BIOSAFETY MANUAL

Valdosta State University

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I. Introduction

In conjunction with the U.S. Patriot Act and Valdosta State University's Campus Homeland Security (www.valdosta.edu/legal/chs), the following policy outlines the practices embodied in the aforementioned authority as they relate to selected agents/chemicals. This policy defines the responsibilities of certain individuals, as well as protocol/practices. Its purpose is to provide guidelines for safe handling of biohazardous materials (including biological agents, toxins, and recombinant DNA) by employees and students at Valdosta State University. These guidelines are offered for the protection of students and employees at Valdosta State University as well as the general community.

II. Primary Policy and Procedures Source for VSU Biosafety Manual Development

This Biosafety Manual is based largely on the Biosafety Manual of the University of Georgia and the relative contents contained therein are used with expressed permission. Biosafety policies and procedures at Valdosta State University conform to policies and procedures as prescribed by the Centers for Disease Control and the National Institute of Health and published in Biosafety in Microbiological and Biomedical Laboratories, CDC/NIH, Fourth Edition, U.S. Department of Health and Human Services, U.S. Government Printing Office, May 1999. (Document is available at

http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm).

III. Resources on Biosafety

Many excellent resources are available on various aspects of biosafety; several are listed below.

· Office of Health and Safety Information System, CDC. (http://www.cdc.gov/od/ohs/default.htm)

· CDC Office of Health and Safety, Biosafety Documents http://www.cdc.gov/od/ohs/biosfty/biosfty.htm

· Biosafety Resources on the Internet, American Biological Safety Association (http://www.absa.org/resources/resource.htm).

· American Society for Microbiology, Resources Related To Biological Weapons Control And Bioterrorism Preparedness http://www.asmusa.org/pasrc/bioprep.htm

· *Biosafety in Microbiological and Biomedical Laboratories, CDC/NIH*, Fourth Edition, U.S. Department of Health and Human Services, U.S. Government Printing Office, May 1999. (available at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm).

· Risk Group Classification for Infectious Agents (available at the web site of the American Biological

· Safety Association, http://www.absa.org/riskgroups/default.htm).

· Bloodborne pathogens (29CFR, 1910.1030), Occupational Safety and Health Administration (available at **http://www.osha-slc.gov/OshStd_data/1910_1030.html**)

· *Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets*, U.S. Dept. of Health and Human Services, September 1995. (available at

· **http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm**).

· Interstate Shipment of Etiologic Agents (42CFR Part 72), Federal Register, July 21, 1980. (available at **http://www.cdc.gov/od/ohs/biosfty/shipregs.htm**).

· Additional requirements for facilities transferring or receiving select agents; Final Rule (42 CFR Part 72.6), Federal Register, Oct. 24, 1996. (available at **http://www.cdc.gov/od/ohs/lrsat/regmat.htm**).

· Select Agent Rule http://www.phppo.cdc.gov/nltn/sar.asp

· Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens, World Health Organization, 1997. (available at **http://www.absa.org/resources/who-97.pdf**).

· Notification of Possession of Select Agents or High Consequence Livestock Pathogens and Toxins. Federal Register Vol 67, No. 151, Tuesday, August 6, 2002

· Agricultural Bioterrorism Protection Act: Biological agents and toxins; possession. Federal Register Vol. 67, No. 155, Monday, August 12, 2002

· USDA Animal and Plant Health Inspection Service, Veterinary Services, National Center for Import and Export http://www.aphis.usda.gov/vs/ncie/

· USDA Animal and Plant Health Inspection Service, Plant Protection and Quarantine http://www.aphis.usda.gov/ppq/

· Agricultural Permits, U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine, (http://www.aphis.usda.gov/ppq/permits/index.html)

· U.S. Regulatory Oversight in Biotechnology, U.S. Department of Agriculture, U.S. Environmental Protection Agency, and U.S. Food and Drug Administration (Unified Homepage)

· (http://www.aphis.usda.gov/biotech/OECD/usregs.htm#fdalaw)

· *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines),* April 2002. (available at http://www4.od.nih.gov/oba/rac/guidelines_02/NIH_Guidelines_Apr_02.htm)

· Code of Federal Regulations (U.S. Government) http://www.access.gpo.gov/nara/cfr/index.html

· Federal Register (1995-present) http://www.gpo.gov/su_docs/aces/aces140.html

· U.S. Congress, 1988. *Medical Waste Tracking Act of 1988.* H.R. 3515, 42U.S.C. 6992-6992k.

· *Biosafety in the Laboratory: Prudent Practices for the Handling and Disposal of Infectious Materials.* 1989. National Research Council. National Academy Press, Washington, DC.

· Fleming, D.O. and D.L. Hunt (Editors) 2000. *Biological Safety, Principles and Practices,* Third Edition. ASM Press, Washington, DC.

IV. General Information about Biosafety

"Containment" refers to safe procedures for handling biohazardous material so that such materials do not pose a hazard to people and/or the environment. Primary containment focuses

on protecting workers in the immediate area; whereas, secondary containment deals with protection of the environment and people outside the immediate area. Containment is accomplished through: (1) the use of appropriate procedures, (2) the use of safety equipment, and (3) conducting work in an appropriately designed facility.

