other than the animals themselves should be conducted with all the Biosafety 1 practices in effect.

11.6 Work with high risk animals (i.e. monkeys or other primates) may require more stringent procedures which are equivalent to the requirements of Biosafety 2 procedures.

11.7 The IACUC will conduct periodic inspections of all animal facilities to determine compliance with federal standards.

12.0 LABORATORY BIOSAFETY MANUAL ADDENDUMS

Where the hazard associated with a particular agent cannot be controlled using the guidance of

this manual and those of the NIH's *Guidelines for Research Involving Recombinant DNA Molecules* and/or CDC's *Biosafety in Microbiological and Biomedical Laboratories* or by requirements levied by the IBC, the PI will develop safety protocols for the process or agent in question. The protocols will serve as an addendum to this manual. Where required, special practices will be included in the manual as well as emergency response procedures for handling spills. Staff members and students must read and understand the lab specific protocols, this manual as well as the Chemical Hygiene Plan.

13.0 BL 3 FACILITY: TESTS AND CERTIFICATIONS

13.1 The minimum certification requirements necessary before operations in a BL3 facility can begin are as follows:

1. Access Control - May be a single laboratory module or a complex of modules within a building or an entire building. The facility must be separated by a controlled access zone from areas open to the public and other laboratory personnel who do not work in the BL3 facility.
2. Penetration Seals -The openings in walls, floors and ceiling through which utility services and air ducts penetrate must be sealed to permit space decontamination
3. Directional Airflow - The ventilation system supporting the containment facility must be capable of controlling air movement. The direction of airflow is to be from areas of lower contamination potential to areas of higher contamination potential. The system is balanced so that there is infiltration of air into the facility from the adjacent corridors. The infiltration rate should be at least 50 cubic feet per minute. Certification of directional air movement should be accomplished at least quarterly or whenever it is suspected that a deficiency exists. Annual verification will be completed by RM&S. RM&S, in conjunction with the PI, will develop procedures for the directional air movement tests.
4. Exhaust Air Ducts - No cross connection between supply air duct and exhaust air ducts is permitted.

13.2 For safe practice guidelines for BL3 refer to BL3 SOPs. (After Appendix E)

14.0 BIOSAFETY CABINETS

14.1 Class II (vertical laminar flow) biological safety cabinets (BSC) provide a partial containment system for the safe handling of pathogenic microorganisms. To ensure safety, BSCs must be used correctly with good microbiological techniques and be in proper mechanical working order. Cabinets must be certified for performance upon installation using National Sanitation Foundation (NSF) Standard #49, section 6. Recertification must be conducted annually or during the interim if the cabinet is moved or if a problem is suspected.

14.2 General Guidelines

1. The cabinet should be left running.
2. If adjustable, the window should be lowered to 8 inches, with a $\geq$ 75 ft/min face velocity.
3. Keep the amount of equipment used or stored in the cabinet to a minimum.
4. Before work is started, everything needed for the procedures should be placed in the cabinet, and let cabinet air exhaust for a few minutes.
5. Nothing should be placed on or blocking the front or rear grilles.
6. Contaminated items should be segregated from clean ones and located so that they never have to pass over clean items.
7. Avoid disrupting the air barrier in a safety cabinet by frequent and rapid arm movements and bringing hands in and out of the cabinet.
8. Waste containers should be placed inside the cabinet to avoid breaking the air barrier and bringing contaminated items out into the room.
9. Do not use a burner inside a Class II BSC because the air currents induced counter to the normal air flow, can cause contamination of the work surface and ignite ethanol and other materials in the cabinet and damage the HEPA filter.
10. When working with biohazards, keep absorbent towels and decontaminating solutions in the cabinet and wipe down work surface with ethanol prior to and at the completion of each session, and after any small spills.
11. Decontaminate all equipment removed from the cabinet. Pipetting aids and tools that are used repeatedly should remain in the cabinet.
12. Decontamination of the entire cabinet (filter, plenums, work surfaces and the fan) is achieved by exposing these areas to a paraformaldehyde vapor. This type of decontamination must be performed only by a certified professional.

14.3 Electricity failure during the use of a BSC

Should the power to the unit fail during use, stop work with biohazardous agents immediately, seal all cultures securely, and decontaminate the work area with a suitable disinfectant.

14.4 Horizontal Laminar Flow Hoods are present in a number of laboratory facilities.

These clean benches provide a very clean environment but must be used only for the manipulation of non-hazardous materials. Since the operator sits in the downstream exhaust from the clean bench, this equipment must never be used for the handling of toxic, infectious, or sensitizing materials, including volatile

chemicals, cell culture materials (except plant cell cultures), or drug formulations.

15. 0 INCUBATORS

Incubators can become the inadvertent and undesired repository of microorganisms. Although they may present a hazard to laboratory workers, most often they are a source of contamination of laboratory cultures. Besides the moist surfaces, rubber gaskets, the humidity trough if present, and the fan mechanism are areas in which contaminating microorganisms concentrate.

15.1 It is recommended that an anti-microbial agent such as Zepharin chloride be added to the humidity source water (do not use sodium azide).

15.2 Panels, trays and other removable parts should be autoclaved and the gaskets and non-removable parts wiped thoroughly with 70% ethanol every 2 months.

16.0 DISPOSAL OF INFECTIOUS MATERIALS (See Appendix C)

17.0 REPORTING OF EXPOSURE INCIDENTS AND SPILLS

17.1. Exposure Incidents - PI's are required to report all incidents which result in a spill of infectious material and/or an exposure to individuals which could result in illness or disease. For infectious materials RM&S must be notified. *Occupational/Nonoccupational Exposure to Blood-Borne Pathogen*s protocols must be followed.

17.2 Handling Spills of Infectious Materials -Containment and cleanup procedures for spills of infectious materials are contained in the *Infectious Waste Disposal Guide.* A copy of the guide is contained in Appendix C of this manual.

19.0 PACKAGING AND SHIPMENT OF BIOLOGICAL MATERIALS

19.1 Shipping packages containing hazardous materials, biological materials or agents, or dangerous goods will always be an important part of the freight business. Likewise, the use of hazardous materials will always be an important part of scientific research. On occasion, it becomes necessary to ship hazardous materials to another researcher, to another university, to another research facility, or even to a manufacturer. These hazardous materials can include compressed gases, flammable liquids and solids, oxidizers, poisons, corrosive materials, radioactive and biological materials and even dry ice.

19.2 Federal hazardous materials regulations (49 CFR parts 171-180) have outlined specific shipping requirements for these hazardous materials. If these materials are offered for transport by a commercial carrier (FedEx, Airborne, UPS), the shipment becomes regulated by the Department of Transportation (DOT) and

sometimes by international agencies. When materials are shipped out of the country, items that may not be considered hazardous in the United States may be classified as hazardous in other countries. To comply with shipping regulations, these hazardous materials must be properly classified, documented, packaged, and handled. For shipments of biological and radioactive materials, transport or export permits and/or authorization may be required prior to shipment.

19.3 Failure to meet these regulatory requirements may result in citations, fines and/or imprisonment. Fines to the University can range from $250 to $500,000 per violation. In addition, individual researchers and shippers may be subject to criminal penalties of up to $500,000 and five years imprisonment.

Federal law also requires that anyone who is involved in or responsible for preparing or transporting a hazardous material must have DOT training and certification.

No one is exempt from these federal transportation requirements.

19.4 RM&S personnel (1-5037) will provide assistance with package selection, material classification and documentation. Please call at least 48 business hours prior to the date of desired shipment. Additional time may be required for overseas packages. Also, even if we can have a package ready for shipment, prior arrangements must be made with most carriers to have these materials picked up.

Hazardous materials cannot be left at drop-off locations.
They must be received from an individual.

