Auburn University
Biological Safety Manual

(Incorporating by Reference NIH Guidelines for Research Involving Recombinant DNA Materials and CDC/NIH BMBL)

December 07

Preface

This manual, in conjunction with the latest edition of the Centers for Disease Control and Prevention (CDC)/National Institutes of Health (NIH) Biosafety in Microbiological and Biomedical Laboratories (BMBL), provides the requirements for safely working with biohazardous materials at Auburn University (AU). The Institutional Biosafety Committee (IBC) is responsible for the regulation of biohazardous materials on the Auburn University campus. Principal Investigators (PIs) using biohazardous materials within Auburn University facilities must register with the IBC using what is termed a Biological Use Authorization which is included as Appendix A of the manual. The IBC may be contacted through Risk Management and Safety (RMS) at 4-4870. Specific responsibilities of PIs, the IBC, and RMS are included in Section 2.0. Many investigators may work with vertebrate animals and/or radioisotopes in conjunction with their work with some biohazardous agents. The Institutional Review Board for the Protection of Human Subjects in Research (IRB) reviews all human subject research conducted at the University or by Auburn University faculty, staff, or students. All vertebrate animal work conducted at Auburn University must be approved by the Institutional Animal Care and Use Committee (IACUC) which can be contacted at 4-5978. All work with radioisotopes conducted at Auburn University must be licensed through the Radiological Safety Committee which can be contacted at 4-4870.

The primary concerns when working with pathogens is the safety of personnel working with the agent and prevention of the release of that agent. Traditionally, microorganisms have been classified by the biological containment they require. Four levels of containment have been defined and termed Biosafety Levels (BSLs or BLs 1-4). Similarly, organisms are assigned classes dependant on the biosafety level they require. For example, an organism requiring BL2 precautions is designated as a Class 2 agent or organism. The specific requirements of BLs are found in the BMBL, an integral component of the Biological Safety Manual. NIH has introduced the concept of four Risk Groups (RGs) by which agents are classified, based on their relative pathogenicity for healthy adult humans. In almost all instances, the RG correlates with the recommended containment level. Thus, an RG2 organism is handled using BL-2 containment. Under some circumstances, the containment level required may be raised or lowered as a result of a comprehensive risk assessment. Auburn University has adopted the concept of RGs as discussed in Section 2.0 of the manual.

The Biological Use Authorization or BUA (Appendix A) is Auburn University's registration document for use with biohazardous agents and materials. Forms 1 and 5 of the BUA are mandatory; Forms 2-4 should be

completed as applicable. BUAs are required for all instructional, research, and outreach projects involving potentially pathogenic microorganisms; etiologic agents; infectious agents; oncogenic viruses; human tissue and blood borne pathogens; and in vitro construction or propagation of recombinant DNA molecules.

Standard Operating Procedures (SOPs) for BL 1-3 laboratory operations are included in Appendix E of this manual. Work with biohazardous agents or materials at AU facilities shall be performed in accordance with (IAW) these SOPs.

Waste management procedures are discussed in Appendix F. The State of Alabama has defined medical waste as sharps or any items displaying the biohazard symbol or wording to that effect. Therefore, even new red bags, unopened syringes and gas chromatography syringes are medical waste once discarded. In general, red bags or autoclave bags displaying the biohazard symbol or wording to that effect are not to be used at AU. Instead blue autoclave bags are available through the AU Scientific Supply Store. Blue autoclave bags may be discarded as normal trash after autoclaving.

Training requirements are summarized in Appendix H. These include a series of video-tapes on basic laboratory safety; lab and agent specific training provided by the PI; and medical waste and bloodborne pathogen training provided by RMS as necessary. These constitute the minimum training requirements. Contact RMS for assistance in determining further training needs.

The PI has primary responsibility for ensuring the safety of students, faculty, staff, visitors, and the environment with respect to their laboratory operations. Specific questions concerning this manual or the Auburn University Biological Safety Program can be directed to RMS at 4-4870. PIs should be aware of other safety programs applicable to their laboratory operations including the Chemical Hygiene Plan and Chemical Waste Management Program. These references are available on-line at. Risk Management and Safety's web page.

Emergency Phone Numbers and Resources

Risk Management and Safety	(334) 844-4870
After Work Hours	911
City of Auburn Department of Public Safety	911

Other Important Numbers

Centers for Disease Control and Prevention	(404) 639-3534
	(800) 311-3435
State Veterinarian, Alabama	(334) 240-7255
Office of Biotechnology Activities, NIH	(301) 496-9838
AU Office of Animal Resources	(334) 844-5978
AU Office of Human Subjects Research (OHSR)	(334) 844-5966
AU Scientific Supply Store	(334) 844-6940

(Source of Auburn University approved autoclave bags)

Web Resources

Risk Management and Safety	http://www.auburn.edu/administration/rms/
Centers for Disease Control and Prevention	http://www.cdc.gov/
National Institutes of Health	http://www.nih.gov/
National Institute for Occupational Safety and Health	http://www.cdc.gov/niosh/homepage.html

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I. Auburn University Policy for Biohazardous Materials

Auburn University is actively committed to preserving the health and safety of its students, staff, and faculty, and to protecting the environment and community. It is recognized that use of potentially pathogenic microorganisms, infectious agents, human tissue and bloodborne pathogens, and/or organisms containing recombinant DNA (rDNA) is necessary in many university research and teaching laboratories. To ensure the safe handling of these organisms, Auburn University requires compliance with the NIH Guidelines for Research Involving rDNA Materials; BMBL; Occupational Safety and Health Administration (OSHA) Occupational Exposure to Bloodborne Pathogen Standard Title 29, CFR Part 1910.1030 and with the recommendations provided in the Auburn University Biological Safety Manual. This manual will constitute a portion of the written safety programs for those units that work with biohazardous materials. *(Auburn University Safety Program Policy and Administration Manual section IV, approved by resolution of the Auburn University Board of Trustees March 26, 1990)*. The Auburn University Biological Safety Manual is consistent with all federal, state, and local requirements regarding biohazardous materials. Compliance with other applicable federal, state, and local regulations is also required.

Original approved by
William V. Muse
President
Date 8/8/00

2008 Revision:

Ralph Zee
Interim Vice President
for Research
Date:

II. Scope and Applicability

The consideration of biological safety issues is a campus-wide concern. With the ever-broadening interest in genetic engineering, agricultural production, and food safety, the need for standard biological safety policies and procedures is critical. A clear, consistent understanding of the organisms involved is an important first step in program organization.

This manual provides general guidelines and operating procedures for research involving the use of biological agents and toxins at Auburn University. All work involving these materials shall be performed in accordance with this manual. In addition the following materials shall be subject to the provisions of this manual.

Generation of rDNA

All experiments involving the generation and/or use of rDNA require IBC registration and may require approval by the IBC (see Appendix A). The National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules is the definitive reference for rDNA research in the United States. There may be rDNA experiments which are not covered by the guidelines that require review and approval by outside agencies before initiation or funding. These experiments are common in the agricultural and environmental sciences. If the experimental protocol is not covered by the guidelines, contact RMS at (334) 844-4870 for determination of further review.

If you have specific questions about a particular host-vector system not covered by the guidelines, please contact the Office of Recombinant DNA Activities (ORDA), National Institutes of Health at (301) 496-9838 or FAX to (301) 496-9839. Updates to the NIH rDNA guidelines are published in the Federal Register and are available on the internet at the NIH website

Human Research

All research conducted by faculty staff or students that involves the use of human subjects must be reviewed and approved under Institutional review Board (IRB) procedures prior to the involvement or recruitment of any subject.

Use of Animals

All animal experiments involving the use of rDNA; infectious or transmissible agents; human blood, body fluids, tissues, or toxins must be submitted to both the IBC and Institutional Animal Care and Use Committee (IACUC) for review and approval. IACUC guidelines are available from the Office of Animal Resources. Investigators who are uncertain how to categorize agents should contact RMS.

Transgenic Animals and Plants

Experiments to genetically engineer animals and plants require IBC registration (BUA - Appendix A.) The NIH rDNA guidelines provide specific biocontainment recommendations for experiments involving the creation and/or use of genetically engineered animals and plants.

Biosafety Levels

The essential elements of the four biosafety levels for activities involving infectious microorganisms and laboratory animals are summarized in Appendix B of this manual. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community.

Biosafety Level 1

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

Biosafety Level 2

Biosafety Level 2 is similar to Biosafety Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs from BSL-1 in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by scientists competent with this level of research/investigation; (2) access to the laboratory is limited when work is being conducted; (3) extreme precautions are taken with contaminated sharp items; and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

Biosafety Level 3

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 scientists competent and experienced in working with these agents.

The Auburn University Institutional Biosafety Committee strictly limits and regulates the work with BL3 agents and the space required for these operations.

Plant Biosafety Levels

There are four plant biosafety levels, designated Plant Biosafety Level 1 through 4 (BL1-P through BL4-P), for work with plant diseases in whole plants or transgenic plants. The levels designate combinations of practices, safety equipment and facilities for experiments on plants infected with agents that produce or may produce disease in cultivated crops or transgenic plants. In general, the biosafety level recommended for working with an infectious agent in vivo and in vitro are comparable. At this time, Auburn University policy does not allow work requiring BL3-P or BL4-P containment.

The use of any greenhouse for research with transgenic plants must be approved by the IBC and responsible greenhouse management personnel.

Plant Biosafety Levels are discussed in Appendix C.

Animal Biosafety Levels

All animal use in AU facilities will comply with IACUC and IBC requirements. Work at Animal Biosafety Levels 1 and 2 are permitted. Facilities do not currently exist for work at higher biocontainment levels.

Animal Biosafety Level 1 (ABSL1) is suitable for work involving well characterized agents that are not known to cause disease in healthy adult humans, and that are of minimal potential hazard to laboratory personnel and the environment.

Animal Biosafety Level 2 (ABSL2) involves practices for work with those agents associated with human disease. It addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure. ABSL2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL1.

Risk Groups

Traditionally, microorganisms have been classified according to the biological containment they require. Four levels of containment have been defined and termed Biosafety Levels (BSLs or BLs 1-4). More recently, the NIH has introduced the concept of Risk Groups (RGs) based on their relative

pathogenicity for healthy adult humans. The following table lists the four groups and the basis for classification.

Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk)
Risk Group 4 (RG4)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk)

For consistency, in this manual all organisms will be termed as Risk Group organisms. In almost all instances, the risk group correlates with the recommended containment level. Thus, a RG2 organism is handled using BL2 containment. Under some circumstances, the containment level required may be raised or lowered as a result of a comprehensive risk assessment as detailed in Section II-A-3 of the NIH Guidelines for Research Involving Recombinant DNA Molecules. If you have any questions or concerns regarding the Risk Group or appropriate level of containment for an organism consult the NIH Guidelines or call RMS.

Definitions

Biohazardous Material

For the purposes of this policy, a biohazardous material is any biological material capable of causing harm to humans, animals or plants, including both biohazardous agents and non-replicating materials such as toxins. In addition, this term may also be used to refer to material that harbors a biohazardous agent. A biohazardous agent is a pathogen capable of replication and is a disease-causing microorganism (bacteria, fungi, parasites, prions, rickettsiae, viruses, etc.) capable of causing diseases in humans, animals, or plants.

A list of biological agents classified according to risk may be found in Appendix D.

Human Blood and Other Potentially Infectious Materials

Experimentation or manipulation of human blood or other materials of human origin, including, but not limited to: excreta, secreta, blood and its components, unfixed tissue, and tissue fluids, all of which may or may not contain an infectious agent, may place the worker at risk of exposure to bloodborne pathogens. Examples which could result in exposure would be clinical laboratories performing tests or analyses on human blood or other

potentially infectious materials, research labs performing experiments and/or manipulations with human blood or unfixed tissues or organs. Any work with human blood draws may need to be approved by the IRB.

Infectious Agents and Materials

Infectious agents, pathogens or substances are defined as those substances containing viable microorganisms or their toxins which are known or suspected to cause disease in animals, plants or humans. Pathogens are classified as bacteria, fungi, rickettsiae, viruses, parasites, oncogenic viruses, and prions. Any materials which come in contact with infectious agents or their byproducts must be handled and disposed of in the same manner appropriate for disposal of the infectious agents themselves.

Recombinant DNA Molecules and Products

Research involving experiments with rDNA materials includes, but is not limited to: commonly used host-vector systems such as E. coli; recombinant DNA experiments using whole animals or plants; recombinant DNA or RNA experiments involving infectious animal or plant viruses; the production of transgenic animals; and the deliberate transfer of DNA or RNA into human subjects (requires IRB approval and approval from federal agencies, including NIH). Regardless of the cloning method utilized, precautions must be taken to assure that the systems neither cause disease in the operator nor release recombinant molecules into the environment.