V. Responsibilities

A. Faculty and Professional Staff (Principal Investigators, Laboratory Directors, Teaching Supervisors and Project Supervisors)

Developing and maintaining a healthful and safe work environment depends on the day-to-day supervision of investigative practices by personnel with a positive safety attitude. The teaching supervisor, principal investigator (PI), laboratory director, or project supervisor, is responsible for complying fully with Valdosta State University, Board of Regents, State, and Federal rules, regulations, and/or standards. The teaching supervisor, principal investigator, and/or laboratory supervisor of both laboratory and field work shall:

1. Determine the known or potential biohazards associated with the proposed work.
2. For recombinant DNA experiments, the principal investigator shall not initiate or modify those experiments requiring approval of the Biosafety Committee until that proposed research or modification has received approval from the Biosafety Committee and has met all other requirements of the appropriate governing State and/or Federal agencies.
3. Submit a Biohazardous Activities Checklist with each appropriate class activity description, research activity description, or grant application, or as directed by the Department Head, and submit a Memorandum of Understanding and Agreement (MUA) as requested by the Biosafety Officer/Biosafety Committee or otherwise required by applicable guidelines, regulations, or standards.
4. Provide those individuals under his/her supervision with knowledge of biohazards to which they may be exposed and safety procedures to be followed. This is to be accomplished by:

a. the supervisor being knowledgeable of good laboratory and field safety practice and having a positive safety attitude.

b. making available to the individuals being supervised copies of the protocols that describe potential biohazards and the precautions to be taken. These protocols as well biosafety concerns should be produced in the form of a standard operating procedure for the work.

c. providing the individuals with formal and informal instruction and training in the practices and techniques required to ensure safety and in the procedures for dealing with laboratory and field accidents.

d. informing the individuals of the reasons and provisions for any precautionary medical practices (e.g. physical examinations, serum collection, and vaccinations).

e. supervising the performance of the individuals to ensure that required safety practices and techniques are employed.

1. Report in writing to the Department Head and Biosafety Officer any laboratory or field accident, exposure of personnel, suspected illness, escape from containment of biohazardous agents, and significant problems pertaining to the operation and implementation of containment practices and procedures.
2. Provide physical examinations and other medical surveillance of personnel when required by the nature of the experiments/activities.
3. Insure the integrity of the physical containment (e.g. biological safety cabinets) and biological containment (e.g. purity and genotypic and phenotypic characteristics).
4. Maintain a knowledge of and adhere to the permit requirements of federal and state agencies for interstate and international movement of biohazardous agents.

B. Department Head

The Department Heads has the following responsibilities:

1. To insure that prior to initiation of the activity or application for a grant, each supervisor using a biohazardous agent files a Biohazardous Activities Checklist and Memorandum of Understanding and Agreement with the Biosafety Committee through the Biosafety Officer when this is required.

2. To insure that students have had instruction in safety procedures in teaching laboratories or field situations where biohazardous agents are used or encountered.

3. To determine that appropriate facilities and safety equipment are available for the proposed research or instruction involving biohazardous agents.

4. To provide leadership in laboratory safety at the management level in the department.

5. To recommend to the University Administration appropriate sanctions for non-compliance with

biosafety standards, guidelines, or regulations.

C. Institutional Biosafety Committee

The Institutional Biosafety Committee serves to advise the Department Heads on policies pertaining to biohazardous research, teaching, and service activities. The committee recommends standards under which biohazardous activities should be conducted and reviews projects for compliance with appropriate federal guidelines and regulations. Other specific responsibilities include:

1. Review for appropriateness and adequacy the containment levels and safety measures proposed and/or used in research and teaching.
2. Assess the adequacy of containment facilities for biosafety level 3 agents and rDNA molecules as required by NIH or other funding or regulatory agencies.
3. Develop with the Biosafety Officer informational and training seminars and workshops

on biohazards for the University community.
4. Review periodically biohazardous research being conducted at the University to insure that the requirements of the University, funding sources, and regulatory agencies are being fulfilled.
5. Recommend to the University Administration appropriate sanctions for non-compliance with biosafety standards, guidelines, or regulations.
6. Adopt emergency plans covering accidental spills and personnel contamination resulting from biohazardous research.

The minimum composition of the Institutional Biosafety Committee (IBC) is specified in the NIH "Guidelines for Research Involving Recombinant DNA Molecules". The Institutional Biosafety Committee shall have at least 5 members selected to have expertise and experience in recombinant DNA technology and capable of assessing the safety of rDNA research experiments and any potential risks to public health and the environment. The IBC shall include at least 2 members who are not affiliated with the University by other than their committee membership. In addition, when experiments using animals or plants require prior IBC approval, there shall be at least one scientist with expertise in plant pathogens or plant pest containment and one scientist with animal containment expertise on the IBC.

D. Biosafety Officer

The University's Biosafety Officer (Coordinator for Biosafety) has responsibility for the daily administration of standards set by the Institutional Biosafety Committee and acts as the agent of the committee in their implementation. Other responsibilities include, as resources allow:

1. receiving Biohazardous Research Checklists and Memorandum of Understanding and Agreement for preliminary screening and assignment to the Biosafety Committee or special subcommittee thereof for review.
2. arranging for initial and periodic inspections of laboratories used in biohazardous research to insure that standards set by the Institutional Biosafety Committee are followed.
3. providing technical advice to Principal Investigators and to the Biosafety Committee on research safety procedures.
4. organizing and conducting informational and training seminars and workshops on biohazards for the University community.
5. arranging with the University Health Service for appropriate medical surveillance of personnel working with certain biohazardous agents or as required by the Institutional Biosafety Committee.
6. providing technical advice to the University regarding biohazard safety needs and requirements for projects involving the renovation or construction of laboratory or other facilities in which biohazards will be used.

E. Valdosta State University

The University and its administrative officers are ultimately responsible for the following:

1. developing and maintaining appropriate policies regarding the conduct of potentially biohazardous research, education, and service activities.
2. developing mechanisms for insuring adherence to biosafety policies.
3. providing the resources necessary for the construction of safe research and teaching facilities and for the implementation of the biosafety program.
4. providing adequate resources for the dissemination of information on biohazards and biosafety procedures, including training programs and workshops.
5. providing resources for appropriate medical surveillance measures to protect the health and safety of employees.
6. providing appropriate and sufficient legal protection for faculty and staff members who conduct activities in compliance with appropriate regulations and guidelines.