19.0 MAINTENANCE/REPAIR AND DISPOSITION OF EQUIPMENT

19.1 It is the PI's responsibility to ensure that equipment and work surfaces which are contaminated or potentially contaminated with chemical, infectious and/or radiological materials are properly decontaminated before clearance is given for any maintenance/repair or custodial activities. This includes, but may not be limited to the activities of Maintenance (maintenance/repair and custodial), Machine Shop, contract personnel or factory representatives. Appendix D provides insight into the method of choice for disinfecting various equipment and surfaces.

19.2 Disposition of Equipment- PI must document decontamination procedures taken prior to disposal. RM&S must be notified prior to any equipment leaving the University.

APPENDIX A REFERENCES

1. National Institute of Health publication, *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines),* January 1996 or as periodically updated

2. CDC publication, *Biosafety in Microbiological and Biomedical Laboratorie*s, 4th Edition

3. NIH publication, *Laboratory Safety Monograph, A Supplement to NIH Guidelines for Recombinant DNA Research*, January 1979

4. Risk Management and Safety, *Chemical Hygiene Plan*, February 2002

5. Occupational Safety and Health Act (OSHAct), Part 1910, Subpart Z, Section1910.1030, *Blood-borne Pathogens*, December 1991

6. National Research Council, *Biosafety in the Laboratory,* National Academic Press, Washington DC (1989).September 1996 B-1

7. The Rockefeller University, *The Safety Manual*

8. Risk Management and Safety, Hazardous Material/Dangerous Goods Shipping Policy

Appendix B Bloodborne Pathogens Exposure Plan

Table of Contents

1.0 Purpose

To ensure adequate protection for University employees, faculty and staff against exposure to potentially infectious blood-borne materials. The requirements of this plan are designed to meet or exceed the Federal requirements defined in 29 CFR 1910.1030.

2.0 Scope

This plan applies to all applicable activities that involve the potential exposure to blood or potentially infectious materials. Potentially infectious materials include all bodily fluids or non-intact tissue of the body. There are a number of occupational positions and laboratory personnel that are covered by this plan. They include:

1. University Health Services employees, including Physicians, Nurses, Nursing Assistants, Housekeeping Staff who are assigned to University Health Services and other medical assistants.
2. Fire Department employees, including Captains, Firefighters and on-call Firefighters.
3. Athletic Department Employees, including Athletic Trainers and Assistant Athletic Trainers.
4. Security employees, including Police Officers and Security Officers.
5. Food Services employees, of any position, who are designated to clean up blood or other potentially infectious material spills or who have First Aid duties. This includes the North Dining Hall, South Dining Hall, Food Service Support Facility and all satellite operations on campus.
6. St. Michael's Laundry employees who may be responsible for handling soiled linens which have a potential to be contaminated with potentially infectious materials or who clean up potentially infectious material spills.
7. Athletic Grounds employees who are responsible for vomit and bodily fluid clean up duties during or following athletic events.
8. RecSports employees who are responsible for assisting injured guests or who clean up blood or potentially infectious materials at the facility.
9. Custodial and Building Services employees of the University (including those at the Joyce Center, Loftus, Rolf's RecSports Center and LaFortune) who may be responsible for the clean up of blood or other potentially infectious materials at their facility.

10. Laboratory personnel who work with blood or potentially infectious materials through their research. This includes Technicians, Graduate Students, Assistants, Principal Investigators and Undergraduate Students.

3.0 Terminology:

Bloodborne Pathogen:
A bloodborne pathogen is a virus found in human blood which can be transmitted from person to person and causes diseases in humans.

Potentially Infectious Materials:
All bodily fluids and non-intact tissue of the body.

Exposure Incident:
A specific eye, nose, mucous membrane or open lesion contact with blood or other potentially infectious materials.

Occupational Exposure:
An exposure incident which occurs while the person is performing job tasks.

Regulated Waste:
Waste that contains blood, semen or vaginal secretions. These discarded materials shall be labeled as Biohazardous Waste and shall not be discarded into the regular trash. Biohazardous Waste shall also be double bagged.

4.0 Methods of Protection:

Universal Precautions, as defined in Appendix A, shall be practiced to prevent contact with blood or other potentially infectious materials that may result in an Occupational Exposure. The following methods of prevention are consistent with the Universal Precautions and shall be followed when encountering such substances:

1. Engineering Controls:

Engineering controls shall include, but are not limited to:

a) The use of devices or equipment for purposes of making physical contact with blood or potentially infectious materials without putting the person at risk of exposure. These devices or equipment will need to be sanitized or discarded following contact with blood or other potentially infectious materials. Examples may include mops, tongs, tweezers, tools, etc.
b) Hand washing facilities or antiseptic hand cleanser or towlettes and cloth or paper towels. These can be used to ensure proper hygiene.

c) Practices following the handling of blood or other potentially infectious materials.
d) Appropriate sharps containers and storage devices to minimize the risk of accidental cuts or punctures.
e) Appropriate pipetting devices which minimize potential exposure to the mouth and face and hands.
f) The use of designated blood clean up kits.

2. Workplace Practices:

Workplace practices should be conducive in minimizing unanticipated exposure to blood or other potentially infectious materials including;

a) Proper hand washing practices.
b) Proper identification and awareness of potentially infectious sources.
c) Proper laundering of contaminated clothing. All contaminated clothing shall be properly labeled and sent to St. Michael's Laundry for safe cleaning.
d) Treating every bodily fluid and every person as if they were potentially infectious.
e) Proper communications between people who will be handling potentially infectious sources (signs, labels, etc).

3. Personal Protective Equipment:

When engineering controls are not enough to insure that there is no potential for an exposure incident, personal protective equipment shall be used as a barrier. Personal protective equipment will be provided to employees at no cost to them and shall be selected by Risk Management and Safety. Such personal protective equipment includes, but is not limited to:

a) Latex or Nitrile Gloves
b) Goggles
c) Face shields
d) Tyvek suites or equivalant
e) CPR barrier devices

4. Signs and Labels:

Signs and labels shall be used as a communications tool for all people who may come into contact with potentially infectious materials. These signs and labels shall be used when potentially infectious materials are being passed on to another party. Red is the universal color that represents Biohazards. Also, see Appendix B for the Universal symbol. The following circumstances would require the use of signs or labels:

a) Regulated waste containers.
b) Items being sent to be laundered that are soiled with potentially infectious materials.

c) Items being stored which are potentially infectious.
d) Evidence items to be analyzed or held for later use.
e) Items of potentially infectious materials being mailed out or shipped to another person or location.

5. Vaccines:

Currently there are no vaccinations for HIV, Hepatitis C or most other bloodborne pathogens. However, there currently is a vaccination available for protection against Hepatitis B. The University will make available and will pay for the Hepatitis B Vaccination series for all employees who are considered to be at high risk for exposure (those who may be reasonably expected to come into physical contact with people while they perform medical treatment activities, such as healthcare professionals, ambulatory professionals, athletic trainers and law officers). If such employees elect not to receive the vaccination series, they shall complete the "declination form" (See Appendix C).

a) Hepatitis B vaccinations will be made available to the "high risk" employees as follows:
    1) At no cost to the employee.
    2) New employees within 10 days of initial assignment.
    3) Antibody testing when necessary to determine employee immunology.
    4) Supervisors of new employees who are candidates for the vaccination shall contact Risk Management and Safety upon hiring them to initiate the vaccination and training process and to obtain the necessary forms.

5.0 Occupational Exposure Procedures

1. The procedure for employees who experience an occupational exposure is as follows:
    a) Wash thoroughly the site of the exposure with anti-microbial soap and water immediately following the exposure.
    b) Notify his/her supervisor and explain what happened.
    c) Obtain a completed "Supervisors Report of Injury" form from his/her supervisor
    d) Seek medical attention at University Health Services.