Miscellaneous Biohazardous Materials

These include materials not directly covered by the above definitions, such as; allergens, cultured animal cells and their potentially infectious agents, tissues from experimental animals (including animal dander), plant viruses, bacteria and fungi, toxins (bacterial, plant, etc.), and those as yet unnamed elements or agents which may produce disease. In regard to allergens, it is not the intent of Auburn University to require a BUA for research projects involving only allergens (i.e., projects involving allergens not listed as biohazardous agents or materials). However, it is important that PIs consider the hazard associated with allergens because manipulations of these allergens, or materials containing allergens, may result in human exposures. The control of allergens in any teaching, research or outreach project must be an important consideration in experimental/procedural design, because severe allergenic reactions can be life-threatening. Prior to initiation of work, personnel must be instructed on the appropriate use of safety devices and procedures used to minimize exposures.

Related Documents

Work involving chemicals or radioactive materials shall comply with the requirements of the Chemical Hygiene Plan, and/or the Radiation Safety

Manual available on the AU Risk Management and Safety web page.

All work involving the use of recombinant DNA and genetically modified organisms shall be performed in accordance with the NIH Guidelines for Research Involving rDNA Materials.

The latest edition of the Biosafety in *Biomedical and Microbiological Laboratories* (BMBL) is an integral component of the Auburn University Biological Safety Manual. All descriptions of containment and handling of specific agents contained in the BMBL have been adopted as Auburn University.

Any work with human pathogens shall comply with the Auburn University Exposure Control Plan.

III. Roles and Responsibilities

Institutional Biosafety Committee

The Institutional Biosafety Committee (IBC) is charged by the President of Auburn University to formulate policy and procedures related to the use of biohazardous agents, including: human, animal, and plant pathogens, other infectious agents, toxins, and recombinant DNA (rDNA). As mandated by the NIH, experiments involving human gene therapy, formation of transgenic animals or plants, and the generation and/or use of rDNA must be reviewed and approved by the IBC. Auburn University also requires IBC review and approval for use of RG2 or higher biohazardous agents.

IBC Organization Structure

The President of Auburn University will appoint members to the IBC and designate one member to serve as chairperson. In order to provide the quality of input needed for in-depth consideration of research activities presenting real or potential hazards, the membership shall be composed of faculty, laboratory staff, community members, and continuing members. The specific composition and membership terms of the IBC shall be as follows:

Faculty: Six faculty members on rotating three year terms staggered such that two rotate on/off each year. Faculty shall be selected on the basis of experience and expertise in infectious disease research, experience and expertise in rDNA technology, the capability to assess the safety of biological research and to identify any potential risk to public health or the environment. At all times one faculty member will be a plant, plant pathogen or plant pest containment expert. At all times one faculty member will be an animal pathogen containment expert. The chair shall be selected from those faculty members entering their second or third year of committee service.

Community Members Not Otherwise Affiliated with Auburn University: Two outside members who represent the interest of the surrounding community with respect to health and protection of the environment (e.g. officials of state or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the community). These will be rotating, staggered, three year memberships.

Administrative/Professional: One member representing the administrative/professional career ladder such as research associate/research assistant, medical technologist, etc. or staff career ladders such as laboratory technician. This will be a rotating three year membership.

Continuing Members: The following will be continuing committee members:

- Director, Animal Resources
- Associate Director, Risk Management and Safety (will serve as secretary to the committee)
- Biological Safety Officer (Note: the Associate Director of Risk Management and Safety may function as the de facto Biological Safety Officer if the BSO cannot fulfill his/her duties.)

Responsibilities

The IBC has the responsibility of assessing risks and potential environmental impact associated with investigations involving biological agents and will make recommendations for safe conduct of such studies. The IBC functions on behalf of the institution to ensure that experimental work is performed in compliance with applicable policies, guidelines, and regulations for work with biohazardous materials and agents.

The IBC does not monitor activities which are appropriately the concern of other established institutional groups, e.g., Radiological Safety Committee, University Safety Committee, Institutional Review Board, and Institutional Animal Care and Use Committee. However, the IBC will closely interact with these groups in a concerted effort to minimize health risks for Auburn University personnel, students, and the general public.

All requests for action by the IBC shall be put in writing and submitted to the committee's secretary. The registration document is the Biological Use Authorization Form (BUA). The BUA and instructions for completion are included as Appendix A and are available on the Risk Management and Safety web page.

Associate Director of Risk Management and Safety

The Associate Director of RMS is primarily responsible for implementation of the biological safety program. Day-to-day management of the program may be delegated to others within RMS, including a Biological Safety Officer. Major duties or activities of the Associate Director include the following:

- Monitor compliance with University Safety practices and procedures regarding potentially infectious and biohazardous materials.
- Assist in the preparation and periodic updating of a biosafety manual which is in accordance with University policy and consistent with government regulatory guidelines.

- Provide consultation to investigators on matters relating to laboratory safety, appropriate handling and containment of biohazardous agents, decontamination, and disposal of biohazardous wastes.
- Aid investigators in the development of appropriate emergency measures for dealing with accidental spills and contamination.
- Conduct surveillance of laboratories in which biohazardous agents are employed to ensure compliance with approved protocol, prescribed safety guidelines, safety training, and correction of deficiencies.
- Investigate incidents involving biohazardous agents to determine probable cause and identify any violation of the approved protocol, safety guidelines, or breech of containment. Upon completing the investigation, the Associate Director of RMS will prepare a report of findings for review and action by the IBC. Copies will be provided to the Vice President for Research. Reports involving rDNA will be forwarded to the NIH.
- Monitor intra-campus transport to ensure compliance with the rigorous containment procedures described herein. Provide information for off-campus shipment of biohazardous materials.
- Serve on building and renovation committees. Review plans for new facilities and modifications of existing structures where potentially pathogenic microorganisms; etiologic agents of RG2, RG3; infectious agents; chemical carcinogens; and/or rDNA materials will be used.
- Develop and conduct training programs for laboratory personnel using biohazardous agents, specifically to promote techniques for the safe handling and disposal of biohazardous materials.
- Serve as liaison between the University and outside regulatory agencies concerned with the use of biohazardous agents.
- Coordinate the off-site treatment of infectious wastes.
- Coordinate with the PI on the close out of a laboratory when a protocol using potentially pathogenic microorganisms is completed, when the PI moves laboratories or when the investigator leaves the University. These agents include:
    - etiologic agents of RG2, RG3
    - infectious agents
    - human tissue and blood borne pathogens
    - organisms containing rDNA.

Biological Safety Officer

NIH guidelines require that a Biological Safety Officer be appointed whenever an institution engages in rDNA research at either BL-3 or large

scale as defined by NIH. The Biological Safety Officer is responsible for the day-to-day management of the biological safety program. Responsibilities include assumption of duties and activities listed for the Associate Director of RMS. The Biological Safety Officer shall also serve as a continuing member of the IBC.

Principal Investigators

Ultimate responsibility for the safe conduct of research involving biohazardous agents rests with the PI. The PI shall comply with the requirements contained in this manual and implement all necessary precautions to prevent undesirable consequences of experimental work conducted within the laboratory.

The PI shall:

- monitor daily operations of the laboratory
- inform/train persons who enter the laboratory
- file a BUA and obtain written approval of the IBC before performing work involving the use of biohazardous agents and toxins
- establish standard operation and decontamination procedures
- establish emergency procedures
- report any incident involving spill/release, injury, exposure, etc.
- arrange for immunization and/or health surveillance of laboratory personnel if deemed appropriate for the research project
- cooperate with the Associate Director and other members of RMS during inspection visits, provide correction of noted deficiencies

Department Heads/Chairpersons, Research Institute and Center Directors

The chief administrator of each department, research institute, center or unit is responsible for the general safety of faculty, staff, and in his/her overall area. The chief administrator shall insure that prior to initiation of work; each PI using a biohazardous agent files the appropriate registration forms.

The chief administrator is mutually responsible, along with the PI, for informing the IBC of work involving biohazardous agents and for reporting accidents or incidents to the Biological Safety Officer.

The chief administrator insures that students have received instruction regarding safety procedures in teaching laboratories or field situations where biohazardous agents are used.

The chief administrator is responsible for providing appropriate facilities and

safety equipment for proposed research or instruction involving biohazardous agents.

The chief administrator is mutually responsible, along with the faculty members, for supervising teaching laboratories, informing students of proper precautions for working with pathogenic microorganisms, and for assuring that proper precautions are taken.

Laboratory Personnel

All laboratory personnel shall adhere to established safety practices/guidelines.

Laboratory personnel are responsible for keeping themselves informed of the risks involved with working in a laboratory and the risks associated with the specific agents they will be working with. Laboratory personnel will participate in laboratory safety training and are encouraged to request additional training if they feel they are unequipped to deal safely with the risks.

Laboratory personnel shall report all unsafe practices to the PI.

Laboratory personnel must report all accidents and injuries to the PI.

IV. Practices and Procedures

Administrative Controls

Review and Approval of Biohazardous Studies

The IBC can effectively carry out its designated functions only if it has adequate prior knowledge of potentially hazardous research projects. Therefore, all instructional, research, and outreach projects involving potentially pathogenic microorganisms; etiologic agents of RG2, RG3; infectious agents; oncogenic viruses; human tissue and bloodborne pathogens; and in vitro construction or propagation of rDNA molecules, must be reviewed and approved in writing by the IBC.

It is not the purpose of the IBC to pass judgment on scientific merits, or to consider "risk" versus "expected benefits" of potentially hazardous research. Rather, it is the concern of this committee that the safety precautions proposed for the experimental work are adequate for the protection of personnel and environment. In general, the review process will focus on (1) qualifications of the PI, (2) agents to be employed, (3) risks presented by experimental procedures, (4) adequacy of containment equipment and facilities, (5) training level of persons directly associated with the work, (6) the need for health surveillance of laboratory personnel, and (7) other factors relevant to safe conduct of the study.

The PI and the IBC must concur on all matters relating to containment requirements, safe practices, and handling and disposal procedures for biohazardous agents. In the event of nonconcurrence, recommendations of the IBC shall prevail until such time as they are modified or rescinded by appellate decision of an administrative review. Administrative review may include outside reviewers, chaired by the Vice President for Research. Questions relating to rDNA studies not covered by the NIH Guidelines will be referred to the NIH Office of Recombinant DNA Activities for resolution.

RG3 Agents: Normally require BL3 containment due to the potential risk to workers and the environment. Therefore, the IBC will perform a more stringent evaluation procedure for oversight of this type of work. The IBC reserves the right to request full justification of all RG3 protocols including issues of scientific merit, compliance with regulatory requirements (including all surveillance, data reporting and adverse event reporting requirements; required containment levels for the research; assessment of the facilities, procedures, practices and training and expertise of personnel involved in the research; and risk/benefit of these projects.)

RG 4 Agents: Organisms or biohazardous materials requiring BL4

containment **are not permitted** in Auburn University facilities.

Clinical and Diagnostic Labs: Clinical and diagnostic labs are exempt from the BUA process.

Archival Samples/Cultures: Some laboratories maintain cultures and/or archival samples of biohazardous agents. In these instances, a generic BUA for maintenance and storage of reference, client and other samples/cultures should be submitted. An inventory of agents should be attached and updated whenever significant changes are made. Significant changes include additions or deletions of additional species or strains of inventoried agents that demonstrate a need for more stringent containment. If the responsible PI or individual utilizes any of those same agents in a specific teaching, research or outreach project, an additional BUA must be submitted.

Biological Use Authorization (BUA)

All requests for action by the IBC shall be put in writing and submitted using the Biological Use Authorization Form (BUA). The BUA and instructions for completion are included as Appendix A and are available on the Risk Management and Safety web page. Standard Operating Procedures for the BUA are included as Appendix E. (PIs) utilizing CDC Select Agents must complete the Identification Form in Appendix F. A discussion of the CDC Select Agents is in Section VII. Registrations approved by the IBC will be active for three years from the date of approval. Within the last quarter of the third year, the PI will be given the opportunity to renew the registration for an additional three years. After six years have elapsed, the investigator will be required to submit a new BUA for review and approval by the IBC. The IBC shall meet as often as necessary to conduct business, typically on a monthly basis. It will be the responsibility of the chairperson to: (1) determine if additional meetings are needed; and (2) officially inform appropriate person(s) of the actions of the committee. The Secretary of the IBC has the responsibility to see that a complete file is kept of minutes, documents, and reports received.

It is important for faculty and staff members to understand that certain information, as described below, may be subject to public scrutiny under a disclosure provision of current NIH guidelines and applicable state and federal laws. This disclosure provision requires Auburn University, upon request, to make available to the public, all minutes of IBC meetings pertaining to recombinant DNA activities, and any documents or reports submitted or received from federal funding agencies, which the latter are required to make public e.g., Memoranda of Understanding and Agreement, reports of guidelines violations and significant research related accidents, facilities inspection reports, and agency directives to modify projects (NIH Guidelines for Research Involving Recombinant DNA Materials). All IBC

members will be required to sign an annual Confidentiality Agreement for service on the committee.