VI. Biohazardous Research

A. General Definitions

1. Institutional Biosafety Committee (IBC) - The University Committee appointed by the President of Valdosta StateUniversity and which meets the requirements specified by NIH in its "Guidelines for Research Involving Recombinant DNA Molecules". The Committee also reviews, approves, activities involving recombinant DNA as well as other biohazards identified in this manual.
2. Biohazard - infectious agents, or parts or products thereof, presenting a real or potential risk to the well-being of humans, other animals, or plants directly through infection and/or toxicity or indirectly through disruption of the environment; and venomous vertebrate or invertebrate animals and other toxic organisms presenting a real or potential risk to humans.
3. Class 2 Agents - are those agents that are to be handled using Biosafety Level 2 or greater containment facilities and practices.
4. Class 3 Agents - are those agents that are to be handled using Biosafety Level 3 or greater containment facilities and practices.
5. Class 4 Agents - are those agents that are to be handled using Biosafety Level 4 containment facilities and practices.
6. Genetic Engineering - the genetic modification of organisms by recombinant DNA techniques.
7. Plant Pests - Any living stage (including active and dormant forms) of insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses; or any organisms similar to or allied with any of the foregoing; or any infectious agents or substances, which can directly or indirectly injure or cause disease or damage in or to any plants or parts thereof, or any processed, manufactured, or other products of plants. (USDA - 7 CFR 340.1)
8. Regulated Article - any organism which has been altered or produced through genetic engineering, if the donor organism, recipient organism, or vector or vector agent belongs to any genera or taxa designated in 7 CFR 340.2 and meets the definition of plant pest, or

is an unclassified organism and/or an organism whose classification is unknown or any product altered or produced through genetic engineering which the Deputy Administrator (USDA) determines is a plant pest or has reason to believe is a plant pest. (USDA - 7 CFR 340.1)

9. Restricted Animal Pathogens - nonindigenous pathogens of domestic livestock and poultry that may require special containment strategies and facilities not generally discussed in this manual.

B. Classification of Biohazardous Research

The University's Biosafety Committee has identified the following types of research as potentially biohazardous. References used in designating specific classes or agents or biohazardous potential are included as appropriate.

Type I - In Vitro Construction and/or Propagation of Recombinant DNA Molecules. The process of genetic engineering is covered by regulations or guidelines promulgated by several federal agencies. The Biosafety Committee is to receive an MUA for all rDNA projects (covered and exempt) for record purposes.

References:

· *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*, January 2001. (available at http://www4.od.nih.gov/oba/rac/guidelines/guidejan01.htm)

· USDA, Animal and Plant Health Inspection Service (APHIS), "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which are Plant Pests or Which There is Reason to Believe are Plant Pests", Federal Register, 52:115:22892-22915, June 16, 1987.

· EPA, Federal Insecticide, Fungicide, Rodenticide Act and the Toxic Substances Control Act; both acts are found in the Code of Federal Regulations 40.

· Office of Science and Technology Policy, "Coordinated Framework For Regulation of Biotechnology: Announcement of Policy and Notice for Public Comment", Federal Register, 51:123:23301-23393, June 26, 1986.

Type II - Experiments with Organisms (or Parts or Products of Organisms, Such as Toxins) of Demonstrated Human Pathogenicity and/or Toxicity. Any infectious agent (or part or product, as defined in the preceding sentence) that may cause serious illness or death is to be reviewed by the Biosafety Officer and/or the Biosafety Committee based on the biosafety level of the agent. MUAs are required for biosafety level 3 agents and may be required for biosafety level 2 agents based on the proposed experiments.

References:

· Occupational Safety and Health Administration, "Occupational Exposure to Bloodborne

Pathogens", Federal Register, 56:235:64004-64174, December 6, 1991.

· *Biosafety in Microbiological and Biomedical Laboratories, CDC/NIH*, Fourth Edition, U.S. Department of Health and Human Services, U.S. Government Printing Office, May 1999. (available at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm).

· Additional requirements for facilities transferring or receiving select agents; Final Rule (42 CFR Part 72.6), Federal Register, Oct. 24, 1996. (available at **http://www.cdc.gov/od/ohs/lrsat/regmat.htm**).

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Type III - Experiments Involving Oncogenic (cancer-causing) Animal Viruses. Oncogenic viruses should be handled similar to the Human Immunodeficiency Virus (HIV) at Biosafety Levels 2 or 3.

References:

· *Biosafety in Microbiological and Biomedical Laboratories, CDC/NIH*, Fourth Edition, U.S. Department of Health and Human Services, U.S. Government Printing Office, May 1999. (available at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm).

· Occupational Safety and Health Administration, "Occupational Exposure to Bloodborne Pathogens", Federal Register, 56:235:64004-64174.

· Bloodborne pathogens (29CFR, 1910.1030), Occupational Safety and Health Administration (available at **http://www.osha-slc.gov/OshStd_data/1910_1030.html**)

Type IV - Studies Involving Human or Other Primate Tissue or Cell Culture. The principal hazards associated with culturing tissue or cells are inherent virus contamination, agents being researched using the cell/tissue culture, and/or contamination of the culture by outside sources. In general, cell/tissue culture is to be conducted at BSL 2 but may be increased by the University Biosafety Committee depending on the agent(s) used. Whenever there is a high potential for aerosol or droplet production, a biosafety cabinet will be used. The Biosafety Committee does not recommend the use of vertical or horizontal laminar flow clean benches for biological work.