2. Post Exposure Evaluation and Follow-up shall be consistent with the following:
    a) The supervisor shall also complete a "First Report of Employee Injury/Illness" form and send it to Risk Management & Safety.
    b) The supervisor shall insure that he/she provides to University Health Services the identification of the source individual if possible.
    c) It will be at the discretion of the medical professional as to the need for further evaluation, such as follow up testing of the employee's blood and the need for counseling.
    d)

6.0 Clean up Responsibilities

1. Each area of the University has an appropriately trained person or person's who are responsible for the clean up of blood or potentially infectious materials based on the following:
    a) Employees and students who have been through the University's Bloodborne Pathogens Training, provided by Risk Management & Safety or University Health Services, are designated to clean up blood or potentially infectious material spills that occur in their work areas.
    b) Blood or potentially infectious material spills which occur inside University buildings, in an area outside of a trained person's work or research area, shall be cleaned up by the designated Building Services person (trained custodial employee) who is on duty at that time or who is designated to do so by his/her supervisor.
    c) Blood or potentially infectious material spills which occur outside of a building, but on University property shall be cleaned up by a trained person in consultation with Risk Management & Safety.
    d) Blood or potentially infectious materials that are on a person's body shall only be cleaned up by a trained first responder, althletic trainer, University Health Services employee or a designated first aid responder who is trained. Employees who are not trained in bloodborne pathogens protection are not expected to perform CPR or First Aid on accident victims or injured employees.

2. Only approved cleaning products may be used to clean up blood or potentially infectious materials. Specific products and instruction will be provided for Building Service personnel and other designated spill clean up employees during training provided by the department of Risk Management and Safety. All products must be approved by Risk Management & Safety.

7.0 Record Keeping

1. The university will establish and maintain accurate records for each employee with occupational exposures as required in 29 CFR 1910.20(1) and for training as required in 29 CFR 1910.20(2).

8.0 Training

1. The University will provide information and training as required in 29 CFR 1910.1030(6)(2) to employees and students listed in section 2.0 of this policy. This training will include, but is not limited to the following:
    a) An accessible copy of this policy shall be made available along with an explanation of its contents.

b) A general explanation of the epidemiology and symptoms of bloodborne diseases.
c) An explanation of the modes of transmission of bloodborne pathogens.
d) An explanation of the appropriate methods for recognizing tasks and other activities that may involve exposure to blood and other potentially infectious materials.
e) An explanation of the use and limitations of methods that will prevent or reduce exposure, including appropriate engineering controls, work practices, signs and labels and personal protective equipment.
f) An explanation of the basis for the selection of personal protective equipment, including when it should be used, how to don, doff, adjust and wear it, it's limitations and the proper care, maintenance and disposal of it.
g) Information on the hepatitis B vaccine, including information on it's efficiency, safety, methods of administration, the benefits of being vaccinated, and that the vaccine will be offered free of charge to them.
h) Information on the appropriate actions to take and persons to contact in an emergency involving blood or other potentially infectious materials.
i) An explanation of the procedures to follow if an exposure incident occurs, including the method of reporting the incident and the medical follow-up that will be made available.
j) Information on the post-exposure evaluation and follow-up that the employer is required to provide for the employee following an exposure incident.
k) An explanation of the signs and labels and/or color coding required by paragraph (g)(1).
l) An opportunity for interactive questions and answers with the person conducting the training session.
m) Information on proper collection, handling and disposal of infectious waste (see Appendix D).

Appendix A Universal Precautions

According to the concept of Universal Precautions, all human blood and certain body fluids are treated as if known to be infectious for HIV (Human Immunodeficiency Virus), HBV (Hepatitis B Virus) and other bloodborne pathogens. These body fluids include semen, vaginal secretions, cerebrospinal fluid (brain and spine), synovial fluid (joint), pleural fluid (lung), pericardial fluid (heart), peritoneal fluid (abdominal), amniotic fluid (pregnancy), saliva, any body fluid that is visibly contaminated with blood and all body fluids in situations where it is difficult or impossible to differentiate between body fluids.

Even though other body substances are not known to transmit HIV, HBV or other bloodborne diseases, we shall always take the following precautions (an example of these substances may include urine, stool, respiratory secretions, vomit and wound drainage):

1. *Blood and body fluid precautions must be used consistently for ALL patients and accident victims.*

2. *Appropriate barrier precautions must be used routinely to prevent skin and mucous membrane exposure when contact with blood or potentially infectious materials is anticipated.*

    A. *Latex or Nitrile gloves must be worn when it is reasonably anticipated that there may be hand contact with blood or other potentially infectious materials, mucous membranes and non-intact skin;*

    B. *Single use latex or nitrile gloves must always be replaced as soon as practical when visibly contaminated, torn, or when their ability to function as a barrier is compromised. They must not be washed or decontaminated for re-use.*

    C. *Masks and protective eyewear or face shields must be worn during procedures that are likely to generate droplets of blood or other body fluids to prevent exposure of mucous membranes of the mouth, nose and eyes.*

    D. *Gowns must be worn during procedures that are likely to generate splashes of blood or other potentially infectious materials.*

E. *Mouthpieces, resuscitation bags and other respiratory equipment must be available so that mouth to mouth resuscitation can be avoided.*

F. *All contaminated items must be disposed of into designated infectious waste bags.*

3. *Hands and other skin surfaces must be washed immediately and thoroughly with soap and water or flush mucous membranes with water immediately or as soon as possible following contact with blood or other potentially infectious materials. Hands must also be washed following removal of gloves.*

4. *Safety precautions must be followed to prevent injuries caused by needles or other sharp instruments during procedures, when cleaning used instruments, during disposal of used needles and when handling sharp instruments after procedures.*

5. *Specimens of blood or other potentially infectious materials must be placed in a container that prevents leakage during collection, handling or transporting. Specimens are considered to be biohazard and the container must be labeled or color coded to identify it as a "Biohazard."*

6. *Contaminated laundry must be handled as little as possible.*

    A. *All contaminated laundry must be placed and transported in bags or containers appropriately labeled or color coded.*

    B. *All contaminated laundry must not be rinsed.*

7. *All equipment and working surfaces must be cleaned and decontaminated after contact with blood or other potentially infectious materials.*

8. *Broken glassware that may be contaminated must not be picked up directly with the hands.*

In Summary:

- Every person is a potential source of infection.

- Treat all body fluids as if infectious.

- Use precautions on everyone.

- Protect yourself through workplace practices and personal protective equipment

- Protect others through the use of appropriate containers and signs and labels.

- Universal precautions must be observed to prevent contact with blood or other potentially infectious materials and are mandatory.

Appendix B

Universal Symbol
For
Biohazard

Appendix C / BBP Policy
Hepatitis B Vaccination Declination (Mandatory)

I understand that due to my potential occupational exposure to blood or other potentially infectious materials, I may be at risk of acquiring hepatitis B virus (HBV) infection. I have been given the opportunity to be vaccinated with a hepatitis B vaccine, at no charge to myself. However, I decline the hepatitis B vaccine at this time. I understand that by declining this vaccine, I continue to be at a greater risk of contracting the HBV virus through my occupational exposure to blood or other potentially infectious materials and that this is a serious disease. If, in the future, I continue to have this potential exposure to blood or other potentially infectious materials and I want to be vaccinated with the HBV vaccine, I may receive the vaccination series at no charge to me.

Employee Signature	**Date**
Employee printed name	**Witness Signature**

APPENDIX C INFECTIOUS WASTE DISPOSAL GUIDE

The University of Notre Dame, in its continual efforts to protect the health and safety of its employees, students, and guests, has instituted an infectious waste disposal program. The program is required under Indiana State Board of Health regulations, Title 410.

The University recognizes that the various researchers and departments involved with infectious material and waste go to great extremes to insure that the material is handled as safely as possible and protection is afforded at all times. The University also recognizes that the disposal of infectious waste has become a national problem and it is with this understanding adopts the following guidelines. These guidelines are instituted in order to further safeguard the employees and students who handle infectious waste products and include the responsibilities of individuals, researchers, and departments in the safe handling of disposal of infectious waste.