In accordance with NIH guidelines, Auburn University will forward public comments regarding IBC actions and the IBC response to those public comments to the NIH.

Stop Work Procedures

The Associate Director of RMS, with concurrence from the Chair of the IBC, or with concurrence of three (3) members of the IBC if the Chair is unavailable, may stop any work with microbial agents or any hazardous research project that creates an unreasonable hazard to personnel or involves experiments prohibited by the University. The entire Committee then will review the situation and will complete the review within a working week and forward written recommendation(s) to the President of Auburn University or his designee, the Provost and Vice President for Research, for final action which will occur within two weeks of receipt of the recommendations. A copy of the final action determination will be maintained in the researcher's BUA file.

Planned/Unplanned Shutdowns

The Associate Director of RMS must be notified immediately in the event of:

- any mechanical malfunction
- any systems breakdown
- shutdowns of any nature
- preventative maintenance of primary containment equipment or components.

In the case of an unplanned event and if Facilities Division staff is not already on the scene, the Associate Director of RMS will notify appropriate Facilities Division staff. Proper precautions must be taken immediately. All experiments must be halted and the biological agents secured (e.g., containers sealed or containers placed in freezer or refrigerator). The area must be cordoned off during the entire time of the shut down. No further activities will be allowed until the Associate Director of RMS certifies that the facility is safe to use.

Biohazard Warning Signs and Posting

Each laboratory must clearly display signage that provides safety information to visitors and service personnel. Signs must contain designations for all

laboratory hazards in use within the laboratory (carcinogens, acutely toxic agents, reproductive hazards, biohazards, radioactive materials, lasers, and magnetic fields). Contact RMS for more information.

All areas and laboratories which contain biohazardous agents must be posted with a biohazard sign. The sign must be red/orange in color with a biohazard symbol (Figure 1) and lettering in black. Appropriate Personal Protective Equipment required for entry into the lab shall be specified on the biohazard sign.

Figure 1

All areas and laboratories which contain biohazardous or toxic agents must clearly display signs stating "EATING, DRINKING, SMOKING AND APPLYING COSMETICS ARE PROHIBITED IN THIS AREA."

There are no Biosafety Level 4, Animal Biosafety Level 3, and Animal Biosafety Level 4, Plant Biosafety Level 3 or Plant Biosafety Level 4 facilities on Auburn University campuses. Use of Risk Group 4 organisms is prohibited at Auburn University facilities.

Occupational Safety and Health Program

Personnel having substantial animal contact shall participate in the Occupational Safety and Health/Medical Monitoring Program, administered through the Office of Animal Resources.

Vaccines will be offered to all clearly identified “at-risk personnel” where the benefits clearly exceed the risk. Immunoprophylaxis may provide an additional level of protection. For more information see Appendix B of the BMBL. Vaccination costs will be the responsibility of the home department.

The AU Exposure Control Plan provides guidelines and procedures for personnel who are occupationally at-risk of exposure to bloodborne pathogens. In accordance with this plan, a Hepatitis B vaccination will be

made available, at no cost to the individual (charged to home department). Post-exposure evaluation and follow-up will also be provided.

For a more detailed explanation of this program, refer to the University's Exposure Control Plan and Office of Animal Resources Occupational Health and Safety Program.

Waste Management

Infectious and Biological Waste Management

Disposal of medical waste, infectious waste, autoclave bags, pipettes, sharps, and biological waste must be performed in accordance with Alabama Department of Environmental Management (ADEM) medical waste regulations. Auburn University has developed, "A Guide to the Handling and Disposal of Medical Waste," Appendix G, that lists compliance procedures and University policies.

Only specified autoclave bags and sharps disposal containers may be used.

NOTE: All cultures of microorganisms should be inactivated, using appropriate procedures, before disposal. This includes cultures of RG1 organisms. This is considered good laboratory practice.

Mixed Waste

Mixed wastes are potentially infectious materials contaminated with other types of hazardous materials, e.g., radioisotopes or toxic/carcinogenic compounds. Because waste disposal is controlled by more than one set of requirements and/or regulatory agencies, it is critical that provisions be made for proper management prior to the initiation of any research resulting in mixed waste. Mixed wastes may require special containers, labeling, storage, etc. Contact RMS prior to initiation of any research that might generate mixed waste.

Animals

The Institutional Animal Care and Use Committee must approve methods of disposal for research animals and animal parts considered to be infectious waste. RMS will be consulted when appropriate.

Biohazard Spill Clean Up Procedures

The following procedures are provided as a guideline to biohazardous spill cleanup. Additional information regarding emergency plans is in Appendix F of the BMBL.

Inside The Biological Safety Cabinet (BSC):

Wear lab coat, safety goggles, and gloves during cleanup.

- Allow cabinet to run during cleanup.
- Apply disinfectant and allow a minimum of 20 minutes contact time.
- Wipe up spillage with disposable disinfectant-soaked cloth.
- Wipe the walls, work surface, and any equipment in the cabinet with a disinfectant-soaked cloth.
- Discard contaminated disposable materials in an appropriate biohazardous waste container(s) and autoclave before discarding IAW A Guide to the Handling and Disposal of Medical Waste.
- Place contaminated reusable items in autoclave bags, autoclavable pans with lids, or wrap in newspaper before autoclaving and before cleanup.
- Expose non-autoclavable materials to disinfectant, 20 minute contact time, before removal from the BSC.
- Remove protective clothing used during cleanup and place in an autoclave bag for autoclaving.
- Run cabinet 10 minutes after cleanup before resuming work or turning cabinet off.

In The Lab, Outside The BSC

Clear the area of all personnel. Wait for aerosol to settle before entering spill area. The time required will depend on ventilation within the area, but a general rule of thumb is 30 minutes.

Remove any contaminated clothing and place in autoclave bag to be autoclaved.
Wear a disposable gown, safety goggles, and gloves at all times when pathogenic organisms are present

Initiate cleanup with disinfectant as follows:

- Soak paper towels in disinfectant and place over spill.
- Encircle the spill with additional disinfectant, being careful to minimize aerosolization while ensuring adequate contact.
- Decontaminate all items within spill area.
- Allow 20 minutes contact time to ensure germicidal action of disinfectant.
- Wipe equipment with 1:10 part bleach solution, followed by water, and then a 70% alcohol solution.
- Place disposable contaminated spill materials in a biohazardous waste container(s) appropriate for autoclaving.
- Place contaminated reusable items in autoclave bags, autoclavable pans with lids, or wrap in newspaper before autoclaving and cleanup.

Inside the centrifuge

If a centrifuge tube breaks while the centrifuge is running, turn off the motor and allow the machine to rest for 30 minutes before opening. If breakage is discovered after the machine has stopped, close the lid immediately and allow the unit to rest for 30 minutes.

- Unplug centrifuge before initiating clean-up.
- Don strong, thick, rubber gloves and other Personal Protective Equipment (PPE) before proceeding with clean-up.
- Flood centrifuge bowl with germicidal disinfectant. Place paper towels soaked in disinfectant over the entire spill area. Allow 20 minutes of contact time.
- Use mechanical means, such as forceps, to remove broken tubes and glass fragments. Place broken tubes and glass in sharps container for autoclaving and disposal as infectious waste IAW A Guide to the Handling and Disposal of Medical Waste.
- Remove buckets, trunnions, and rotor then place in disinfectant for 24 hours or autoclave. Place disinfectant soaked paper towels over equipment if transport is necessary.
- Unbroken, capped tubes may be placed in disinfectant and recovered after 20 minute contact time, or these tubes can be autoclaved.
- Use mechanical means to remove remaining disinfectant soaked materials from centrifuge bowl and discard as infectious waste IAW Appendix G.
- Place disinfectant soaked paper towels in the centrifuge bowl and allow soaking overnight. Wipe down again with disinfectant, wash with water, and dry. Discard disinfectant soaked materials as infectious waste IAW Appendix G.
- Remove protective clothing used during cleanup and place in a biohazard bag for autoclaving. Wash hands after removing gloves.

Outside The Lab, During Transport

Transport biohazardous material in an unbreakable, well-sealed, primary container placed inside an unbreakable, lidded container labeled with the biohazard symbol. (cooler, plastic pan or pail)

Should a spill occur in a public area, do not attempt to clean it up without appropriate PPE.

As an interim measure, with gloved hands, place disinfectant soaked paper towels directly on spilled materials to prevent spread of contamination. To assure adequate contact, surround the spill with disinfectant, if available,

taking care to minimize aerosols.

Call RMS at (334) 844-4870 for assistance with cleanup. Call 911 for assistance if spill takes place outside of normal working hours.

Standard Operating Procedures

Auburn University has developed general practices and procedures for work in a laboratory containing biohazardous agents. These are included in Appendix E - Standard Operating Procedures.

VI. Transportation and Shipping

Outgoing shipments of hazardous materials, biological, chemical, and/or radioisotopes, must be coordinated through RMS. Shippers of hazardous materials must have documented, DOT/IATA/ICAO mandated training. The University Mail Room will not accept hazardous materials shipments. Transporters will not pick up any hazardous materials without specific Hazardous Materials documents which are available through RMS. Packaging should be approved by RMS before use. Please call (334) 844-4805 for further information. Packaging and shipping requirements can be found in Appendix C of the BMBL.

NOTE: There are significant **PERSONAL** fines/possible imprisonment associated with improper packaging and shipping of hazardous materials.

Transport of Hazardous Materials by Research Personnel

Diagnostic and clinical specimens, infectious materials, and rDNA molecules need to be packaged in a sealed, leak proof, primary container (e.g., glass tube), which is securely positioned in a secondary leak proof and closable container (e.g., cooler, ice chest) clearly labeled with the biohazard symbol. A list of contents as well as emergency information (e.g., PI's phone number) needs to accompany the material (e.g., attached to the cooler in a plastic pouch). The use of private cars for the transportation of such materials on or off campus is highly discouraged. University vehicles are available upon request through individual departments. In case of an emergency (e.g., car accident), contact police and safety personnel and inform them of the presence of biohazardous materials and contact RMS.

Import/Export of Etiologic Agents

Import/export requirements for etiologic agents are included in Appendix C of the BMBL.

Transport of Select Agents

Any transport of Select Agents off campus requires a Commercial Drivers License (CDL). Please contact RMS for more information.

VII. Select Agents

CDC and USDA APHIS Select Agents

"The Antiterrorism and Effective Death Penalty Act of 1996," established provisions to regulate transfer of government-defined hazardous agents termed "select agents." A list of select agents is contained in Appendix C of the BMBL. Commercial suppliers of select agents, as well as government agencies, universities, research institutions, individuals, and private companies that transfer or obtain these agents, must register with the CDC/USDA. Registration is done on both an individual laboratory basis as well as an institutional basis. RMS has registered Auburn University and all labs currently shipping and receiving select agents. Contact RMS for registration forms necessary for the transfer of select agents.

Law and regulations require that Auburn University identify a responsible facility official that has demonstrated their ability to work safely with select agent(s) and keep records of the transfer of select agents between University facilities. The Biosafety Officer has been designated the responsible facility official. RMS maintains the required records. PIs must ensure their laboratories are equipped and their staff is able to demonstrate an ability to work safely with "select agents." Demonstration is accomplished by annual inspections of laboratories working with "select agents" to ensure facilities and procedures provide the level of containment and personnel training necessary. Inspections are performed with the use of a questionnaire entitled Laboratory Assessment Instrument developed by the CDC. Please call RMS at (344) 844-4870 with questions concerning the Select Agent Program.

Forbidden Agents

Appendix H lists agents which cannot by policy or statute be brought onto the Auburn University campus. Any clinical specimens determined to contain one or more of these agents must immediately be destroyed or transported off-campus for verification. The only exclusion to this policy is reference material necessary for identification purposes. Reference material must be labeled as such and stored in an inactive state, either frozen or lyophilized. All freezers and storage cabinets must be clearly labeled with signs that include the biohazard symbol, the name of the organism, and emergency information (e.g., PI's phone number). A list of all such viable reference material must be provided to the IBC.

Appendix A Biological Use Authorization Form (BUA)

The most current versions of the forms can be found at:

Auburn University - Risk Management and Safety

Appendix B Recommended Biosafety Levels for Infectious Agents

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults	Standard Microbiological Practices	None required	Open bench top sink required
2	Associated with human disease, hazard = percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: Limited access Biohazard warning signs "Sharps" precautions Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPEs: laboratory coats; gloves; face protection as needed	BSL-1 plus: Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practice plus: Controlled access Decontamination of all waste Decontamination of lab clothing before laundering Baseline serum	Primary barriers = Class I or II BCSs or other physical containment devices used for all open manipulations of agents; PPEs: protective lab clothing; gloves; respiratory protection as needed	BSL-2 plus: Physical separation from access corridors Self-closing, double-door access Exhausted air not recirculated Negative airflow into laboratory
4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission	BSL-3 practices plus: Clothing change before entering Shower on exit All material decontaminated on exit from facility	Primary barriers = All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: Separate building or isolated zone Dedicated supply and exhaust, vacuum, and decon systems Other requirements outlined in the text

Source: *Biosafety in Microbiological and Biomedical Laboratories,* 4th ed.