References:

· Biohazards in Biological Research, Proceedings of a Conference sponsored by the National Science Foundation, National Cancer Institute, and American Cancer Society, January 22-24, 1973, Cold Spring Harbor Laboratory.

· *Biosafety in Microbiological and Biomedical Laboratories, CDC/NIH*, Fourth Edition, U.S. Department of Health and Human Services, U.S. Government Printing Office, May 1999. (available at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm).

· Fleming, D.O. and D.L. Hunt (Editors) 2000. *Biological Safety, Principles and Practices*, Third Edition. ASM Press, Washington, DC.

Type V - Studies Involving Venomous Invertebrates or Vertebrates. Federal and state agencies including but not limited to the United States Departments of Agriculture, Interior, and Health and Human Services and Georgia Departments of Agriculture and Natural Resources restrict the movement of certain species of venomous vertebrates and invertebrates and in special instances specify containment levels.

Type VI - Activities Involving the Movement Into Georgia of: 1) pathogens that adversely affect plants or animals; 2) any non-indigenous species of plants or animals (including offspring); 3) any indigenous species of plants or animals infected with pathogenic organisms outside the state.

Joel W. Hedgpeth in a column titled "Foreign Invaders" reminds us that with "All things considered, man, whether by intent or inadvertence, is the principal agent responsible for introducing organisms of all sorts to North America and elsewhere.(and) Some of these introductions are pleasant reminders of faraway places, but many are detrimental to their new ecosystems." (Science 261:34-35, July 2, 1993). With this in mind, federal and state agencies regulate the international and interstate movement of specified animals, plants, cells and tissues, and microorganisms. Permits are generally required to move regulated organisms and may also involve the inspection of the receiving laboratory for appropriate containment and safe operation. It is essential that the investigator carefully follow the requirements/recommendations for shipping. Pathogens will be handled according to the appropriate criteria as identified by the University Biosafety Committee and/or the University Biosafety Officer.

References:

· Agricultural Permits, U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine, (http://www.aphis.usda.gov/ppq/permits/index.html)

· Interstate Shipment of Etiologic Agents (42CFR Part 72), Federal Register, July 21, 1980. (available at **http://www.cdc.gov/od/ohs/biosfty/shipregs.htm**).

· Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens, World Health Organization, 1997. (available at **http://www.absa.org/resources/who-97.pdf**).

· Additional requirements for facilities transferring or receiving select agents; Final Rule (42 CFR Part 72.6), Federal Register, Oct. 24, 1996. (available at **http://www.cdc.gov/od/ohs/lrsat/regmat.htm**).

Type VII - Activities Involving the Transfer or Receipt of Select Agents as Defined in the Code of Federal Regulations (42 CFR Part 72.6). It is essential that the investigator carefully follow the requirements/recommendations for shipping. Select agents will be handled according to the appropriate criteria as identified by the University Biosafety Committee and/or the University Biosafety Officer.

Reference:

· Additional requirements for facilities transferring or receiving select agents; Final Rule (42 CFR Part 72.6), Federal Register, Oct. 24, 1996. (available at **http://www.cdc.gov/od/ohs/lrsat/regmat.htm**).

VII. Biohazardous Wastes

It is expected that investigators using biohazardous agents and/or producing biomedical wastes as

defined below will comply with the rules promulgated by the Georgia Environmental Protection Division, Chapter 391-3-4 section .15 "Solid Waste Management" (reference: http://www.state.ga.us/dnr/environ/). The waste streams generated by biological laboratories should be separated into non-hazardous waste (trash), biohazardous waste, chemical waste, and radioactive waste. Biohazardous waste must be sterilized before or as a part of disposal.

A. General Definitions

"Biomedical Waste" means any solid waste which contains pathological waste, biological waste, cultures, and stocks of infectious agents and associated biologicals, contaminated animal carcasses (body parts, their bedding, and other wastes from such animals), chemotherapy waste, discarded medical equipment and parts, not including expendable supplies and materials, which have not been decontaminated, as further defined in Rule 391-3-4-.15.

1. Pathological Waste - This term refers to all recognizable human tissues and body parts except teeth which are removed during surgery, obstetrical procedures, autopsy, and laboratory procedures.
2. Biological Waste - This term means blood and blood products, exudates, secretions, suctionings, and other body fluids which contain free liquids and cannot be or are not directly discarded into a municipal sewer system.
3. Cultures and Stocks of Infectious Agents and Associated Biologicals - includes cultures from medical and pathological laboratories, cultures and stocks of infectious agents from research and industrial laboratories, wastes from the production of biologicals, discarded live and attenuated vaccines, and culture dishes and devices used to transfer, inoculate and mix cultures.
4. Contaminated Animal Carcasses - includes body parts, the bedding and other wastes from animals which are infected with or have been exposed to infectious agents capable of causing disease in humans.
5. Sharps - this term means any discarded article that may cause punctures or cuts. This waste includes, but is not limited to, items such as needles, IV tubing and syringes with needles attached, and scalpel blades.
6. Chemotherapy Waste -means any disposable material which has come in contact with cytotoxic/antineoplastic agents and/or antineoplastic agents during the preparation,

handling, and administration of such agents. Such wastes include, but are not limited to, masks, gloves, gowns, empty IV tubing, bags and vials and other contaminated materials. The above wastes must first be classified as empty (a quantity remaining that is not subject to other federal or state waste management regulations) prior to being handled as biomedical waste.

7. Discarded Medical Equipment and Parts - not including expendable supplies and materials which have not been decontaminated, that were in contact with infectious agents.