Infectious waste, while a very broad term, is generally accepted to include waste that epidemiological evidence indicates is capable of transmitting dangerous communicable diseases.

A. **Definition of infectious waste includes but is not limited to:**

1. Cultures and stocks of infectious agents and associated biologicals including cultures from medical and pathological laboratories; cultures and stocks of infectious agents from research and industrial laboratories, waste from the production of biologicals; discarded live and attenuated vaccines and culture dishes and devices used to transfer, inoculate, and mix cultures.
2. Pathological wastes which includes body tissues and their containers.
3. Human or animal blood and blood products including liquid waste, human and animal blood, products of blood, items saturated or dripping with human or animal blood; items that were saturated and dripping that are now caked with dried human or animal blood; serum, plasma, and other blood components and their containers which were used or intended for use either in patient care, testing, laboratory analysis, research, or the development of pharmaceuticals. Intravenous bags are also included in this category.
4. Used and unused sharps that have been used in animal or human patient care, treatment or medical research, or industrial laboratories; including hypodermic needles, syringes (with or without the attached needle), Pasteur pipettes, scalpel blades, blood vials, needles with attached tubing, and culture dishes (regardless of presence of infectious agents). Also included are other types of broken or unbroken glassware that were in contact with infectious agents such as used slides and cover slips.
5. Contaminated animal carcasses including whole contaminated animal carcasses, body parts, and bedding of animals that were know to have been exposed to

infectious agents during research (including research in veterinary hospitals), production of biologicals or testing of pharmaceuticals.

6 Other waste that has been intermingled with infectious waste including any material (i.e., paper products, plastic products, and disposables) that has at any time been in contact with or believed to have been in contact with any infectious agent.

B. Infectious Waste Generator Responsibilities

It shall be the responsibility of the infectious waste generator (i.e., individual, researcher, department) to adhere to the following guidelines and procedures as listed:

1. All infectious waste (except sharps and sharps containers, animal carcasses and contaminated bedding) shall be rendered innocuous through autoclaving or treatment with bleach (5% sodium hypochlorite solution) prior to discard as regular trash.
2. Any autoclaved or treated waste suitable for disposal as regular trash must be BROWN bagged PRIOR to disposal. Disposal of brown bag waste into a solid waste dumpster is the responsibility of the researcher or department.
3. For infectious wastes that cannot be autoclaved or treated, the waste shall be segregated and labeled as follows:

    (1) Used and unused sharps shall be placed in the container identified as <u>USED AND UNUSED SHARPS</u> or similar wording which indicates the type of waste.

    (2) Infectious cultures and stocks, pathological waste, and human blood products should be placed in the container labeled <u>CULTURES, PATHOLOGICALS, BLOOD,</u> or similar wording which indicates the type of waste.

    (3) Contaminated animal bedding shall be placed in the container labeled <u>ANIMAL BEDDING</u> or similar wording which indicates the type of waste.

    (4) Contaminated animal carcasses shall be properly packaged, frozen and secured.

4. It shall be the individual department's responsibility to provide protective garments as necessary to persons involved in infectious agent research or infectious waste handling. See the University's Personal Protective Equipment Policy for guidance or contact Risk Management and Safety.
5. All infectious wastes must be located in the appropriate container, the container lids are kept on, and the general area is maintained in a clean and sanitary condition.
6. Keep infectious waste storage areas secured or otherwise protected from unauthorized entry.

C. Risk Management and Safety Responsibilities

It is the responsibility of Risk Management and Safety to collect, treat (if necessary) and dispose of infectious waste generated at the University of Notre Dame. Representatives of Risk Management and Safety will collect the various containers of infectious waste and animal

carcasses on a scheduled basis and transport these materials to the department's waste processing facility.

It is also the responsibility of Risk Management and Safety to maintain records indicating the amount (in pounds) of each class of waste material and notation of researcher or department prior to final disposal. Risk Management and Safety shall also maintain responsibility for the final disposal of infectious waste by the following means:

1. Contract with a licensed infectious waste disposer to transport and dispose of infectious waste at licensed incinerators.
2. Utilize other available technology and approved means of waste disposal as appropriate.

Risk Management and Safety will provide, at cost to the individual researcher or department, adequate containers with appropriate labeling to be located in secured building or department areas (as selected upon mutual agreement between Risk Management and Safety and the researcher or department). The biohazard symbol will be displayed at all storage areas. As required, the containers will be as follows:

1. Sharp Containers
    a. Leakproof, rigid and puncture resistant
    b. Labeled with the biohazard symbol
2. Other infectious Waste
    a. Impervious to moisture
    b. Of sufficient strength
    c. Secured
    d. Labeled with the biohazards symbol

At all times prior to disposal, infectious waste will be stored in closed containers in a secure area, protected from adverse environmental conditions and identified with the biohazard label.

It shall be the responsibility of Risk Management and Safety to provide necessary instruction and training in Universal precaution procedures and all other applicable guidelines regarding the safe handling or transportation of infectious waste. Records of instruction including an attendance record shall be maintained by Risk Management and Safety.

All records, including waste shipments, weights, departments, disposal, instruction, training attendance and any other pertinent or necessary records shall be maintained by Risk Management and Safety.

D. Violations of the Guidelines

Violations of any of the above guidelines will be handled as any violation of University guidelines by Human Resources in conjunction with the individual's supervisor and Risk Management and Safety.

Appendix D Use of Disinfectants

		Final			
	Disinfectant	Concentration**	Effective on:	Ineffective on:	Comments
	Phenolics: *e.g.* LysolTM*		Bacteria, most viruses, TB,	Spores, polio, Coxsackie	Relatively insensitive to
		1/20	HIV	VlrUses.	high protein
					concentrations. Corrosive.
	Chlorine Bleaches: *e.g.*		Bacteria, some spores, viruses, TB t, HIV		Prepare once a week. It
	Chlorox TM*	1:10		Some spores	takes 20 minutes to
					disinfect. Corrosive.
					A surface disinfectant.
	Iodophors: *e.g.*	1:100	Bacteria, most viruses, TB	Spores	Iodine is insoluble so it's
	WescodyneTM*				not good in solutions.
					Corrosive.
	Alcohols: e.g. ethanol,	70%	Bacteria, most viruses	Spores, TB	At 100% alcohols are a
	isopropanol				preservative!! Flammable.
*	The use of brand names does not imply a recommendation.				
**	Concentration of named brands.				
t	Use 1/5 dilution				

APPENDIX E Laboratory Biosafety Level Criteria per CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL)

Biosafety Level 1

Practices, safety equipment, and facilities are appropriate for undergraduate and secondary educational training and teaching laboratories and for other facilities in which work is done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. Many agents not ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and in immunodeficient or immunosuppressed individuals.

Biosafety Level 1 is suitable for work involving agents of no known or of minimal potential hazard to laboratory personnel and the environment. The laboratory is not separated from the general traffic patterns in the building. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science. The following standard and special practices, safety equipment, and facilities apply to agents assigned to

Biosafety Level 1:

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. Work surfaces are decontaminated once a day and after any spill of viable material.

3. All contaminated liquid or solid wastes are decontaminated before disposal.

4. Mechanical pipetting devices are used; mouth pipetting is prohibited.

5. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only. Food storage cabinets or refrigerators should be located outside of the work area.

6. Persons wash their hands after they handle viable materials and animals and before leaving the laboratory.

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

8. It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.

B. Special Practices

1. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leakproof container which is closed before being removed from the laboratory .

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

C. Special containment equipment is generally not required for manipulations of agents assigned to Biosafety Level 1.

D. Laboratory Facilities

1. The laboratory is designed so that it can be easily cleaned.

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

3. Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.