Appendix C Plant Biosafety Level Criteria

Research involving rDNA-containing plants, plant-associated microorganisms, and small animals shall be conducted in accordance with Appendix P. Physical And Biological Containment For Recombinant DNA Research Involving Plants as contained in the latest copy of the document Guidelines For Research Involving Recombinant DNA Molecules (NIH Guidelines) published by the National Institutes of Health (NIH).

The NIH Guidelines specify physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving rDNA-containing plants, plant-associated microorganisms, and small animals. These guidelines supersede standard rDNA containment requirements when research plants are of a size, number, or have growth requirements that preclude the use of standard laboratory containment conditions. Plants covered in the NIH guidelines include, but are not limited to, mosses, liverworts, macroscopic algae, and vascular plants including terrestrial crops, forest, and ornamental species. Plant-associated microorganisms include viroids, virusoids, viruses, bacteria, fungi, protozoans, certain small algae, and microorganisms that have a benign or beneficial association with plants, such as; certain Rhizobium species and microorganisms known to cause plant diseases. These guidelines apply to microorganisms which are modified with the objective of fostering an association with plants. Plant-associated small animals include those arthropods that:

- are in obligate association with plants,
- are plant pests,
- are plant pollinators, or
- transmit plant disease agents.

Other small animals include nematodes for which tests of biological properties necessitate the use of plants. Microorganisms associated with such small animals (e.g., pathogens or symbionts) are included.

Researchers handling similar organisms (plants, plant-associated microorganisms, and small animals) for which a USDA, APHIS, U.S. Department of the Interior or U.S. Public Health Service permit is required must notify the IBC of its research by memo with a copy of the permit attached.

Appendix D Classification of Human Etiologic Agents on The Basis of Hazard

This appendix includes those biological agents and selected animal agents known to infect humans and pose theoretical risks if inoculated into humans. Included are lists of representative genera and species known to be pathogenic. Mutated, recombined, and non-pathogenic species and strains are not considered. Non-infectious lifecycle stages of parasites are excluded.

This appendix reflects the current state of knowledge and should be considered a resource document. The list includes more commonly encountered agents and is not meant to be all inclusive. Information on agent risk assessment may be found in the Agent Summary Statements of the BMBL. Further guidance on agents not listed in this Appendix may be obtained through: CDC; National Institutes of Health; U.S. Department of Agriculture.

Risk Group 1 (RG1) Agents

RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include asporogenic Bacillus subtilis or Bacillus licheniformis.

Those agents not listed in RGs 2, 3 and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

Risk Group 2 (RG2) Agents

RG2 agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.

Risk Group 2 (RG2) - Bacterial Agents

Acinetobacter baumannii (formerly Acinetobacter calcoaceticus)
Actinobacillus
Actinomyces pyogenes (formerly Corynebacterium pyogenes)
Aeromonas hydrophila
Amycolata autotrophica
Archanobacterium haemolyticum (formerly Corynebacterium haemolyticum)
Arizona hinshawii - all serotypes
Bacillus anthracis
Bartonella henselae
Bartonella Quintana
Bartonella vinsonii
Bordetella
Mycobacterium bovis BCG vaccine strain Mycobacterium chelonei
Mycobacterium fortuitum
Mycobacterium kansasii
Mycobacterium leprae
Mycobacterium malmoense
Mycobacterium marinum
Mycobacterium paratuberculosis
Mycobacterium scrofulaceum
Mycobacterium. simiae
Mycobacterium szulgai
Mycobacterium ulcerans
Mycobacterium xenopi
Mycoplasma, except M. mycoides and M. agalactiae which are restricted

animal pathogens
Neisseria gonorrhoeae
Neisseria meningitidis

Risk Group 2 (RG2) - Bacterial Agents (cont'd)

Bordetella pertussis
Bordetella burgdorferi
Borrelia recurrentis
Burkholderia (formerly Pseudomonas species) except those listed in RG3
Campylobacter coli
Campylobacter fetus
Campylobacter jejuni
Chlamydia psittaci
Chlamydia trachomatis
Chlamydia pneumoniae
Clostridium botulinum
Clostridium chauvoei
Clostridium haemolyticum
Clostridium histolyticum
Clostridium novyi
Clostridium septicum
Clostridium tetani
Corynebacterium diphtheriae
Corynebacterium pseudotuberculosis
Corynebacterium renale
Dermatophilus congolensis
Edwardsiella tarda
Erysipelothrix rhusiopathiae
Escherichia coli - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including E. coli O157:H7
Haemophilus ducreyi
Haemophilus influenzae
Helicobacter pylori
Klebsiella - all species except K. oxytoca (RG1)
Legionella
Legionella pneumophila
Leptospira interrogans - all serotypes
Listeria
Moraxella
Mycobacterium (except those listed in RG3)
Mycobacterium avium complex
Mycobacterium asiaticum
Nocardia asteroides
Nocardi otitidiscaviarum
Nocardi brasiliensis
Nocardi transvalensis
Rhodococcus equi
Salmonella
Salmonella arizonae
Salmonella cholerasuis
Salmonella enteritidis
Salmonella gallinarum-pullorum
Salmonella meleagridis
Salmonella paratyphi, A, B, C
Salmonella typhi
Salmonella typhimurium
Shigella
Shigella boydii
Shigella dysenteriae, type 1
Shigella flexneri
Shigella sonnei
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus
Streptococcus pneumoniae
Streptococcus pyogenes
Treponema carateum
Treponema pallidum
Vibrio cholerae
Vibrio parahemolyticus
Vibrio vulnificus
Yersinia enterocolitica

Risk Group 2 (RG2) - Fungal Agents

Blastomyces dermatitidis
Cladosporium bantianum
Cladosporium (Xylohypha) trichoides
Cryptococcus neoformans
Dactylaria galopava (Ochroconis gallopavum)
Epidermophyton
Exophiala (Wangiella) dermatitidis
Fonsecaea pedrosoi
Microsporum
Paracoccidioides braziliensis
Penicillium marneffei
Sporothrix schenckii
Trichophyton

Risk Group 2 (RG2) - Parasitic Agents

Ancylostoma human hookworms
Ancylostoma duodenale
Ancylostoma ceylanicum
Ascaris
Ascaris lumbricoides suum
Babesia
Babesia divergens
Babesia microti
Brugia filaria worms
Brugia malayi
Brugia timori
Coccidia
Cryptosporidium
Cryptosporidium parvum
Cysticercus cellulosae (hydatid cyst, larva of T. solium)
Echinococcus
Echinococcus granulosis,
Echinococcus multilocularis
Echinococcus vogeli
Entamoeba histolytica
Enterobius
Fasciola
Fasciola gigantica
Fasciola hepatica
Giardia
Giardia lamblia
Heterophyes
Hymenolepis
Hymenolepis diminuta
Hymenolepis nana
Isospora
Leishmania
Leishmania braziliensis
Leishmania donovani
Leishmania ethiopia
Leishmania peruvania
Leishmania tropica
Loa loa filaria worms
Microsporidium
Naegleria fowleri
Necator human hookworms
Necator americanus
Onchoerca filaria worms
Onchoerca volvulus
Plasmodium including simian species
Plasmodium cynomologi
Plasmodium falciparum
Plasmodium malariae
Plasmodium ovale
Plasmodium vivax
Sarcocystis
Sarcocystis sui hominis
Schistosoma
Schistosoma haematobium
Schistosoma intercalatum
Schistosoma japonicum
Schistosoma mansoni
Schistosoma mekongi
Strongyloides
Strongyloides stercoralis
Taenia solium
Toxocara
Toxocara canis
Toxoplasma
Toxoplasma gondii
Trichinella spiralis
Trypanosoma
Trypanosoma brucei brucei
Trypanosoma brucei gambiense
Trypanosoma brucei rhodesiense
Trypanosoma cruzi

Leishmania major
Leishmania mexicana
Wuchereria bancrofti filaria worms

Risk Group 2 (RG2) - Viruses

Adenoviruses, human - all types
Alphaviruses (Togaviruses) - Group A Arboviruses
- Eastern equine encephalomyelitis virus
- Venezuelan equine encephalomyelitis vaccine strain TC-83
- Western equine encephalomyelitis virus

Arenaviruses
- Lymphocytic choriomeningitis virus (non-neurotropic strains)
- Tacaribe virus complex
- Other viruses as listed in the reference source

Bunyaviruses
- Bunyamwera virus
- Rift Valley fever virus vaccine strain MP-12
- Other viruses as listed in the reference source

Calciviruses

Coronaviruses
Flaviviruses (Togaviruses) - Group B Arboviruses
- Dengue virus serotypes 1, 2, 3, and 4
- Yellow fever virus vaccine strain 17D
- Other viruses as listed in the reference source

Hepatitis A, B, C, D, and E viruses
Herpesviruses - except Herpesvirus simiae (Monkey B virus) (see Risk Group 4 (RG4) -Viral Agents)
- Cytomegalovirus
- Epstein Barr virus
- Herpes simplex types 1 and 2
- Herpes zoster
- Human herpesvirus types 6 and 7

Orthomyxoviruses
- Influenza viruses types A, B, and C

Other tick-borne orthomyxoviruses as listed in the reference source (consult RMS)

Papovaviruses
All human papilloma viruses
- Paramyxoviruses
- Newcastle disease virus
- Measles virus
- Mumps virus
- Parainfluenza viruses types 1, 2, 3, and 4
- Respiratory syncytial virus

Parvoviruses
- Human parvovirus (B19)

Picornaviruses
- Coxsackie viruses types A and B
- Echoviruses - all types
- Polioviruses - all types, wild and attenuated
- Rhinoviruses - all types

Poxviruses - all types except Monkeypox virus (see Risk Group 3 (RG3) - Viruses and Prions) and restricted poxviruses including Alastrim, Smallpox, and Whitepox
Reoviruses - all types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)
Rhabdoviruses
- Rabies virus - all strains
- Vesicular stomatitis virus - laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow

Togaviruses (see Alphaviruses and Flaviviruses)
- Rubivirus (rubella)

Risk Group 3 (RG3) Agents

RG3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.

Bartonella
Brucella
Brucella abortus
Brucella canis
Brucella suis
Burkholderia (Pseudomonas) mallei
Burkholderia pseudomallei
Coxiella burnetii
Francisella tularensis
Mycobacterium bovis (except BCG strain, see RG2 - Bacterial Agents)
Mycobacterium tuberculosis
Pasteurella multocida type B -"buffalo" and other virulent strains
Rickettsia akari
Rickettsia australis
Rickettsia canada
Rickettsia conorii
Rickettsia prowazekii
Rickettsia rickettsii
Rickettsia siberica
Rickettsia tsutsugamushi
Rickettsia typhi (Rickettsia mooseri)
Yersinia pestis

Risk Group 3 (RG3) - Fungal Agents

Coccidioides immitis (sporulating cultures; contaminated soil)
Histoplasma capsulatum
Histoplasma capsulatum var. duboisii

Risk Group 3 (RG3) - Parasitic Agents

None

Risk Group 3 (RG3) - Viruses and Prions

Alphaviruses (Togaviruses) - Group A Arboviruses
- Semliki Forest virus
- St. Louis encephalitis virus
- Venezuelan equine encephalomyelitis virus (except the vaccine strain TC-83, see RG2)
- Other viruses as listed in the reference source

Arenaviruses
- Flexal
- Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)

Bunyaviruses
- Hantaviruses including Hantaan virus
- Rift Valley fever virus

Flaviviruses (Togaviruses) - Group B Arboviruses

Poxviruses
- Monkeypox virus

Prions
- Transmissible spongioform encephalopathies (TME) agents (Creutzfeldt-Jacob disease and kuru agents)(contact RMS for containment instruction)

Retroviruses
- Human immunodeficiency virus (HIV) types 1 and 2
- Human T cell lymphotropic virus (HTLV) types 1 and 2
- Simian immunodeficiency virus (SIV)

Rhabdoviruses

- Japanese encephalitis virus
- Yellow fever virus
- Other viruses as listed in the reference source
- Vesicular stomatitis virus

Risk Group 4 (RG4) Agents

RG4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.