B. VSU Procedures for handling biomedical wastes on campus

1. Biomedical/biohazardous waste shall be segregated by separate containment from other waste at the point of origin. These wastes, except for sharps, are to be placed in orange or red plastic bags clearly identified with the universal biohazard symbol or clearly marked with the work "BIOHAZARD". The bags are to have strength sufficient to preclude ripping, tearing, or bursting under normal conditions of use.
2. Sharps (needles and syringes, Pasteur pipettes, etc.) must be placed in puncture proof and leak proof containers which are closed and transported to the autoclave for sterilization prior to disposal. Sharps may be rendered unrecognizable by grinding (contact the VSU Biosafety Officer), incineration, or the "Isolyser" system (or similar EPD approved system). Contract arrangements for pick-up and disposal of biomedical wastes including sharps may also be used. Broken glass may or may not be considered biomedical waste - glassware that has been contaminated with biohazardous agents must be decontaminated (autoclaving or chemical disinfection) prior to disposal with broken glass.
3. Contaminated combustible wastes and animal carcasses should be collected in leak- proof closed containers and transported to a properly operating, pathological waste incinerator for disposal by incineration. If an incinerator is not available, these wastes should be sterilized by autoclaving prior to disposal by burial or in an approved landfill.
4. Liquid biohazardous materials are to be properly inactivated or sterilized prior to disposal in the community sewage treatment system. Garbage disposal units are not to be used with contaminated materials because of the aerosols generated. Chemotherapy waste may not be disposed of in the community sewage; it must be incinerated in a permitted incinerator. Contract disposal services may be used - contact the Biosafety Officer.
5. Biomedical wastes may be treated so as to render it non-biomedical wastes. Properly treated wastes may be combined and handled with regular solid wastes. Biomedical wastes may be treated by autoclaving in a recording autoclave. Recording of the temperature during each complete cycle shall be used to assure the attainment of 121oC or 250oF for a minimum of 30 minutes in order to achieve decontamination of the entire load. Monitoring of the autoclave process through the use of bio logical or other approved indicators is to by accomplished by the investigator and maintained along with the temperature recording as proof of decontamination.

Several factors affect the steam sterilization process including load size, distribution and compaction, altitude above sea level; and heat penetration. The investigator or personnel responsible for sterilization may have to determine the appropriate time at standard autoclave temperature and pressure for certain loads of biohazardous materials. Barbeito and Gremillion in

their article "Microbiological Safety Evaluation of an Industrial Refuse Incinerator" (Applied Microbiology 16:2:291-95) reported on various times required for autoclaving selected animal carcasses, animal bedding materials, and eggs. With some loads, even extended times did not provide for sterilization.

Biomedical wastes may be treated by incineration in an incinerator which provides complete combustion of waste to render it nonpathogenic.

C. Georgia Regulations

Georgia Department of Natural Resources/Environmental Protection Division - rules on solid waste management covering biomedical waste (391-3-4-.15) start on page 77. This document requires the Adobe Acrobat Reader 3.0 and is available at http://www.state.ga.us/dnr/environ/

VIII. Containment of Biohazardous Research

Reference: *Biosafety in Microbiological and Biomedical Laboratories, CDC/NIH*, Fourth Edition, U.S. Department of Health and Human Services, U.S. Government Printing Office, May 1999. (available at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm).

Physical Containment of Experiments

Within each type of biohazard there are different degrees of risk which require different levels of

containment. The term "containment" is used in describing safe methods for managing biohazardous agents in the laboratory environment where they are being handled or maintained. Primary containment, the protection of personnel and the immediate laboratory environment from exposure, is provided by good technique and the use of appropriate safety equipment that has been properly designed, located, installed, and maintained. Secondary containment, the protection of the environment external to the laboratory from exposure to biohazardous agents, is provided by a combination of facility design and operational practices. The four biosafety levels are discussed under Laboratory Biosafety Levels.

Biosafety Level 1 / Laboratory

Biosafety Level 1 (BSL 1) is suitable for working with agents having no known or minimal hazard to laboratory personnel and the environment (including plants and other animals). The laboratory practices and techniques, safety equipment, and physical facilities are those appropriate for undergraduate and secondary educational training and teaching. When assessing the risk of an experiment and determining the appropriate containment level it is important to remember that BSL 1 depends entirely upon good laboratory practice and using agents with no known hazards. The use of standard microbiological practice and techniques is basic for laboratory safety/containment; however, the PI must recognize that special precautions may be needed. Research laboratories in which biological agents are used should be at BSL-2.

BSL 1 Standard Microbiological Safety Practices

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director. Limiting access to control the in/out traffic when experiments are in progress reduces sources of distraction and disturbance which may result in accidents. Closing laboratory doors during experiments is one method of controlling in/out traffic. This also allows for the exclusion of special category persons (children, immunosuppressed persons, etc.) during times of potential exposure.
2. Work surfaces are decontaminated at least once a day and following any spill of viable material. 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 or surplused. This practice assists in the control of general contamination of the laboratory and reduces infection potential among laboratory personnel as well as repair people and others in contact with with laboratory equipment.
3. All contaminated liquid and solid wastes are decontaminated prior to disposal. Disposal of biomedical wastes shall be accomplished so as to comply with state and federal laws and regulations, see Part VI.
4. Mechanical pipetting devices are used; mouth pipetting is prohibited. Mechanical pipetting is easy and accurate, and prevents the ingestion of the materials being pipetted. Several older publications referred to human infection and death associated with the mouth pipetting of pathogens.
5. Eating, drinking, smoking, and the application of cosmetics are not permitted in the work area. Food may be stored cabinets and refrigerators designated and used for this purpose only. Food storage cabinets and refrigerators should be located outside the work area. Storage and/or consumption of food and drink and application of cosmetics in biohazardous work areas may result in exposure to laboratory personnel via the contamination of these products.
6. Personnel wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory or animal facility. This practice reduces the potential for ingestion and/or absorption of harmful microorganisms and hazardous chemicals. It also reduces the likelihood of carrying materials from one project to another, to other laboratories or facilities outside laboratory, or into the home.
7. All procedures are performed carefully to minimize the creation of aerosols. Aerosols may be generated by several routine laboratory procedures and gain entry to lab personnel via inhalation, ingestion, and absorption. Aerosols have been associated with many laboratory acquired infections. They are, however, controllable with the use of safety procedures and containment equipment.
8. Personal protective equipment (PPE) are worn as appropriate when working with viable microorganisms, animals, and chemicals. Laboratory coats, gowns, uniforms, gloves, eye protection, etc. are examples of personal protective equipment. This procedure reduces the possibility of contaminating or soiling street clothing and carrying potentially hazardous agents out of the laboratory.
9. An insect and rodent control program is in effect.
10. No special safety procedures are identified at BSL 1. Special containment equipment (i.e. biological safety cabinets) is not required; laboratory facilities are basic teaching labs