4. Each laboratory contains a sink for handwashing.

5. If the laboratory has windows that open, they are fitted with fly screens.

Biosafety Level 2

Practices, equipment, and facilities are applicable to clinical, diagnostic, teaching and other facilities in which work is done with the broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing aerosols is low. Hepatitis B virus, the salmonellae, and Toxoplasma spp. are representative of microorganisms assigned to this containment level. Primary hazards to personnel working with these agents may include accidental autoinoculation, ingestion, and skin or mucous membrane exposure to infectious materials. Procedures with high aerosol potential that may increase the risk of exposure of personnel must be conducted in primary containment equipment or devices.

Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that:

a. laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists,

b. access to the laboratory is limited when work is being conducted, and

c. certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment.

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

A. Standard Microbiological Practices

1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents or recombinant DNA molecules is in progress.

2. Work surfaces are decontaminated at least once a day and after any spill of viable material.

3. All infectious liquid or solid wastes are decontaminated before disposal.

4. Mechanical pipetting devices are used; mouth pipetting is prohibited.

5. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only. Food storage cabinets or refrigerators should be located outside of the work area.

6. Persons wash their hands after handling infectious materials or organisms containing recombinant DNA molecules, animals, and when they leave the laboratory.

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

8. Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

B. Special Practices

1. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leakproof container which is closed before being removed from the laboratory.

2. The laboratory director limits access to the laboratory. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

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

4. When the infectious agent(s) or organisms containing recombinant DNA molecules are in use in the laboratory require special provisions for entry (e.g.,vaccination), a hazard warning sign, incorporating the universal biohazard symbol, is posted on the access door to the laboratory work

area. The hazard warning sign identifies the infectious agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

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

6. Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before leaving the laboratory for non-laboratory areas (e.g., cafeteria, library administrative offices), this protective clothing is removes and left in the laboratory or covered with a clean coat not used in the laboratory.

7. Animals not involved in the work being performed are not permitted in the laboratory.

8. Special care is taken to avoid skin contamination with infectious materials or organisms containing recombinant DNA molecules; gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable.

9. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

10. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organism that contain recombinant DNA molecules or infectious agents. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the sheath or guard or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

11. Spills and accidents which result in overt exposures to infectious materials or organisms that contain recombinant DNA molecules are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

12. 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.

13. A biosafety manual is prepared or adopted. Personnel are advised of special hazards are required to read instructions on practices and procedures and to follow them.

C. Containment Equipment

Biological safety cabinets (Class I or II) (see Appendix A in CDC *BMBL*) or other appropriate personal protective or physical containment devices are used whenever:

1. Procedures with a high potential for creating infectious aerosols 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 eggs.

2. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed heads or centrifuge safety caps are used and if they are opened only in a biological safety cabinet.

D. Laboratory Facilities

1. The laboratory is designed so that it can be easily cleaned.

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

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

4. Each laboratory contains a sink for handwashing.

5. If the laboratory has windows that open, they are fitted with fly screens.

6. An autoclave for decontaminating infectious laboratory wastes is available.

Biosafety Level 3

Practices, safety equipment, and facilities are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents where the potential for infection by aerosols is real and the disease may have serious or lethal consequences. Autoinoculation and ingestion also represent primary hazards to personnel working with these agents. Examples of such agents for which Biosafety Level 3 safeguards are generally recommended include Mycobacterium tuberculosis, St. Louis encephalitis virus, and Coxiella burnetii.

Biosafety Level 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 material are

conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal protective clothing and devices. The laboratory has special engineering and design features. It is recognized, however, that many existing facilities may not have all the facility safe-guards recommended for Biosafety Level 3 (e.g., access zone, sealed penetrations, and directional airflow, etc.). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in laboratories where facility features satisfy Biosafety Level 2 recommendations provided the recommended "Standard Microbiological Practices," "Special Practices,"and "Containment 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. Work surfaces are decontaminated at least once a day and after any spill of viable material.
2. All infectious liquid or solid wastes are decontaminated before disposal.
3. Mechanical pipetting devices are used; mouth pipetting is prohibited.
4. Eating, drinking, smoking, storing food and applying cosmetics are not permitted in the work area.
5. Persons wash their hands after handling infectious materials,animals, organisms that contain recombinant DNA molecules and when they leave the laboratory.
6. All procedures are performed carefully to minimize the creation of aerosols.
7. Persons under 16 years of age shall not enter the laboratory.
8. If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring BL3 level physical containment, they shall be conducted in accordance with all BL3 level laboratory practices.

B. Special Practices

1. Laboratory doors are kept closed when experiments are in progress.
2. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak proof container which is closed before being removed from the laboratory.

3. The laboratory director controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

4. 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.

5. When infectious materials or organisms containing recombinant DNA molecules or experimental 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.

6. All activities involving infectious materials or organisms containing recombinant DNA molecules 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.

7. The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with infectious materials or organisms containing recombinant DNA molecules is finished. Plastic-backed paper toweling used on non-perforated work surfaces within biological safety cabinets facilitates clean-up.

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

9. Laboratory clothing that protects street clothing (e.g., solid front or wrap-around gowns, scrub suits, coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated before being laundered.

10. Special care is taken to avoid skin contamination with infectious materials or organisms containing recombinant DNA molecules; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.

11. Molded surgical masks or respirators are worn in rooms containing infected animals.

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

13. Laboratory animals held in a BL3 area shall be housed in partial-containment caging systems such as Horsfall units, open cages placed in ventilated enclosures, solid-wall and bottom cages covered by filter bonnets on holding racks equipped with ultraviolet in radiation lamps and reflectors. Note: Conventional caging systems may be used provided that all personnel wear appropriate personal protective devices. These shall include at a minimum wraparound gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.

14. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

15. Vacuum lines are protected with high efficiency particulate air (HEPA) filters and liquid disinfectant traps.

16. Hypodermic needles and syringes are used only for parenteral injection aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the infection or aspiration of infectious fluids or organisms containing recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the sheath or guard or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

17. Spills and accidents which result in overt or potential exposures to infectious materials or organisms containing recombinant DNA molecules are immediately reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.

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

19. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read instructions on practices and procedures and to follow them.

20. Alternate Selection of Containment Equipment- Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified can be conducted in a BL3 laboratory using containment equipment specified for the BL2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than

that specified can be conducted in a BL3 laboratory using containment equipment specified for the BL4 level of physical containment.

C. Containment Equipment

Biological safety cabinets (Class I, II, or III) or other appropriate combinations of personal protective or physical containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals) are used for all activities with organisms containing recombinant DNA molecules which pose a threat of aerosol exposure. These include manipulation of cultures and of those clinical or environmental materials which may be a source of aerosols; the aerosol challenge of experimental animals; and harvesting of infected tissues or fluids from experimental animals and embryonate eggs; and necroscopy of experimental animals.

D. Laboratory Facilities

1. The laboratory is separated from areas which are open to unrestricted personnel traffic within the building. Passage through two sets of doors is the basic requirement for entry into the animal room from access corridors or other contiguous areas. Physical separation of the high containment laboratory from access corridors or other activities may also be provided by a double doored clothes change room (showers may be included), airlock, or other access facility which requires passage through two sets of doors before entering the laboratory.

2. The interior surfaces of walls, floors, and ceilings are water resistant so that they may be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area.

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

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

5. Each laboratory contains a sink for handwashing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.

6. Windows in the laboratory are closed and sealed.

7. Access doors to the laboratory or containment module are self-closing.

8. An autoclave for decontaminating laboratory wastes is available, preferably within the laboratory.

9. A duct exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The building exhaust can be used for this purpose if the exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from occupied areas and air intakes.

Personnel must verify that the direction of the airflow (into the laboratory) is proper. The exhaust air from the animal room that does not pass through biological safety cabinets or other primary containment equipment can be discharged to the outside without being filtered or otherwise treated.