Risk Group 4 (RG4) - Bacterial Agents

None

Risk Group 4 (RG4) - Fungal Agents

None

Risk Group 4 (RG4) - Parasitic Agents

None

Risk Group 4 (RG4) - Viral Agents

Arenaviruses

- Guanarito virus
- Lassa virus
- Junin virus
- Machupo virus
- Sabia

Bunyaviruses (Nairovirus)

- Crimean-Congo hemorrhagic fever virus

Filoviruses

- Ebola virus
- Marburg virus

Flaviruses (Togaviruses) - Group B Arboviruses

- Tick-borne encephalitis virus complex including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses

Herpesviruses (alpha)

- Herpesvirus simiae (Herpes B or Monkey B virus)

Paramyxoviruses

- Equine morbillivirus

Hemorrhagic fever agents and viruses as yet undefined

Animal Viral Etiologic Agents in Common Use

The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents are associated with disease in healthy adult humans; they are commonly used in laboratory experimental work.

A containment level appropriate for RG1 human agents is recommended for their use. For agents that are infectious to human cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.

Adenoviruses

Paramyxoviruses

- Avian adenovirus, including CELO virus
- Bovine adenovirus
- Canine adenovirus
- Equine adenovirus

Baculoviruses

Coronaviruses
- Bovine coronavirus
- Canine coronavirus
- Equine coronavirus
- Feline coronavirus

Herpesviruses
- Bovine herpesvirus
- Canine herpesvirus
- Channel catfish herpesvirus
- Equine herpesvirus
- Feline herpesvirus
- Herpesvirus ateles
- Herpesvirus saimiri
- Marek's disease virus
- Murine cytomegalovirus
- Pseudorabies virus

Papovaviruses
- Bovine papilloma virus
- Polyoma virus
- Shope papilloma virus
- Simian virus 40 (SV40)
- Bovine parainfluenza
- Bovine respiratory syncytial virus
- Canine distemper virus
- Canine parainfluenza

Parvoviruses
- Bovine parvovirus
- Canine parvovirus
- Equine parvovirus
- Porcine parvovirus

Retroviruses
- Avian leukosis virus
- Avian sarcoma virus
- Bovine leukemia virus
- Feline leukemia virus
- Feline sarcoma virus
- Gibbon leukemia virus
- Mason-Pfizer monkey virus
- Mouse mammary tumor virus
- Murine leukemia virus
- Murine sarcoma virus
- Rat leukemia virus

Restricted Foreign Animal Pathogens

The importation, possession or use of the following agents is prohibited or restricted by law or USDA regulation or administrative policy.

African horse sickness virus
African swine fever
Akabane virus
Besnoitia besnoiti
Borna disease virus

Bovine infectious petechial fever
Bovine spongiform encephalopathy
Brucella melitensis
Camel pox virus
Cochliomyia hominivorax(screw worm)
Ephemeral fever virus
Foot and mouth disease virus
Mycoplasma agalactiae (contagious agalactia of sheep)
Mycoplasma mycoides (contagious bovine pleuropneumonia)
Nairobi sheep disease virus
Newcastle disease virus
Peste des petits ruminants (Pest of small ruminants)
Pseudomonas ruminatium (heart water)
Rift Valley fever virus
Rinderpest virus
Sheep pox virus
Swine vesicular disease virus
Teschen disease virus

Fowl plague virus (lethal avian influenza)
Goat pox virus
Histoplasma (Zymonema) farciminosum
Hog cholera virus
Louping ill virus
Lumpy skin disease virus
Theileria annulata
Theileria bovis
Theileria hirci
Theileria lawrencei
Trypanosoma vivax (Nagana)
Trypanosoma evansi
Vesicular exanthema virus
Wesselsbron disease virus

NCI CLASSIFICATION SCHEME FOR ONCOGENIC AGENTS

The National Cancer Institute (NCI) has prepared minimum safety guidelines for research involving oncogenic viruses which are designed to protect the laboratory worker and his/her experiments, to minimize hazards to anyone else who might enter the laboratory area, and to insure the safety of the surrounding community. NCI strongly recommends that the guidelines be practiced in all research laboratories where oncogenic viruses are present.

It is assumed that oncogenic viruses vary in their potential hazard to man. Criteria have been developed to identify oncogenic viruses of moderate and high risk. All other oncogenic viruses are considered low risk. The criteria are not absolute, but are subject to modification as research continues.

In addition to the listed criteria, the extent to which there is prior experience with the virus without indication of any harmful effect on man must also be considered when evaluating risk. Listed below are some known low-risk and moderate-risk oncogenic agents, respectively.

In general, low-risk oncogenic viruses may be handled at BL1 and moderate risk oncogenic viruses may be handled at BL2. All viruses of human or primate origin must be handled at BL2, 3 or 4. For assistance in determination of the appropriate containment level, please contact RMS at (344) 844-4870.

Low-Risk Oncogenic Viruses

Rous sarcoma
SV-40
CELO
Ad7-SV40
Polyoma
Bovine papilloma
Rat mammary tumor
Rat leukemia
Hamster leukemia
Bovine leukemia
Dog sarcoma*
Mason-Pfizer monkey virus
Marek's
Guinea pig herpes

Avian leukosis	Lucke (Frog)
Murine leukemia	Adenovirus
Murine sarcoma	Shope fibroma
Mouse mammary tumor	Shope papilloma

*Although listed by NCI, it is questionable whether a dog sarcoma virus has been isolated.

Criteria for Moderate Risk Oncogenic Viruses

- Suspected oncogenic virus isolate from man
- Virus that transforms human cells in vitro, as evidenced by a morphological and/or functional alteration that is transferred genetically
- Virus that produces cancer without the aid of experimental host modification in either a subhuman primate at any age or across another mammalian species barrier in juvenile or adult animals
- A genetic recombinant between an animal oncogenic virus and a microorganism infectious for man shall be considered moderate risk until its oncogenic potential for man is determined.
- Any concentrated oncogenic virus or infectious transforming viral nucleic acid

Moderate-Risk Oncogenic Viruses

Ad2-SV40	GaLV
FeLV	HV ateles
HV Saimiri	Yaba
EBV	FeSV
SSV-1	

Criteria for High Risk Oncogenic Viruses

A virus proved to induce cancer in man shall be classified as high risk until its complete hazard potential can be determined. At the present time, there are no known oncogenic viruses classified as high risk.

Appendix E Standard Operating Procedures

Personal Protective Equipment (PPE): PPE such as gloves, safety glasses, and a laboratory coat must be worn whenever biological work is conducted in the laboratory. No sandals or open-toed shoes are allowed in the laboratory. Long pants/skirt are recommended.

Handwashing: Hands must be washed immediately or as soon as feasible after removing gloves or other personal protective clothing.

Use of Sharps: Minimize the use and exposure to sharps in the workplace. Never recap, bend, or shear needles. When possible, replace glassware with less damaging materials such as plastic. Keep sharps containers readily available in all locations where sharps waste may be generated.

Clean Areas: Eating, drinking, smoking, applying cosmetics or lip balm, and handling contact lenses in the laboratory are prohibited in work areas where there is a reasonable likelihood of occupational exposure.

Aerosol Generation: Any procedures that could potentially generate aerosols or other inhalation hazards must be performed in a manner that will minimize airborne pathogen transmission.

Proper Labeling: Place a color-coded label incorporating the universal biohazard label on the work surface of any potentially contaminated equipment or work surface to warn others of biohazard contamination which may not be easily visible. This includes freezers, refrigerators, and incubators.

Autoclave Safety: Always wear heat-resistant gloves, goggles or safety glasses, and a laboratory coat when opening an autoclave. Be sure to allow the superheated steam to exit before attempting to remove the contents.

Spills: Always clean spills from the periphery of the spill towards the center. All cleaning materials must be disposed of in an appropriate manner.

Mouth Pipetting: Mouth pipetting may lead to accidental ingestion of biological specimens and is strictly prohibited.

Decontamination Procedures: A 0.5% sodium hypochlorite (a freshly prepared 1:10 dilution of household bleach) must be used to decontaminate equipment and work surfaces. In locations where bleach would cause corrosion, an iodophor (e.g., Wescodyne) must be used to decontaminate.

Local Transport of Infectious Materials: All infectious materials transported to and from the laboratory must be enclosed in a primary container with sealed lid, which must then be enclosed in a secondary leak-proof, non-breakable container, appropriately labeled with the biohazard symbol. Any specimens transported to and from off-campus satellite facilities must be escorted by a responsible lab employee.

Storage: All infectious materials to be stored must be clearly labeled with the universal biohazard symbol. The storage space (e.g., freezer, refrigerator) must also be similarly labeled.

Bloodborne Pathogens: All PI's using human or non-human primate blood or blood products, unfixed tissue, body fluids, or organ or cell cultures of human or non-human primate origin, must follow the procedures outlined in the Auburn University Bloodborne Pathogen Exposure Control Plan.

Human Organ and Cell Culture: All PI's using human organ or cell cultures (primary cultures, cell strains, cell lines), must handle all such cultures under BL2 conditions and in accordance with the Bloodborne Pathogen Standard, unless the IBC has specifically approved a lower standard of containment.

Transport of "Select Agents"/Toxins: RMS must be notified of all transfers.

Waste Disposal: All biological waste must be autoclaved before disposal in accordance with Auburn University's Medical Waste Management Guide. Blue autoclave bags are to be used for this purpose.

All biohazardous spills must be handled using the following procedure:
For spills which staff is able to clean up safely, a person wearing protective equipment (gloves, goggles, long-sleeved lab coat) must first disinfect the area with a 1:10 dilution of household bleach or an iodophor before wiping up the spill with disposable paper towels and disposing of all spill materials properly. Broken glass must be handled only by remote means such as tongs, forceps or a broom/dustpan combination.

For spills which staff may not be able to clean up safely, the room must be evacuated and personnel must be prevented from entering the area. The lab director or supervisor and RMS (344) 844-4870 must be contacted immediately. After 5:00 p.m., dial 911 immediately.

Injuries: All injuries and accidental autoinoculation, ingestion or inhalations of infectious agents, must be reported immediately to the lab director or supervisor and RMS (344) 844-4870. Affected employees should be sent to the Auburn University Medical Clinic (AUMC) for evaluation, possible treatment and/or referral. Outside AUMC hours of operation, send affected employees to East Alabama Medical Center Emergency Room. Dial 911 immediately for any medical emergency.

Shipments: All domestic and international shipments of biological materials must follow University policy and all applicable federal and international regulations. Proper permits/licenses must be obtained before importing or exporting biological materials. Contact RMS prior to assist with shipments.

Emergency Preparedness: In case of natural disasters, fires, or power failure the following precautions must be taken:

- In power failures, immediately discontinue all work until power is restored. If a tissue culture hood is being used, then all open containers must be closed, gas turned off, and hood sash closed.
- In natural disasters, personnel must immediately follow standard emergency procedures (911; 4-4870 (RMS)). Upon return to facility, Personal Protective Equipment (PPE) must be used when entering a lab to decontaminate any disaster-related release of infectious material. Contain released material using spill procedures. Emergency personnel should don PPE before entering lab and/or areas housing infected animals.
- In case of fire, personnel must immediately follow standard emergency procedures (911; 4-4870 (RMS)). Temperatures sufficient to ignite materials will inactivate infectious agents used in the laboratory. However, emergency personnel should don PPE before entering the lab and follow disinfecting procedures described above for decontaminating any released infectious materials not involved in the fire.

ENGINEERING CONTROLS

Biological Safety Cabinets (BSCs)

BSCs are designed to contain aerosols generated during work with infectious material through the use of laminar air flow and high efficiency particulate air (HEPA) filtration. Consult CDC/NIH BMBL for a discussion of the types and uses of BSCs.

Safe and Effective Use of Biosafety Cabinets

In general:

- Ensure your BSC is certified when it is installed, after it is moved, and annually thereafter. For information on cabinet certification, call RMS at (344) 844-4870. Check the magnahelic gauge regularly for indication of a problem.
- Understand how your cabinet works.
- Do not disrupt the protective airflow pattern of the BSC. Such things as rapidly moving your arms in and out of the cabinet, people walking

rapidly behind you, and/or open lab doors may disrupt the airflow pattern and reduce the effectiveness of the BSC.

- Plan your work.
- Minimize the storage of materials in and around the BSC.
- Always leave the BSC running.

Operational directions:

- Before using, wipe work surface with 70% alcohol. Wipe off each item you need for your procedures and place in cabinet.
- DO NOT place objects over the front air intake grille. DO NOT block the rear exhaust grille.
- Segregate contaminated and clean items. Work from "clean to dirty."
- Place a pan with disinfectant and/or a sharps container inside the BSC for pipette discard. DO NOT use vertical pipette discard canisters on the floor outside cabinet.
- It is not necessary to flame items; this creates turbulence in airflow and will compromise sterility; heat buildup may damage the filters.
- Move arms slowly when removing or introducing new items into the BSC.
- If you use a piece of equipment that creates air turbulence in the BSC (such as a centrifuge, blender) place equipment in the back 1/3 of the cabinet; stop other work while equipment is operating.
- Protect the building vacuum system from biohazards by placing a HEPA cartridge filter or its equivalent between the vacuum trap and the source valve in the cabinet.
- Clean up all spills in the cabinet immediately. Wait 10 minutes before resuming work.
- When work is finished, remove all materials and wipe all interior surfaces with 70% alcohol.
- Remove lab coat and wash hands thoroughly before leaving laboratory.