at BSL 1.

Biosafety Level 2 / Laboratory

Biosafety Level 2 is suitable for work involving agents of moderate potential hazard to personnel and the environment (including plants and other animals). The practices, equipment, and laboratory design are appropriate for clinical, diagnostic, teaching, and basic research with a broad spectrum of indigenous moderate-risk agents associated with human disease and/or which may negatively impact the environment. Laboratory procedures which generate aerosols may increase the risk and therefore are to be conducted in a biological safety cabinet and/or other primary containment equipment.

Biosafety Level 2 facilities and procedures are those that are basic in a good quality laboratory working with microorganisms, genetic materials, cell/tissue cultures, and carcinogens. In addition to the BSL 1 Standard Microbiological Safety Procedures, the following Special Practices are implemented:

1. Access to the laboratory is limited or restricted by the laboratory supervisor when work with biohazardous agents is in progress. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal rooms. Keeping laboratory doors closed during experiments is recommended. Persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections.
2. The principal investigator is responsible for providing training of laboratory personnel in the potential hazards and safety procedures. Knowledgeable personnel work more efficiently and effectively in the laboratory by reducing the risks of accidents that could result in personal injury or loss of research effort. Georgia Law "Public Employee Hazardous Chemical Protection and Right to Know Act of 1988" (The Official Code of Georgia Annotated Title 45 Chapter 22) and the Department of Labor regulations (Chapter 300-3-19) provide for training of employees using hazardous chemicals. It only makes sense that investigatory also provide training to employees using biohazards.
3. When research involves working with or storing biohazardous agents in the laboratory a hazard warning sign incorporating the universal biohazard symbol is posted on the access door. The principal investigator is ultimately responsible for informing persons, including emergency personnel, of any special requirement for entering the laboratory.
4. Before leaving the laboratory areas, protective clothing (lab coats, aprons, etc.) is removed and left in the laboratory. This practice helps prevent infectious agents from being carried from the laboratory on contaminated clothing.
5. Animals not involved in the work being performed are not permitted in the laboratory. Pets or other noninvolved animals may bring unwanted organisms into the laboratory or may carry infectious agents from the laboratory into the home, into other areas of the building, or into the community.
6. Special care is taken to avoid contamination of skin and mucous membranes with infectious materials; appropriate personal protective equipment (gloves, goggles, face

shield, etc.) should be worn when handling infected animals or infectious materials. (See X. Bloodborne Pathogens; Universal Blood and Body Fluid Precautions)

7. Spills and accidents which result in exposure of people or the environment to infectious materials and/or rDNA molecules are immediately reported to the principal investigator, to the appropriate Department Head, and to the Biosafety Officer. Exposure may require medical evaluation, treatment, and surveillance. Accident investigation may assist in the prevention of similar types of accidents in the future.
8. When it is deemed appropriate by the principal investigator and/or the Biosafety Committee, baseline serum samples for laboratory and other at-risk personnel are collected and stored at the University Health Services. Additional samples may be collected periodically. Serum samples are useful for biological monitoring of workplace exposures in the effort to reduce occupational risks. Stored serum samples are used only to compare pre and post occupational exposure of serum components. Any use of stored samples for any purpose other than those associated with occupational exposures requires the informed consent of the individuals involved.
9. Laboratory personnel are to read and become familiar with the VSU Biosafety Manual and specific standard operating procedures of the laboratory. The principal investigator is responsible for providing supplemental safety training and information for personnel in his/her laboratory.
10. NIH rDNA Guidelines and OSHA Bloodborne Pathogens regulations are examples of two federal regulations requiring appropriate biosafety training for laboratory personnel. Since personnel who are trained and use appropriate biosafety procedures are less likely to lose research time from injuries, providing safety training for personnel is prudent.
11. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Needles and syringes should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids for laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

    1. Only needle-locking syringes or disposable syringe-needle units (i.e. needle is integral to the syringe) are used for injection or aspriation 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 puncture-resistant containers used for sharps disposal.
    2. Syringes which re-sheathe the needle, needle-less systems, and other safe devices should be used when appropriate.
    3. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, of forceps.
    4. Disposal of biohazardous materials covered under the Georgia Environmental Protection Division regulations on Biomedical Wastes is to be accomplished according to those regulations. See Part VI in this manual.