10. The HEPA filtered exhaust air from Class I or Class II biological safety cabinets or other primary containment devices is discharged directly to the outside or through the building exhaust system. Exhaust air from these primary containment devices may be recirculated within the laboratory if the cabinet is tested and certified at least every 12 months. If the HEPA filtered exhaust air from Class I or Class II biological safety cabinets is discharged to the outside through the building exhaust system, it is connected to this system in a manner (e.g., thimble unit connection) that avoids any interference with the air balance of the cabinets or building exhaust system

General Laboratory Practices for BL-3
SOP #: BL#-101
Date: January 25, 2005
Purpose: To provide safe handling procedures and operations for personnel working in the BL3 facility.

1.0 Restricted Access

1.1 Entry into the BL3 facility is restricted to authorized individuals who have obtained raining specific for BL3 operations and emergency procedures.
1.2 It is emphasized upon certification that any breach of the safety manual procedures will result in temporary suspension from the BL3 facility.
1.3 Visitors are not permitted in the BL3 facility.

2.0 Entry

2.1 Sign Up: To reserve time to use the BSC put your name and the time you need on the sign up calendar in advance. If an operator has reserved time in advance they have priority to use the facility.
2.2. Before entering the anteroom, the operator must first empty the autoclave if necessary and enter all details required in the log-in book (name, date, time in, procedures in use).
2.3 A biosafety protocol for the procedure to be performed must be on file before work can begin.
2.4 Once inside the anteroom the operator dons a full body Tyvek suit with feet, a mask, head covering and gloves fastened appropriately to overlap suit and when appropriate a Racal hood and respirator unit. The operator is then ready to enter the laboratory.
2.5 BL3 users must undergo mask-fitting training annually administered by a representative of RM&S.

3.0 Biosafety Cabinet Use

3.1 Before working in the biosafety cabinet (BSC), the fluorescent light is switched on, a biohazard bag, a paper towel, a Lysol squeeze bottle and a fresh absorbent sheet are placed in the cabinet. If the Vacuum line is to be used be sure an in line filter is in place.
3.2 All materials needed to complete the experiment are placed in the cabinet to limit the number of times hands pass through the air barrier. Equipment is not to be placed on the intake grills at the front of the cabinet, nor against the exhaust opening at the back of the cabinet.
3.3 A second pair of gloves is always worn when working in the BSC and are always removed before removing hands from the BSC.
3.4 A biohazard bag should be present in the cabinet. Absorbent material is placed in the bottom of the biohazard bag. This bag is used for discarding pipets replaced in their wrappers, contaminated Pipetman tips, gloves, plastic waste and wrappers. Contaminated liquid should be put into a roller bottle containing sufficient concentrated Lysol. The outside of the bottle should then be wiped with Lysol and removed from the hood to the waste tray by the sink. The waste tray will be autoclaved when full.

3.5 Anything removed from the BSC during the work session is to be decontaminated by wiping with Lysol while still in the BSC.

3.6 At the end of each work session, culture tubes, DNA tubes, racks and other material to be removed from the cabinet are decontaminated by wiping with Lysol while still within the cabinet.

3.7. The absorbent sheet, other absorbent materials used during cleaning and gloves are placed into a biohazard bag while still within the cabinet.
The bag is closed with a rubber band while still in the cabinet. Do not twist or tie the bag as it will blow open in the autoclave.

3.8 A fresh pair of gloves is donned and the hood is now wiped down completely with Lysol followed by 70% Ethanol. A second biohazard bag is used to double bag the waste bag while still in the hood. Cleaning cloths and gloves used to wipe down the BSC surfaces are put into this second bag. The bag is closed with a rubber band and removed from the BSC to the Autoclave Bin.

3.9 Nothing should be left in the Biosafety cabinet when leaving the facility.

4.0 Exiting

4.1 If autoclaving is necessary, the operator is to follow autoclaving procedures detailed in section 5.0. If others are working in the BL3 at the time the operator should coordinate with them on autoclaving. The last person at the end of the day should run the autoclave.

4.2 Rinse hands with Lysol prior to entering the anteroom.

4.3 Once inside the anteroom, Tyvek coveralls, head cover, mask, and gloves are removed in that order and put in a biohazard bag. The bag is closed with a rubber band and the bag put through the hatch.

4.4 The operator exits through the outer door and notes his/her time out in the logbook.

5.0 Autoclaving

5.1. All waste material leaving the BL3 facility must first be autoclaved.

5.2 A double-door autoclave is located in the laboratory.

5.3 It is the responsibility of all BL3 operators to know correct autoclaving procedures; instruction in such procedures is included in the operator training schedule.

5.4 A steam sterilization indicator is attached to each bag by a length of autoclave indicator tape prior to loading into the autoclave.

5.5 After the completed cycle, the autoclave is unloaded through the door outside the BL-3 facility. Autoclave debris is put into MPW boxes and glassware is removed for cleaning. Autoclaved liquids are released into a sink. Autoclaved chemicals are disposed of according to protocol for the specific chemical.

Title: Shipping and Receiving Biohazardous Materials
SOP #: BL3-102
Date: January 25, 2005
Purpose: To ensure that shipping and receiving of specimens and cultures which harbor or are suspected of harboring live mycobacteria is performed in a controlled and dedicated manner, thereby providing containment of infectious agents. Risk Management and Safety will handle all shipping of biohazardous materials using the following procedure.

1.0 Shipping Biohazardous materials

1.1 A Saf-T-Pak infectious shipper should be used to ship all infectious agents. The STP-100 is used to ship samples not requiring temperature control. For samples requiring cold packs or dry ice an STP-100 inside an STP-300 is used. Consult instruction included in each box.

1.2 The general approach to shipping cultures is to first protect the etiologic agent (either on a agar slant or in a plastic propylene screw top tube) with padding/ bubble wrap inside an approved secondary sealing screw cap container capable of withstanding 95 Kpa pressure Differential. An absorbent gauze pad is placed in each container to control any potential spills.

1.3 The secondary container is placed in a cardboard coil and then into the outside shipping box and the box closed appropriately.

1.4 Shipments requiring dry ice or freezer packs are put into the polystyrene freezer box of the STP – 300 with dry ice or freezer packs and the polystyrene box placed in the outside cardboard box.

1.5 A letter explaining the contents and their hazard is placed inside.

1.6 Labels are affixed for hazards both biohazard and dry ice as appropriate. Infectious substance sticker must be in place and the infectious materials proper shipping name, technical name and UN number must be written on the package.

1.7 Make sure full name, addresses and telephone numbers are written on the package.

2.0 Receiving Biohazardous Material

2.1. Open exterior box and remove secondary container. Take secondary container into BL3 and open in the biosafety hood.

2.2. Remove primary container and inspect for damage.

2.3. Wipe outside of Primary container with Lysol and store as appropriate.

2.4. Put Secondary container with lid off and internal packaging in biohazard bag and autoclave.

2.5. Receipt of Clinical Isolates:

2.5.1. An LJ slant will be grown and sent off to Specialty Labs immediately to determine Drug Resistance unless the isolates have already been tested.

3.0 Transport between rooms

3.1 If it is necessary to transport samples between rooms, a Plexiglass transport box will be used appropriately.

3.2 Alternatively, samples may be transported in the secondary (orange cap) container of the STP-100 shipping container.

Title: Spill Response in BL-3 Facility
SOP #: BL3-103
Date: January 25, 2005
Purpose: To provide safe procedures for spill response in the BL3 facility.

1.0 Spills occurring while working in the biosafety cabinet

1.1. When a spill occurs, the operator should immediately cover the affected area with absorbent pads, tissues or towels to contain the incident and prevent further aerosolization.
1.2. The absorbent material should then be soaked with Lysol.
1.3. No work should then proceed within the cabinet for at least 15 minutes to allow the cabinet exhaust system to remove aerosols and give sufficient contact time for the germicide to act.
1.4. At the end of this period, the operator should don a second pair of gloves and disposable Tyvek sleeves and place all clean up materials (broken tubes, plates, absorbent towels, etc.) into a biohazard bag.
1.5. Any disposable plasticware or tubes which were within the cabinet at the time of the incident should be disposed of as above.
1.6 Any other non-disposable items should be carefully decontaminated with Lysol followed by 70% ethanol residue.
1.7 The entire cabinet interior (including grills at front and rear) should be wiped down with Lysol.
1.8 All biohazard bags should then autoclaved.