Safety Equipment

Safety equipment includes items for personal protection such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses or goggles. PPE is often used in combination with BSCs and other devices which contain the biohazardous agents, animals or materials. When it is impractical to work in BSCs, PPE may form the primary barrier between personnel and infectious materials. Examples include certain animal studies, animal necropsy, agent production activities, and activities relating to maintenance, service or support of the laboratory facility.

Other safety equipment such as safety centrifuge cups and safety blenders are enclosed containers designed to prevent aerosols from being released during centrifugation or homogenization of infectious material.

Containment controls such as BSCs, safety centrifuge cups, and blenders must be used for handling infectious agents that can be transmitted through the aerosol route of exposure. A description of the effective use of BSCs and information on other safety equipment may be found in the Recommended Work Practices below.

For more information on proper use and selection of a BSC or other safety equipment, call RMS at (344) 844-4870.

Recommended Work Practices

Pipettes and Pipetting Aids

Pipettes are used for volumetric measurements and transfer of fluids that may contain infectious, toxic, corrosive, or radioactive agents. Laboratory associated infections have occurred from oral aspiration of infectious materials, mouth transfer via a contaminated finger, and inhalation of aerosols. Exposures to aerosols may occur when liquid from a pipette is dropped onto the work surface, when cultures are mixed by pipetting, or when the last drop of an inoculum is blown out. A pipette may become a hazardous piece of equipment if improperly used. The safe pipetting techniques which follow are required to minimize the potential for exposure to hazardous materials.

- Never mouth pipette. Always use a pipetting aid.
- If working with biohazardous or toxic fluid, confine pipetting operations to a biosafety cabinet.
- Always use cotton plugged pipettes when pipetting biohazardous or toxic materials, even when safety pipetting aids are used.
- Do not prepare biohazardous materials by bubbling expiratory air through a liquid with a pipette.
- Do not forcibly expel biohazardous material out of a pipette.
- Never mix biohazardous or toxic material by suction and expulsion through a pipette.
- When pipetting, avoid accidental release of infectious droplets. Place a disinfectant soaked towel on the work surface and autoclave the towel after use.
- Use "to deliver" pipettes rather than those requiring "blowout."

- Do not discharge material from a pipette at a height. Whenever possible allow the discharge to run down the container wall.
- Place contaminated, reusable pipettes horizontally in a pan containing enough liquid disinfectant to completely cover them. Do not place pipettes vertically into a cylinder.

Discard contaminated disposable pipettes in an appropriate sharps container. Autoclave the container when it is 2/3 to 3/4 full and dispose as medical waste. See "A Guide to the Handling and Disposal of Medical Waste," Appendix F. Call (344) 844-4870 if you need an additional copy. This document is also available on the RMS web page, http://web6.duc.auburn.edu/administration/rms/pdf/medical-waste-disposal.pdf
Pans or sharps containers for contaminated pipettes should be placed inside the biosafety cabinet, if possible.

Syringes and Needles

Syringes and hypodermic needles are dangerous instruments. The use of needles and syringes should be restricted to procedures for which there is no alternative. Blunt cannulas should be used as alternatives to needles wherever possible (i.e., procedures such as oral or intranasal animal inoculations). Needles and syringes should never be used as a substitute for pipettes. When needles and syringes must be used, the following procedures are recommended:

- Use disposable needle locking syringe units whenever possible.
- When using syringes and needles with biohazardous or potentially infectious agents:
- Work in a biosafety cabinet whenever possible.
- Wear gloves.
- Fill the syringe carefully to minimize air bubbles.
- Expel air, liquid, and bubbles from the syringe vertically into a cotton pledget moistened with disinfectant.
- Do not use a syringe to mix infectious fluid forcefully.
- Do not contaminate the needle hub when filling the syringe. This will help avoid transfer of infectious material to fingers.
- Wrap the needle and stopper in a cotton pledget moistened with disinfectant when removing a needle from a rubber-stoppered bottle.
- Bending, recapping, clipping, or removing needles from syringes is prohibited. The use of needle nipping devices is prohibited.
- Use a separate pan of disinfectant for reusable syringes and needles.

Do not place them in pans containing pipettes or other glassware to eliminate sorting later.

- Used disposable needles and syringes must be placed in appropriate sharps disposal containers and discarded as medical waste. See "A Guide to the Handling and Disposal of Medical Waste", Appendix F, or http://web6.duc.auburn.edu/administration/rms/pdf/medical-waste-disposal.pdf

Cryostats

Frozen sections of unfixed tissue infected with an etiologic agent pose a risk because accidents can occur. Freezing tissue does not necessarily inactivate infectious agents. Freezing propellants under pressure should not be used for frozen sections as they may cause spattering of droplets of infectious materials. Gloves should be worn during preparation of frozen sections. When working with biohazardous material in a cryostat, the following is recommended:

- Consider the contents of the cryostat to be contaminated and decontaminate it frequently with 70% ethanol.
- Consider trimmings and sections of tissue that accumulate in the cryostat to be potentially infectious and remove them during decontamination.
- Defrost and decontaminate the cryostat with a tuberculocidal hospital disinfectant once a week and immediately after tissue known to contain bloodborne pathogens, Mycobacterium tuberculosis or other infectious agents is cut.
- Handle microtome knives with extreme care, stainless steel mesh gloves should be worn when changing knife blades.
- Consider solutions for staining potentially infected frozen sections to be contaminated.

Centrifuge Equipment

Hazards associated with centrifuging include mechanical failure and the creation of aerosols. To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer's instructions. Users should be properly trained and operating instructions that include safety precautions should be prominently posted on the unit.

Aerosols are created by practices such as filling centrifuge tubes, removing plugs or caps from tubes after centrifugation, removing supernatant, and resuspending sedimented pellets. The greatest aerosol hazard is created if a tube breaks during centrifugation. To minimize the generation of aerosols

when centrifuging biohazardous material, the following procedures should be followed:

Use sealed tubes and safety buckets that seal with O-rings. Before use, inspect tubes, O-rings, and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.

Fill and open centrifuge tubes, rotors, and accessories in a BSC. Avoid overfilling of centrifuge tubes so that closures do not become wet. After tubes are filled and sealed, wipe them down with disinfectant.
Add disinfectant to the space between the tube and the bucket to disinfect material in the event of breakage during centrifugation.
Always balance buckets, tubes, and rotors properly before centrifugation.
Do not decant or pour off supernatant. Use a vacuum system with appropriate in-line reservoirs and filters. For more information, call RMS at (344) 844-4870.
Work in a BSC when resuspending sedimented material. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.

Small low-speed centrifuges may be placed in a BSC during use to reduce aerosol escape. High-speed centrifuges pose additional hazards. Precautions should be taken to filter the exhaust air from vacuum lines, to avoid metal fatigue resulting in disintegration of rotors and to use proper cleaning techniques and centrifuge components. Manufacturers' recommendations must be meticulously followed to avoid metal fatigue, distortion and corrosion.

Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials. Celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They distort on boiling and can be highly explosive in an autoclave. If celluloid tubes must be used, an appropriate chemical disinfectant must be used to disinfect them.

Personal Protective Equipment (PPE)

PPE is used to protect personnel from contact with hazardous materials and infectious agents. Appropriate clothing may also protect the experiment from contamination. PPE must be provided without cost to personnel. The following PPE is recommended for regular use:

Face protection

Goggles in combination with masks, or chin length face shields, or other splatter guards are required whenever there is the possibility of splashes, sprays, or splatters of infectious or other hazardous materials to the face.

Laboratory clothing

This category includes: laboratory coats, smocks, scrub suits, and gowns. Long sleeved garments should be used to minimize the contamination of skin or street clothes, and to reduce shedding of microorganisms from the arms. In circumstances where it is anticipated that splashes may occur, the garment must be resistant to liquid penetration (in order to protect clothing from contamination). If the garment is not disposable, it must be capable of withstanding sterilization in the event it becomes contaminated. Additional criteria for selecting clothing are: comfort, appearance, closure types, and location, antistatic properties, and durability. Protective clothing must be removed and left in the laboratory before leaving for non-laboratory areas. Disposables should be available for visitors, maintenance, and service workers in the event it is required. All protective clothing should be either discarded in the laboratory or laundered by the facility. Personnel must not launder laboratory clothing at home.

Gloves

Gloves must be selected based on the hazards involved and the activity to be conducted. Gloves must be worn when working with biohazards, toxic substances and other physically hazardous agents. Temperature resistant gloves must be worn when handling hot material or dry ice. Delicate work requiring a high degree of precision dictates the use of thin walled gloves. Protection from contact with toxic or corrosive chemicals may also be required. For assistance in glove selection consult the selection information in Prudent Practices for Handling Hazardous Chemicals in Laboratories. For further assistance call RMS at (334) 844-4870.

When working with hazardous materials, the lower sleeve and the cuff of the laboratory garment should be overlapped by the glove. A long sleeved glove or disposable arm-shield may be worn for further protection of the garment.

In some instances “double gloving” may be appropriate. If a spill occurs, hands will be protected after the contaminated outer gloves are removed. Gloves must be disposed of when contaminated and removed when work with infectious material is completed. Gloves are not to be worn outside the laboratory. Disposable gloves must not be washed or reused.

Respirators

In certain instances additional PPE may be required. Respirator selection is based on the hazard and the protection factor required. Personnel who require respiratory protection must contact RMS for inclusion in the Auburn University Respiratory Protection Program. The program provides: a physical examination to ensure no health conditions exist that would be exacerbated by respirator usage; annual fit testing to ensure proper respirator

size and type; and training to ensure proper respirator use and maintenance. Under no circumstances shall anyone wear a respirator unless he/she is a participant in the program.

Contact RMS for assistance in selection of respirators or other personal protective equipment.

Blenders, Ultrasonic Disrupters, Grinders and Lyophilizers

The use of blenders, ultrasonic disrupters, grinders, and/or lyophilizers devices may result in considerable aerosol production. Blenders, grinders, and cell-disruption equipment should be used in a BSC when working with biohazardous materials.

Safety blenders, although expensive, are designed to prevent leakage from the bottom of the blender jar, provide a cooling jacket to avoid biological inactivation and to withstand sterilization by autoclaving. If blender rotors are not leakproof, they should be tested with sterile saline or dye solution prior to use with biohazardous material. The use of glass blender jars is not recommended; however, if they must be used, glass jars should be covered with a polypropylene jar to prevent spraying of glass and/or contents if the jar breaks. A towel moistened with disinfectant should be placed over the top of the blender during use. Before opening the blender jar, allow the unit to rest for at least one minute to allow the aerosol to settle. The device should be decontaminated promptly after use.

Lyophilizers and ampoules

Depending on lyophilizer design, aerosol production may occur when material is loaded or removed from the unit. If possible, sample material should be loaded in a BSC. The vacuum pump exhaust should be filtered to remove any hazardous agents or, alternatively, the pump can be vented into a BSC. After lyophilization is complete, all surfaces of the unit that has been exposed to the agent should be disinfected. If the lyophilizer is equipped with a removable chamber, it should be closed off and moved to a BSC for unloading and decontamination. Handling of cultures should be minimized and vapor traps should be used wherever possible.

Opening ampoules containing liquid or lyophilized culture material should be performed in a BSC to control the aerosol produced. Gloves must be worn. To open, nick the neck of the ampoule with a file, wrap it in disinfectant soaked towel, hold the ampoule upright and snap it open at the nick. Reconstitute the contents of the ampoule by slowly adding liquid to avoid aerosolization of the dried material. Mix the contents without bubbling and withdraw it into a fresh container. Discard the towel and ampoule top and bottom as infectious waste.

Ampoules used to store biohazardous material in liquid nitrogen have exploded causing eye injuries. The use of polypropylene tubes eliminates this hazard. These tubes are available dust-free or presterilized and are fitted with polyethylene caps and silicone washers. Heat sealable polypropylene tubes are also available.

Loop Sterilizers and Bunsen Burners

Sterilization of inoculation loops or needles in an open flame generates small-particle aerosols which may contain viable microorganisms. The use of a shielded electric incinerator minimizes aerosol production during loop sterilization. Alternatively, disposable plastic loops and needles may be used for culture work where electric incinerators or gas flames are not available. The loops are semiquantitative and can be used for counting bacteria.

Continuous flame gas burners should not be used in BSCs. These burners can produce turbulence which disturbs the protective airflow patterns of the cabinet. Additionally, the heat produced by the continuous flame may damage the HEPA filter. If a gas burner must be used, one with a pilot light should be selected.

Laundry

All personal protective clothing must be cleaned, laundered and disposed of by the employer at no cost to employees. Apparel contaminated with blood or other potentially infectious materials should be handled as little as possible and decontaminated, preferably by autoclaving, before being sent to the laundry for cleaning. Appropriate PPE must be worn by employees who handle contaminated laundry.