BSL-2 Containment Equipment

Biological safety cabinets (BSC) and other appropriate containment devices are to be used whenever laboratory procedures have a good potential for creating aerosols of infectious materials or rDNA molecules. Procedures that may create aerosols include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers, inoculating animals intranasally, and harvesting tissues from animals or eggs. Biological safety cabinets and other containment devices are to be maintained in good working condition. Certification of biological safety cabinets is to accomplished annually or whenever the cabinet is moved or the HEPA filter is changed or major repair is accomplished (whenever the contaminated plenum is breached). Certification of biological safety cabinets is conducted by trained personnel and is coordinated by the Biosafety Officer.

BSL-2 Laboratory Facilities

Laboratory facilities are similar to those for BSL-1 with the addition of an autoclave which is readily available and easily accessible. A recording autoclave is required for treating biomedical waste.

Biosafety Level 3 / Laboratory

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 inhalation. A greater level of attention to microbiological practice, laboratory containment, safety equipment, and facilities is required. All procedures involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices, by personnel wearing appropriate personal protective clothing and equipment. The laboratory has special engineering and design features. The same standard practices as discussed for BSL-1 are appropriate for BSL-3 with the following additions:

1. Laboratory doors must be kept closed during experiments.
2. Protective clothing is worn with the closed front smock replacing the laboratory coat. All protective clothing is either disposed of in the laboratory or decontaminated by autoclaving prior to laundering.

BSL-3 Special Safety Practices

The same special practices which were appropriate for BSL-2 are appropriate for BSL-3 with the addition of:

1. All activities involving infectious materials or rDNA molecules from BSL-3 organisms are conducted using appropriate containment devices - Biological Safety Cabinets, safety centrifuge cups, etc. No work in open vessels is conducted on the open bench. The significant reduction/prevention of exposure to aerosols is accomplished by a combination of safe work practices and containment equipment.

2. Decontamination of work surfaces is important for both biosafety and quality control. However, special care is to be taken at BSL-3 due to the type work being conducted. Spills of infectious material are to be handled immediately and properly -(see the spill procedures in Part XI.) Contact the University Biosafety Officer if assistance is needed.
3. The need for and type of respiratory protection is based on the research being conducted. The investigator should discuss respiratory protection equipment with the Biosafety Officer.
4. Animals and plants not related to the work being conducted are not permitted in the laboratory. These may provide a means of infectious agents escaping containment and/or carrying organisms into the laboratory which would cause problems of cross contamination.
5. Vacuum lines are protected with high efficiency particulate air (HEPA) filters and liquid disinfectant traps. These safety devices are routinely maintained and replaced as needed. The use of HEPA filters and traps help prevent contamination of vacuum equipment and exposure of personnel who work on that equipment.

BSL 3 Containment Equipment

Biological safety cabinets and/or other physical containment equipment (e.g. centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals) are necessary for all activities with infectious materials which pose a threat of aerosol exposure. Containment equipment may be augmented with personal protective clothing. The types of procedures which may produce aerosol exposure include but are not limited to: laboratory manipulation of cultures or clinical and environmental samples which may contain infectious agents, aerosol challenge of experimental animals, harvesting of tissues or fluids from infected animals and embryonated eggs, and necropsy of infected animals.

BSL 3 Laboratory Facilities

1. The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the high containment laboratory from access corridors or other contiguous areas. Controlled access improves security for the containment laboratory and reduces air turbulence associated with the unrestricted movement of people.
2. Access doors to the high containment laboratory are self closing. This reduces the problem of forgetting to close doors to containment laboratories.
3. Basic laboratory features as discussed for BSL l facilities are standard for the BSL 3 facility. These features are:
    1. The Laboratory is designed so that it can be easily cleaned.
    2. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
    3. Each laboratory contains a sink for hand washing. The sink is foot, elbow, or automatically operated and located near the exit door in BSL-3 facilities.
4. Windows in the high containment laboratory are closed and sealed. Preferably, there are

double windows to prevent possible breach of containment in the case of window breakage.

5. A ducted exhaust air ventilation system is provided in the high containment laboratory. The system complies with the specific guidelines in the following reference: Biosafety *in Microbiological and Biomedical Laboratories, CDC/NIH*, Fourth Edition, U.S. Department of Health and Human Services, U.S. Government Printing Office, May 1999. (available at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm).
6. The exhaust air is not recirculated to any other area of the building and is dispersed away from occupied areas and air intakes. HEPA filtration and other treatment is optional but must be approved by the Biosafety Officer. This provides for a directional flow into the high containment laboratory. Since all work which may generate infectious aerosols is accomplished inside a biological safety cabinet or other containment device which filters exhaust air through HEPA filters, ambient laboratory air should not be contaminated.
7. The HEPA filtered exhaust air from a Class II biological safety cabinet is discharged directly to the outside via exhaust ducting or may be recirculated in the laboratory depending on the nature of the work being conducted, the investigator's history of maintaining containment equipment, and the approval of the Biosafety Officer. HEPA filters must be routinely maintained and certified to reduce the potential of exposure from leaks. Using an outside exhaust adds to the safety margin.
8. A method of decontaminating all laboratory waste is available in the laboratory (autoclave, incineration, chemical disinfection, or other approved method).
9. 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 air.

Operation of Laboratory Equipment

VSU personnel should not operate equipment that they have not been specifically trained and authorized to use. Operating manuals must be consulted for detailed operating instructions for individual pieces of equipment. Equipment known or suspected of being faulty should not be operated. Mechanically or electrically unsafe equipment should be tagged and reported to the laboratory supervisor.

Autoclaves/Steam Sterilizers

Moist heat, in the form of steam under pressure, is the most dependable medium for the destruction of all forms of microbial life. Autoclaves are instruments, which produce superheated steam under high pressure and are used for two processes: decontamination and sterilization.