2.0 Spill outside the biosafety cabinet

2.1. When any quantity of potentially infectious aerosol may be generated outside the BSC (e.g., by breaking a tube or flask of liquid culture), the operator should immediately inform all others working in the facility.
2.2. The entire facility should then be evacuated.
2.3. Once all individuals have entered the anteroom, all disposable safety clothing is removed and disposed of in the biohazard container through the autoclave hatch.
2.4. Once outside the facility, the person responsible for the spill will install warning signs and tape.
2.5. The person responsible for the spill will contact the Facility Manager and Risk Management and Safety.
2.6. After an appropriate length of time, spill responders will gown up and enter the BL3 facility wearing HEPA filtered hoods.
2.7. The spill will be covered with the Universal gel sorbent or other appropriate sorbent and the resulting gel then gathered and scooped into a biohazard bag.
2.8. The area will be sprayed with Lysol solution and left for a contact time of at least 30 minutes.
2.9. BL3 personnel may reenter the facility and complete the cleaning up procedure by mopping the floor and counter tops with disinfectant.
2.10. The BL3 log book record of the incident is then updated with details of all remedial actions undertaken.

Title: Decontamination and Maintenance of the BL3 Facility
SOP #: BL3-104
Date: January 25, 2005
Purpose: To provide safe procedures for decontamination and maintenance of the BL3 facility.

1.0 Service or Contract Maintenance

1.1. Whenever possible, equipment should be removed from the BL3 facility for servicing repair.
1.2. All equipment to be routinely serviced or repaired must first be decontaminated.
1.3. In the case of a refrigerator, freezer or incubator failure, all material contained should be removed to a back-up refrigerator, freezer or incubator and the inside and outside of the equipment are decontaminated with Lysol.
1.4. When maintenance personnel must enter the facility, all infectious material must be secure and no work with infectious agents should proceed during the entire period that the contractors are present.
1.5. All materials and tools brought in must be decontaminated with Lysol before they removed. This procedure should also be followed for any pens, pencils and other items that were used within the facility.
1.6. Outside contractors are advised by the BL3 Facility Manager or designee of mandatory safety measures which must adopted while working in the BL3 facility and are required to wear a complete complement of protective clothing.
1.7. Details of work performed should be entered in the BL3 log book and signed by the contractor(s).
1.8. Routine scheduled maintenance for the entire facility will occur at least annually.
1.9. The procedures followed for complete facility decontamination will be determined in communication with Risk Management and Safety but may include pyrolysis of paraformaldehyde.

2.0 Daily Cleaning and Maintenance of the BL3 facility

2.1 Daily cleaning and routine maintenance is the responsibility of those working in the facility. This includes surface decontamination of biosafety cabinets, counters and all equipment used that day.
2.2 The floors are cleaned monthly with Lysol. Cleaning equipment and supplies are kept under the sink. Monthly mopping of the floor and surface decontamination of all equipment and counter tops is the responsibility of the facility manager or designee.
2.3 When required, fluorescent light tubes should be changed by the facility manager or designee.
2.4 Biosafety Cabinets will be thoroughly cleaned and disinfected monthly by the facility manager or designee.

3.0 Biosafety Cabinet Monitoring and Maintenance

3.1. Biosafety cabinet efficiency will be monitored annually by Risk Management and Safety and certified annually by a certified contractor.
3.2. Cabinet interiors and any equipment contained therein should be wiped down Lysol followed by 70% ethanol.

4.0 BL3 Facility Supply Check List

4.1. Tyvek Suits, gloves, eye protection, masks, and head covering should be stocked in the anteroom and adequate supplies should be maintained by the Facility Manager in the BL3 storeroom.

4.2. A sponge mop, Lysol, absorbent pads and towels should always be present in the Laboratory.

4.3. Autoclave bags, bag ties and autoclave tape and steam sterilization indicators are kept in the lab.

4.4. Other items of safety equipment present in the laboratory include an eyewash (at the sink), chemical spill control kit (pads, rubber gloves, goggles, wiper treatments for solvent, caustic and acid spills), a bucket of powdered gel sorbent and a fire extinguisher.

4.5 A back up supply of disposable plasticware used in the BL3 such as roller bottles, pipets, pipet tips, cuvettes, sterile loops and spreaders, etc should be kept in the anteroom. An ample supply should be kept in the Laboratory itself to avoid unnecessary entry and exit to the anteroom.

Title: **Handling Medical and Facility Emergencies in BL3**
SOP #: **BL3-105**
Date: **January 25, 2005**
Purpose: To provide safe procedures for handling medical and facility emergencies.

1.1 Medical Emergencies

1.1 In case of a medical emergency, dial 911
1.2 If the individual is conscious and can be moved, exit the facility immediately.
1.3 If the individual is unconscious, immediately begin first aid.
1.4 If possible (and if it will cause no further harm) move the unconscious person to the outside of the facility through the anteroom.
1.5 If the victim cannot be moved, instruct the emergency medical personnel in proper gowning procedures to enter the facility.
1.6 Continue first aid until emergency medical personnel arrive and take over.

2.0 Electrical Failures

2.1. In case of a power outage the operator must use his/her own best judgment to assess the situation and act accordingly.
2.2. Loss of power to the building trips the emergency generator so that power should be restored.
2.3. If the blower fan of a Biological Safety Cabinet stops and the blower fan stops working for any length of time any operator working in the cabinet is required to cease all work immediately, discard gloves and then exit from the laboratory.
2.4. The blower must be on for at least thirty minutes before work can resume.
2.5. In case of a blackout, all operators are to evacuate the facility.
2.6. Emergency lights are present to guide personnel towards the anteroom.
2.7. If power has not returned within two minutes, the door facing the main laboratory is to be taped shut.
2.8. In the anteroom, all items of disposable clothing are removed and discarded through the hatch.
2.9. A sign should be placed across the anteroom entrance door and a notice posted to advise persons not to enter the facility.

Title: Training and Certification of BL3 Facility Operators
SOP #: BL3-106
Date: January 25, 2005
Purpose: To provide training and certification for individuals using the BL3 facility and outline safe operating procedures. All BL3 employees must undergo a training and certification process designed to ensure that they are fully cognizant of all health hazards and safety issues associated with the work.

1.0. Training

1.1 All employees working with biohazardous materials will go through annual training provided by RM&S.

1.2. The employee is given a copy of the Biosafety Manual including BL3 safety SOPs by BL3 Facility Manager or designee and instructed to read it carefully.

1.3. The employee is subsequently given the opportunity to discuss any questions or issues relating to the manual during a training session with the Facility Manager.

1.4. The Facility Manager will then:

- 1.4.1. Go through the SOPs with the employee
- 1.4.2. Outline safe working practices which all persons in the facility are expected to follow
- 1.4.3. Outline responses appropriate to spills in the laboratory
- 1.4.4. Outline emergency decontamination procedures
- 1.4.5. Take the employee to the BL3 facility and demonstrate how to correctly put on all disposable protective clothing
- 1.4.6. Walk through the entire facility with the employee, pointing out the location of specific equipment and safety items
- 1.4.7. Work within one of the biological safety cabinets with props to demonstrate safe working practices and show how contaminated items are to be discarded
- 1.4.8. Demonstrate how the autoclave works and correct procedures for autoclaving loads

1.5 Annual refresher training is required.

2.0 Certification

2.1. At the end of the session, both the employee and Facility Manager are required to sign a form to indicate that the employee has received training specific for BL3 work. This form is to be retained by the Facility Manager and a copy sent to Risk Management and Safety.