Housekeeping

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

Laboratory personnel are responsible for cleaning laboratory benches, equipment, and areas that require specialized technical knowledge. Additional laboratory housekeeping concerns include:

- Keeping the laboratory neat and free of clutter; surfaces should be clean and free of infrequently used chemicals, glassware, and equipment. Access to sinks, eyewashes, emergency showers, and fire extinguishers must not be blocked.
- Proper disposal of chemicals and waste - old and unused chemicals

should be disposed of promptly and properly. Call RMS at (344) 844-4805/4870 for details.

- Providing a workplace that is free of physical hazards - aisles and corridors should be free of tripping hazards. Attention should be paid to electrical safety, specifically; as it relates to the use of extension cords, proper grounding, avoidance of overloaded electrical circuits, and the creation of electrical hazards in wet areas.
- Removing unnecessary items from floors, under benches, or in corners.
- Properly securing all compressed gas cylinders.
- Never using fume hoods for storage of chemicals or other materials.
- Practical custodial concerns include:
- Dry sweeping and dusting which may lead to the formation of aerosols is prohibited.
- The usual wet or dry industrial type vacuum cleaner is a potent aerosol generator and, unless equipped with High Efficiency Particulate Air (HEPA) filter, must not be used in the biological research laboratory. Use of these industrial type vacuums that do not have HEPA filters, is prohibited in order to protect personnel as well as the integrity of the experiment. Wet and dry units with HEPA filters on the exhaust are available from a number of manufacturers.

Decontamination

Decontamination is a term used to describe a process or treatment that renders a medical device, instrument, or environmental surface safe to handle. A decontamination procedure can range from sterilization to simple cleaning with soap and water. Sterilization, disinfection, and antisepsis are all forms of decontamination.

Sterilization is the use of a physical or chemical procedure to destroy all microbial life, including highly resistant bacterial endospores.

Disinfection eliminates virtually all pathogenic non-sporeforming microorganisms, but not necessarily all microbial forms on inanimate objects (work surfaces, equipment, etc.). Effectiveness is influenced by the kinds and numbers of organisms, the amount of organic matter, the object to be disinfected, and the chemical exposure time, temperature, and concentration.

Antisepsis is the application of a liquid antimicrobial chemical to skin or living tissue to inhibit or destroy microorganisms. It includes swabbing an injection site on a person or animal and hand washing with germicidal

solutions. Although some chemicals may be utilized as either a disinfectant or an antiseptic, adequacy for one application does not guarantee adequacy for the other. Manufacturers' recommendations for appropriate use of germicides should always be followed.

General Procedures

All infectious materials and all contaminated equipment or apparatus should be decontaminated before being washed, stored, or discarded. Autoclaving is the preferred method. Each individual working with biohazardous material should be responsible for its proper handling.

Biohazardous materials should not be placed in autoclaves overnight in anticipation of autoclaving the next day.

Autoclaves should not be operated unattended or by untrained personnel.

Special precautions should be taken to prevent accidental removal of material from an autoclave before it has been sterilized or simultaneous opening of both doors on a double door autoclave.

Dry hypochlorites, or any other strong oxidizing material, must not be autoclaved with organic materials such as paper, cloth or oil.

OXIDIZER + ORGANIC MATERIAL + HEAT = POSSIBLE EXPLOSION

Methods

There are four main categories of physical and chemical decontamination. They are heat, liquid disinfection, vapors and gases, and radiation. Each category is discussed briefly below.

Heat

Wet heat is the most dependable method of sterilization. Autoclaving (saturated steam under pressure of approximately 15 PSI to achieve a chamber temperature of at least 250º F for a prescribed time) is the most convenient method of rapidly achieving destruction of all forms of microbial life. In addition to proper temperature and time, prevention of entrapment of air is critical to achieving sterility. Material to be sterilized must come in contact with steam and heat. Chemical indicators, e.g. autoclave tape, must be used with each load placed in the autoclave. The use of autoclave tape alone is not an adequate monitor of efficacy. Autoclave sterility monitoring should be conducted on a regular basis using appropriate biological indicators (B. stearothermophilus spore strips) placed at locations throughout the autoclave. The spores, which can survive 250º F for 5 minutes but are killed at 250º F in 13 minutes, are more resistant to heat than most, thereby providing an adequate safety margin when validating decontamination

procedures. Each type of container employed should be individually tested with these spores because efficacy varies with the load, fluid volume, etc. Autoclaves used for sterilization of materials subject to ADEM Medical Waste Rules must comply with those rules. See "A Guide to the Handling and Disposal of Medical Waste", Appendix F for those requirements.

Dry Heat is less efficient than wet heat and requires longer times and/or higher temperatures to achieve sterilization. It is suitable for the destruction of viable organisms on impermeable non-organic surfaces such as glass, but it is not reliable in the presence of shallow layers of organic or inorganic materials which may act as insulation. Sterilization of glassware by dry heat can usually be accomplished at 160 - 170º C for periods of 2 - 4 hours. Dry heat sterilizers should be monitored on a regular basis using appropriate biological indicators [Bacillus subtilis (globigii) spore strips].

Incineration is another effective means of decontamination by heat. As a disposal method incineration has the advantage of reducing the volume of the material prior to its final disposal.

Liquid disinfection

The most practical use of liquid disinfectants is for surface decontamination, and when used in sufficient concentration, to decontaminate liquid wastes prior to final disposal in the sanitary sewer. If liquid disinfectants are used, they must have been shown to be effective against the organism(s) present.

Liquid disinfectants are available under a wide variety of trade names. In general, these can be classified as halogens, acids, alkalis, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydes, ketones, alcohols, and amines. The more active a compound is, the more likely it is to have undesirable characteristics such as corrosivity. No liquid disinfectant is equally useful or effective under all conditions and for all viable agents. Properties of common disinfectants can be viewed at, http://www.ehrs.upenn.edu/programs/bio/bsm/Table3.xls.

Vapors and gases

A variety of vapors and gases possess decontamination properties. Vapors and gases are primarily used to decontaminate biological safety cabinets and associated systems, bulky or stationary equipment not suited to liquid disinfectants, instruments or optics which might be damaged by other decontamination methods, rooms, buildings, and associated air-handling systems. Agents included in this category are: glutaraldehyde and formaldehyde vapor, ethylene oxide gas, peracetic acid, and hydrogen peroxide vapor. When used in closed systems and under controlled conditions of temperature and humidity, excellent disinfection can be obtained. Great care must be taken during use because of the hazardous

nature of many of these compounds. Contact RMS for monitoring requirements if these compounds are to be used.

Radiation

Although ionizing radiation will destroy microorganisms, it is not a practical tool for laboratory use. Nonionizing radiation in the form of ultraviolet radiation (UV) is used for inactivating viruses, bacteria, and fungi. It will destroy airborne microorganisms and inactivate microorganisms on exposed surfaces or in the presence of products of unstable composition that cannot be treated by conventional means.

Because of the low penetrating power of UV, microorganisms inside dust or soil particles will be protected from its action, limiting its usefulness. UV is used in air locks, animal holding areas, ventilated cabinets and laboratory rooms to reduce levels of airborne microorganisms and maintain good air hygiene. Because UV can cause burns to the eye and skin of people exposed for even a short period of time, proper shielding should be maintained when it is in use. UV lamps that are used for space decontamination should be interlocked with the general room or cabinet illumination, so that turning on the lights extinguishes the UV.

UV lamps are not recommended for decontamination unless they are properly maintained. Because UV lamp intensity or destructive power decreases with time, it should be checked with a UV meter yearly. Frequent lamp cleaning (at least every few weeks) is necessary to prevent accumulation of dust and dirt which drastically reduces its effectiveness. If UV must be used, it should be used when areas are not occupied.

Infectious Waste Management

Consult "A Guide to the Handling and Management of Medical Waste" for complete details on the disposal of infectious waste in accordance with ADEM regulations and University policy. A copy is included as Appendix F of the Biosafety Manual. Additional copies are available by request, either call (344) 844-4870 or send a request to RMS, 316 Nuclear Science Center.

Mixed Waste

Mixed wastes are potentially infectious waste contaminated with other types of waste, e.g., radioisotopes or toxic/carcinogenic compounds. Because of the difficulty in disposal of wastes regulated by more than one set of requirements and more than one regulatory agency, it is critical that provision be made for proper management prior to the initiation of any research that might result in mixed waste. Mixed wastes may require special containers, labeling, storage, etc. Contact RMS prior to initiation of any research that might result in potentially infectious waste with multiple hazards.

Animals

The Institutional Animal Care and Use Committee must approve the disposal method for research animals and animal parts that are considered to be infectious waste. RMS will be consulted when necessary.

Important Note: All cultures of microorganisms should be inactivated before disposal. This includes cultures of RG1 organisms. This is considered good laboratory practice.

Appendix F Identification of Investigators Using CDC Select Agents and Toxins

Principal Investigator	Title:
Department:	Phone:
Building:	Room:
E-Mail Address:	Fax:

Please provide a brief description of the agent(s), the quantities that you will be handling and storing, storage locations, security precautions, and mechanisms by which you will ensure their safe usage and disposal.

Please put a check mark next to the viruses, bacteria, rickettsiae, fungi, toxins and/or recombinant organisms/molecules, on the following list, which you have or plan to have in your laboratory.

	HHS SELECT AGENTS AND TOXINS		USDA SELECT AGENTS AND TOXINS
☐	Abrin	☐	African horse sickness virus
☐	Cercopithecine herpesvirus 1 (Herpes B virus)	☐	African swine fever virus
☐	*Coccidioides posadasii*	☐	Akabane virus
☐	Conotoxins	☐	Avian influenza virus (highly pathogenic)
☐	Crimean-Congo haemorrhagic fever virus	☐	Bluetongue virus (Exotic)
☐	Diacetoxyscirpenol	☐	Bovine spongiform encephalopathy agent
☐	Ebola virus	☐	Camel pox virus
☐	Lassa fever virus	☐	Classical swine fever virus
☐	Marburg virus	☐	*Cowdria ruminantium* (Heartwater)
☐	Monkeypox virus	☐	Foot-and-mouth disease virus
☐	Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)	☐	Goat pox virus
		☐	Japanese encephalitis virus
		☐	Lumpy skin disease virus
☐	Ricin		Malignant catarrhal fever virus (Alcelaphine herpesvirus type 1)
☐	*Rickettsia prowazekii*	☐	
☐	*Rickettsia rickettsii*	☐	Menangle virus
☐	Saxitoxin	☐	*Mycoplasma capricolum*/ M.F38/*M. mycoides Capri* (contagious caprine pleuropneumonia)
☐	Shiga-like ribosome inactivating proteins		*Mycoplasma mycoides mycoides* (contagious bovine pleuropneumonia)
☐	South American Haemorrhagic Fever viruses	☐	
☐	Flexal	☐	Newcastle disease virus (velogenic)
☐	Guanarito	☐	Peste des petits ruminants virus

☐	Junin	☐	Rinderpest virus
☐	Machupo	☐	Sheep pox virus
☐	Sabia	☐	Swine vesicular disease virus
☐	Tetrodotoxin	☐	Vesicular stomatitis virus (Exotic)
☐	Tick-borne encephalitis complex (flavi) viruses	**USDA PLANT PROTECTION AND QUARANTINE (PPQ) SELECT AGENTS AND TOXINS**	
☐	Central European Tick-borne encephalitis	☐	*Candidatus* Liberobacter africanus
☐	Far Eastern Tick-borne encephalitis	☐	*Candidatus* Liberobacter asiaticus
☐	Kyasanur Forest disease	☐	*Peronosclerospora philippinensis*
☐	Omsk Hemorrhagic Fever	☐	*Ralstonia solanacearum* race 3, biovar 2
☐	Russian Spring and Summer encephalitis	☐	*Schlerophthora rayssiae* var *zeae*
☐	Variola major virus (Smallpox virus) and Variola minor virus (Alastrim)	☐	*Synchytrium endobioticum*
		☐	*Xanthomonas oryzae* pv. *oryzicola*
☐	*Yersinia pestis*	☐	*Xylella fastidiosa* (citrus variegated chlorosis strain)

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

Signature of
Principle Investigator: _____ Date: _____

Appendix G Medical Waste Management Guide

Appendix F is the latest version of the Auburn University Medical Waste Management Guide available from Risk Management and Safety.

http://www.auburn.edu/administration/safety/MedicalWaste.html

Appendix H Training

As described in Section II, the PI is responsible for the training of everyone working in his/her laboratory. Training is possibly the single most important action a PI can take to promote a safe and healthy working environment/ laboratory. All training must be documented.

At a minimum, training will consist of the following:

- Basic Laboratory Safety Training
- Laboratory - Specific Biosafety Training
- Medical Waste Management Training
- Bloodborne Pathogens Training (if necessary)

Violations of established safety procedures resulting in accident/incident may be followed by recommendations from the IBC that would require additional specialized training. These recommendations would be routed through the Vice President for Research, as well as appropriate deans, directors and department heads.

Basic Laboratory Safety Training consists of a series of short videotapes that can be viewed in approximately one hour. The videotape cassettes can be borrowed from RMS. Alternatively, the tapes can be viewed at that office. Please call (344) 844-4870 to request the tapes or schedule a viewing. The required tapes are:

Practicing Safe Science	29 minutes
Chemical Hazards	10 minutes
Emergency Response	12 minutes
Centrifugation Hazards	12 minutes

Specific Laboratory Biosafety Training will be provided by the PI and will include generalized training for the biosafety level at which the laboratory operates and specialized training for specific hazards present in that laboratory. For workers in laboratories operating at BL1, it is suggested the memorandum included as Appendix H be reviewed with each worker. The memorandum should be signed by the employee or student, Laboratory Supervisor, Laboratory Director/PI and Department Head. This training shall be reviewed annually. If the suggested memorandum is not used, an equivalent instrument must be developed to document that all standard and special practices, safety equipment, and facilities related to work at BL1 have been addressed.

For workers in laboratories operating at BL2, the memorandum included as Appendix H or its equivalent must be reviewed with each worker. The memorandum should be signed by the employee or student, Laboratory Supervisor, Laboratory Director/PI and Department Head. Note that CDC "select agents" may be present and their presence should be noted on training documentation. This training shall be reviewed annually.

For workers in laboratories operating at BL3, it is suggested the memorandum included as Appendix H or its equivalent must be reviewed with each worker. The memorandum should be signed by the employee or student, Laboratory Supervisor, Laboratory Director/PI and Department Head. Note that CDC select agents may be present and their presence should be noted on training documentation. This training shall be reviewed annually.

The signature page(s) should be retained by the PI as explained in the section below titled Documentation. Training regarding the requirements of BL1, BL2 and BL3 must be documented. In addition, additional training shall be provided to any employee or student for any specific hazards present in the laboratory in which they work. Additional training must also be documented.

Medical Waste Management Training is provided by the RMS, without cost. Please call 4-4870 to schedule Medical Waste Management Training. This training must be documented.

Bloodborne Pathogens Training is necessary for any student or employee who works with human blood, human blood components, and products made from human blood, human organs, or human body fluids. Bloodborne Pathogens Training is provided by RMS. Please call (344) 844-4870 to schedule Bloodborne Pathogens Training. This training must be documented.

Violations of established safety procedures resulting in accident/incident may be followed by recommendations from the IBC that would require additional specialized training. These recommendations would be routed through the Vice President for Research, as well as appropriate deans, directors and department heads.

DOCUMENTATION

All training must be documented, to ensure each laboratory worker receives the required training, and to provide written testimony to that effect. Auburn University has developed an example form that may be used for this purpose. Besides documentation, the form provides the PI with a checklist of safety training that might be required by hazards present within his/her laboratory.

The Training Form, signature pages from the memoranda described above and any additional training certifications should be kept together in a central location within each laboratory. The PI is responsible for ensuring that the required training is provided and documented as discussed in Section II of this manual. Documentation will be examined during regular laboratory inspections performed by the RMS.

For the purposes of this program a certified training course is a course that is offered through RMS or a recognized, independent source of training.

Teaching Laboratories

Instructors in teaching laboratories should provide specific training for the hazards expected to be encountered in the laboratory procedures utilized. Because of the brevity of courses, the large number of students involved, and the detail and length of the Training Form, it is recommended that each instructor develop a streamlined training form emphasizing the particular elements required for their course. Both the instructor and student should sign the training form.

Biological Laboratory Certification BSL-1

Date: _____

MEMORANDUM FOR: _____
(Laboratory PI)

FROM: *Risk Management & Safety/Laboratory Safety*
SUBJECT: *Laboratory Certification/Review of Laboratory Safety Guidelines*

The laboratory facilities contained in room/s _____ of the _____ building are designated as Biosafety Level 1 (BSL-1) facilities and therefore potentially contain human and/or animal pathogens. These facilities are subject to the guidelines outlined in the Auburn University Biosafety Manual (BSM) and the Biosafety in Microbiological and Biomedical Laboratories Manual (BMBL) published by the Center for Disease Control and Prevention and the National Institutes of Health. Adherence is mandatory. Failure to comply with these guidelines may result in denied access to the facilities and/or immediate job termination and/or federal prosecution. ****Please have laboratory personnel/students read and sign safety guidelines below. A copy of this certification/these guidelines shall be maintained in the lab and available for review by safety personnel.***

BSL-1 Safety Guidelines

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

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 or work with cultures and specimens are in progress.

2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.

3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the work areas. Persons who wear contact

lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.

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

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

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

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

8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated outside of the immediate laboratory are packaged in accordance with applicable local, state, and federal regulations before removal from the facility.

9. A biohazard sign can be posted at the entrance to the laboratory whenever infectious agents are present. The sign may include the name of the agent(s) in use and the name and phone number of the investigator.

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

B. *Special Practices* None

C. *Safety Equipment (Primary Barriers)*

1. Special containment devices or equipment such as a biological safety cabinet are generally not required for manipulations of agents assigned to Biosafety Level 1.

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

3. Gloves should be worn if the skin on the hands is broken or if a rash is present. Alternatives to powdered latex gloves should be available.

4. Protective eyewear should be worn for conduct of procedures in which splashes of microorganisms or other hazardous materials is anticipated.

D. *Laboratory Facilities (Secondary Barriers)*

1. Laboratories should have doors for access control.

2. Each laboratory contains a sink for handwashing.

3. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.

4. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surface and equipment.

5. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning.

6. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

STATEMENT OF AGREEMENT

I have read and understand this memorandum and agree to adhere to all requirements herein. I understand violation of these requirements could result in one or more of the following actions:

- ***Make me ineligible for access to this laboratory.***
- ***Result in possible job termination.***
- ***Result in possible prosecution under pertinent state and federal regulations.***

_____	_____
Laboratory Personnel Signature	***Date Signed***
_____	_____
Laboratory Personnel Signature	***Date Signed***
_____	_____
Laboratory Personnel Signature	***Date Signed***
_____	_____
Laboratory Personnel Signature	***Date Signed***

Biological Laboratory Certification BSL-2

Date: _____

MEMORANDUM FOR: _____
(Laboratory PI)

FROM: *Risk Management & Safety/Laboratory Safety*
SUBJECT: *Laboratory Certification/Review of Laboratory Safety Guidelines*

The laboratory facilities contained in room/s _____ of the _____ building are designated as Biosafety Level 2 (BSL-2) facilities and therefore potentially contain human and/or animal pathogens. These facilities are subject to the guidelines outlined in the Auburn University Biosafety Manual (BSM) and the Biosafety in Microbiological and Biomedical Laboratories Manual (BMBL) published by the Center for Disease Control and Prevention and the National Institutes of Health. Adherence is mandatory. Failure to comply with these guidelines may result in denied access to the facilities and/or immediate job termination and/or federal prosecution. ***Please have laboratory personnel/students read and sign safety guidelines below. A copy of this certification/these guidelines shall be maintained in the lab and available for review by safety personnel.***

BSL-2 Safety Guidelines

Biosafety Level 2 is similar to Biosafety Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs from BSL-1 in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; (3) extreme precautions are taken with contaminated sharp items; and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

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 at the discretion of the laboratory director when experiments are in progress.

2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.

3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not

permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.

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

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

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

7. Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern.

8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the facility are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.

9. An insect and rodent control program is in effect (see Appendix G).

B. *Special Practices*

1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.

2. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.

3. A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate information to be posted includes the agent(s) in use, the biosafety level, the required immunizations, the investigator's name and telephone number, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.

4. Laboratory personnel receive appropriate immunizations or tests for the agents

handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

5. 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.

6. Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

7. The laboratory director ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.

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

a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

c. Syringes which re-sheathe the needle, needleless systems, and other safety devices are used when appropriate.

d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.

9. Cultures, tissues, specimens of body fluids, or potentially infectious wastes are

placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.

10. Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

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

12. Animals not involved in the work being performed are not permitted in the lab.

C. *Safety Equipment* (Primary Barriers)

1. Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:

a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonate eggs.

b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.

2. Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.

3. Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.

4. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

D. *Laboratory Facilities* (Secondary Barriers)

1. Provide lockable doors for facilities that house restricted agents (as defined in 42 CFR 72.6).

2. Consider locating new laboratories away from public areas.

3. Each laboratory contains a sink for handwashing.

4. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.

5. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.

6. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.

7. Install biological safety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Locate biological safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the biological safety cabinets' air flow parameters for containment.

8. An eyewash station is readily available.

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

10. There are no specific ventilation requirements. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air

without recirculation to spaces outside of the laboratory. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

STATEMENT OF AGREEMENT

I have read and understand this memorandum and agree to adhere to all requirements herein. I understand violation of these requirements could result in one or more of the following actions:

- ***Make me ineligible for access to this laboratory.***
- ***Result in possible job termination.***
- ***Result in possible prosecution under pertinent state and federal regulations.***

_____	_____
Laboratory Personnel Signature	***Date Signed***
_____	_____
Laboratory Personnel Signature	***Date Signed***
_____	_____
Laboratory Personnel Signature	***Date Signed***
_____	_____
Laboratory Principal Investigator	***Date Signed***

Biological Laboratory Certification BSL-3

Date: _____

MEMORANDUM FOR: _____
(Laboratory PI)

FROM: *Risk Management & Safety/Laboratory Safety*
SUBJECT: *Laboratory Certification/Review of Laboratory Safety Guidelines*

The laboratory facilities contained in room/s _____ of the _____ building are designated as Biosafety Level 3 (BSL-3) facilities and therefore potentially contain human and/or animal pathogens. These facilities are subject to the guidelines outlined in the Auburn University Biosafety Manual (BSM) and the Biosafety in Microbiological and Biomedical Laboratories Manual (BMBL) published by the Center for Disease Control and Prevention and the National Institutes of Health. Adherence is mandatory. Failure to comply with these guidelines may result in denied access to the facilities and/or immediate job termination and/or federal prosecution. ****Please have laboratory personnel/students read and sign safety guidelines below. A copy of this certification/these guidelines shall be maintained in the lab and available for review by safety personnel.***

BSL-3 Safety Guidelines

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

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

Safety Equipment for Biosafety Level 3 are rigorously followed. The decision to implement this modification of Biosafety Level 3 recommendations should be made only by the laboratory director.

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

A. *Standard Microbiological Practices*

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.

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

3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the laboratory. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.

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

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

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

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

8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leakproof container and closed for transport from the laboratory. Infectious waste from BSL-3 laboratories should be decontaminated before removal for off-site disposal.

9. An insect and rodent control program is in effect (see Appendix G).

B. *Special Practices*

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

2. The laboratory director controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. Persons who are at increased risk of acquiring infection or for whom infection may have serious

consequences are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. No minors should be allowed in the laboratory.

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

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

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

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

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

8. Laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural changes.

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

techniques.

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

a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

c. Syringes which re-sheathe the needle, needleless systems, and other safe devices are used when appropriate.

d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass should be decontaminated before disposal, and disposed of according to any local, state, or federal regulations.

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

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

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

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

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

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

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

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

C. *Safety Equipment* (Primary Barriers)

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

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

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

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

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

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

D. *Laboratory Facilities (Secondary Barriers)*

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

included in the passageway.

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

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

4. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and those chemicals used to decontaminate the work surfaces and equipment.

5. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.

6. All windows in the laboratory are closed and sealed.

7. A method for decontaminating all laboratory wastes is available in the facility and utilized, preferably within the laboratory (i.e., autoclave, chemical disinfection, incineration, or other approved decontamination method). Consideration should be given to means of decontaminating equipment. If waste is transported out of the laboratory, it should be properly sealed and not transported in public corridors.

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

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

provided at the laboratory entry. Consideration should be given to installing an HVAC control system to prevent sustained positive pressurization of the laboratory. Audible alarms should be considered to notify personnel of HVAC system failure.

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

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

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

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

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

15. The Biosafety Level 3 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified, at least annually, against these procedures as modified by operational experience.

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

STATEMENT OF AGREEMENT

I have read and understand this memorandum and agree to adhere to all requirements herein. I understand violation of these requirements could result in

one or more of the following actions:

- ***Make me ineligible for access to this laboratory.***
- ***Result in possible job termination.***
- ***Result in possible prosecution under pertinent state and federal regulations.***

_____	_____
Laboratory Personnel Signature	***Date Signed***
_____	_____
Laboratory Personnel Signature	***Date Signed***
_____	_____
Laboratory Personnel Signature	***Date Signed***
_____	_____
Laboratory Personnel Signature	***Date Signed***
_____	_____
Laboratory Principal Investigator	***Date Signed***