2.2. The Facility Manager must be present on the first occasion (and subsequent occasions, if deemed necessary) that the employee works in the facility so as to be on hand if problems arise and to ensure that safe working practices are being adhered to.

Title: Equipment Operation
SOP #: BL3-107
Date: January 25, 2005
Purpose: To provide guidance in the operation of equipment that poses a risk of aerosol production during use.

1.0 Centrifuges

1.1 Centrifugation can create aerosols. If a tube is broken during centrifugation opening of the rotor can lead to exposure.

1.2 Table Top Centrifuge

1.2.1 This centrifuge is equipped with a swinging bucket rotor with sealable caps for the tube holders. The tube holders are to be **opened and closed only in the biosafety cabinet**. Before centrifugation make sure the O-ring is in good condition and the rotor seals tightly before removing it from the hood.

1.2.2 After use spray rotor with Lysol followed by 70% Ethanol, wipe well, secure lid and return to centrifuge or storage location.

1.2.3 If a minor leak has occurred wipe tubes well with Lysol and clean rotor thoroughly with Lysol in the biosafety cabinet. Do not leave Lysol in the rotor without wiping completely before leaving the facility.

1.2.4 If a breakage has occurred, soak immediately with Lysol, secure lid and leave in biosafety cabinet for 10 minutes. Clean rotor completely before removing from biosafety cabinet.

1.3 Microcentrifuge

1.3.1 This centrifuge is equipped to hold sealed microcentrifuge tubes. The tubes should be locked to avoid opening during centrifugation. If a tube breaks in the rotor, it should be remobed, placed in a biosafety cabinet and cleaned as described above. Be sure the O-ring is in place and in good condition before use. Follow the same procedures as above with the large centrifuge.

2.0 37^{o}C Shaking Incubator

2.1 Cultures are to be grown only in non-breakable plastic flasks with leak-proof lids.

2.2 Flasks are only to be opened within the biosafety cabinet.

2.3 After use, spray flask with Lysol followed by 70% ethanol, wipe well and set aside for autoclaving. **Do not re-attach lid.**

2.4 If spill occurs within the 37^{o}C shaking incubator, laboratory personnel should be evacuated and RM&S notified. The clean-up protocol should follow the one described in section *Spill outside the biosafety cabinet*.

3.0 37^{o}C Plate Incubator

3.1 M. tuberculosis should only be grown in slabs with sealable lids or in falcon tight-seal Petri dishes (catalog # 351006).

3.2 Slabs or Petri dishes should only be opened in the biosafety cabinet.

4.0 CO_2 Incubator

4.1 Macrophages grown on tissue culture plates will be removed from the CO2 incubator and placed in the biosafety cabinet. The bacilli will be added to the media and macrophages and the infected cells placed back into the incubator.

4.2 At the times indicated by the experiment, the plates containing the infected macrophages are to be removed from the incubator and placed in the biosafey cabinet. All manipulations of the cells and mycobacteria are to be performed in the hood.

4.3 Solid and liquid waste is to be discarded as described above in the *Biosafety Cabinet Use* section.

4.4 Spills inside or outside the biosafety cabinet will be handled as described in the section on *Spill Response in BL-3 Facility***.**

3.0 Platemate

3.1 This equipment is an autopipeting station for 96 well plates. Pipeting causes aerosols. Therefore this equipment is only to be used in the biosafety cabinet and it will remain there permanently.

3.2 Reservoirs

3.2.1 An open reservoir containing TB moving back and forth on the rails has a potential for creating aerosols. Only reservoirs with splash dividers should be the horizontal speed of the carriage must be reduced to less than 80 rpm.

3.2.2 Only use Teflon reservoirs because they are autoclaveable.

3.3 Clean up

3.3.1 A paper towel soaked with Lysol is to be placed under the tips before changing them to catch any droplets released. Tips and paper towel are immediately placed in a biohazard bag.

3.3.2 Concentrated Lysol should be added to any remaining liquid in the reservoir and it is allowed to stand for 10 minutes.

3.3.3 After 10 minutes pour the liquid into the waste bottle.

3.3.4 Put the reservoir on a paper towel and spray with Lysol completely saturating it inside and out. Let stand a few minutes. Wipe completely until dry.

3.3.5 Repeat above step with fresh paper towel.

3.3.6 Spray and wipe with 70% ETOH and dry completely

3.3.7 Spray and wipe with water.

3.3.8 Dry reservoir and return to Platemate carriage.

3.3.9 Finally with a paper towel soaked in Lysol wipe all surfaces of Platemate and biosafety cabinet.

3.3.10 Repeat with 70% ETOH.

Title: Use of MDR TB and Uncharacterized Clinical Isolates
SOP #: BL3-108
Date: January 25, 2005
Purpose: To provide guidelines for the use of MDR TB strains and unknown clinical isolates.

1.0 INH and RIF Drug Resistance

1.1 Use of Strains with resistance to INH and RIF or clinical isolates with probable front line drug resistance will require special handling procedures

2.0. Handling procedures:

2.1. Communication

2.1.1 Scheduling: Operator will sign up on the calendar 24 hours in advance whenever possible. When 24 hour sign up is not possible operator will notify and coordinate with other operators.

2.1.2 Log book: Operator will note MDR use in the procedure section of the log book.

2.1.3 Labeling: all cultures will be visibly labeled with a MDR sticker.

2.1.4 Notification: If anyone is in the Facility when you enter you must notify them of your intent to work on MDR strains.

2.2 Culture Limit

2.2.1 Cultures will have a 50 ml maximum limit. Any user needing to exceed this limit will consult with the PI and Facility Manager to discuss and define further safety precautions and handling procedures.

Title: Radioactivity
SOP #: BL3-109
Date: April 15, 1999
Purpose: To provide guidelines for the use of Radioactivity in the BL3.

1.0 Radioactive Handling procedures:

1.1 Communication

1.1.1 **Log book:** Before entering the user will check the radioactivity box in the procedure section of the log book. After exiting the BL3 the user will check the survey complete box when logging out.

1.1.2 **Labeling:** all cultures will be labeled with a radioactive label.

1.2 Waste

1.2.1 **Short and Long half lives:** Short and long half lives will be separated. Short half-life isotopes have half lives of less than or equal to 89 days and long half-life isotopes have half lives of more than 89 days.

1.2.2 **Aqueous:** All aqueous radioactive waste will be decontaminated and made into solid waste. This is accomplished by:

a. Put all liquid waste in a roller bottle and decontaminate with Lysol for at least 15 min

b. In the BSC add an appropriate amount of Gel Sorbent to solidify the waste.

c. Decontaminated cultures are stored in the radioactive storage area only. Storage of radioactive materials in the BL3 is for decontamination purposes only.

1.2.3 **Solid:** Solid waste must be fully decontaminated before removal from the BL3. This is accomplished by:

a. Put all pipet tips, serological pipets, loops and other plastic items into an appropriate size roller bottle.

b. All eppendorf tubes and cuvettes must be open.

c. The bottle will contain an appropriate amount of Lysol so that all items are submerged. Bottle may be laid on its side to minimize the amount of aqueous waste generated.

d. After decontamination for at least 15 min. the aqueous waste is made into solid waste and removed to the BL3 radioactive storage area

e. If paper waste has been contaminated, make sure it is soaked in Lysol and put into a clear autoclave bag. It may be put into the radioactive storage area for autoclaving.

1.2.4 **Autoclaving:** Autoclaving of radioactive waste is allowed and will be coordinated between the Radioactive users, the Facility manager and the Responsible Investigator.

a. All waste to be autoclaved must be solid.

b. **DO NOT PUT ANY RADIOACTIVE WASTE IN BIOHAZARD BAGS. Put in clear autoclave bags.**

c. After autoclaving, this waste must be sent out as solid radioactive waste.

LAB SPECIFIC SOPs

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Biosafety Manual

February 2005