Autoclave loads should be routinely checked with appropriate indicators to the adequacy of the sterilization or decontamination (for biomedical wastes) processes. Barbeito and Gremillion in their article "Microbiological Safety Evaluation of an Industrial refuse Incinerator" (Applied Microbiology 16:2:291-95) reported on various times required for autoclaving selected animal carcasses, animal bedding materials, and eggs. With some loads even extended times did not provide for sterilization. Investigators or personnel responsible for sterilization may have to

determine appropriate times and maintain appropriate records of the process.

Autoclaves should receive routine inspection to determine the need for maintenance and repair. Autoclave door gaskets may become distorted if the door is tightly shut for prolonged periods resulting in leaks. Doors should be kept open or loosely closed except when the autoclave serves as a barrier between clean and dirty areas.

Effective decontamination and sterilization by steam depends on the adequacy of circulation of the steam; loads packed tightly may not allow for adequate circulation. The steam must penetrate all packaging materials and contact all surfaces to be decontaminated or sterilized. And, finally the packaging must prevent the recontamination of the sterilized materials. To achieve effective and safe use of the autoclave you must be familiar with and follow your laboratory's procedures regarding:

1. Types of packaging – autoclavable pan, bag in pan, double bag, etc.
2. Separating into pans/bags for autoclaving in the lab
3. Adding water/germicidal solutions - Do not autoclave radioisotopes or explosive or volatile chemicals without checking with radiation safety, laboratory safety and biological safety.
4. Uses of specific autoclaves
5. Proper settings for type of cycle, and type and amount of material. Details of proper operation and settings may be contained in the specific device operation manual. Monitor the autoclave process for proper cycle and length of time. Cycle and time depend on what is being sterilized. For example, liquids would require the use of slow exhaust and while most loads require cycle times of 15 to 30 minutes at 121o C, longer times may be needed to meet the thermodynamic needs of special loads. The decontamination of biomedical waste may regularly require 60 minutes at 121o C.
6. When the cycle is completed care must be taken to wear proper personal protective equipment and to use proper unloading procedures. These include: Personal protective equipment – laboratory coat and apron that resists liquids (i.e. rubber/plastic) gloves that are heat and liquid resistive, and goggles and/or face shield.
7. Procedures – Stand away from the autoclave door when opening to avoid a rush of steam and open slowly; do not move boiling liquids; and allow sufficient cooling time before handling superheated solution (i.e., microbiological culture media) to avoid burns and exploding glass.
8. Spill clean-up procedures should be posted in every autoclave room and followed when a spill occurs.

IX. Equipment Use

<u>Biohazard Containment Equipment</u>

Many manipulations of bacterial and viral cultures commonly used in the laboratory generate aerosols of viable organisms. This principle must be remembered when evaluating a person's degree of risk.

Biological Safety Cabinets

Primary biohazard containment devices serve to protect laboratory personnel from exposure to infectious aerosols produced by routine procedures. The biological safety cabinet can be an extremely useful containment device for both personnel and product protection. Please see CDC/NIH Primary Containment of Biohazards: Selection, Installation, and Use of Biological Safety Cabinets.

Before purchasing a biological safety cabinet, horizontal flow clean bench or a vertical flow clean bench an approval form must be completed by the investigator and approved by the Biosafety Officer and the Engineering Department of Physical Plant Division

Centrifuges

Centrifuges are an important tool in the microbiological laboratory and must be treated with respect. Each time you use a centrifuge you make a series of choices: Which centrifuge, which rotor, which tubes and adapters, what speed and for how long. In addition, if you are using infectious agents you must decide on the level of containment and then select the appropriate rotor and tubes. Load the infectious agents inside a biological safety cabinet to prevent aerosol exposure. Your choices will affect both your research and yours and others safety.

Always check your user manual for specific requirements as well as load limitations and speed. Operating procedures for each centrifuge must be established by the laboratory supervisor or principle investigator and followed by each operator. These procedures should follow the information provided in the operation manual and guidelines for centrifugation of infectious agents, chemical hazards and/or radioactive materials. Make sure the load is properly balanced – a minor error may not be a problem at low speed but may be serious at higher speeds.

Centrifuge tubes must be selected with the knowledge of the materials they will contain and the pressures they will be under. Plastic centrifuge tubes should be used whenever possible to minimize breakage. Nitrocellulose tubes should only be used when clear, without discoloration, and flexible. It is advisable to purchase small lots several times a year rather than one large lot. Storage at 4O C extends the shelf life. Nitrocellulose tubes must not be used in angle-head centrifuges.

Tubes to be used in angle-head centrifuges must never be filled to the point that the liquid is in

contact with the lip of the tube when it is placed in the rotor, even though the meniscus will be vertical during rotation. When the tube lip is wetted, high G force drives the liquid past the cap

seal and over the outside of the tube.

Inspect all centrifuge tubes prior to use. Broken, cracked, or damaged tubes are to be discarded. Capped centrifuge tubes should be used whenever possible.

It has been estimated that 80% of centrifuge accidents are operator error. The most common operator errors are failure to secure the rotor to the drive shaft; failure to place lid on the rotor; and failure to secure the lid. Additionally it is very important not to run the rotor above it's rated maximum and not to overfill it.

Cryogenic Liquids

Cryogenic liquids are gases that have been transformed into extremely cold refrigerated liquids, which are stored at temperatures below minus 90o C (-130o F). They are normally stored at low pressures in specially constructed multi-walled, vacuum-insulated containers. The hazard potential presented by cryogenic liquids may result from the extreme cold, extreme pressure (which can result from rapid vaporization), and asphyxiation due to the displacement of air.

Appropriate personal protective equipment (heavy leather gloves/gloves for extreme cold, safety shoes, aprons, and eye protection) is to be worn when handling cryogenic liquids or materials preserved in cryogenic liquids.

Lasers

Lasers are tools of biological research and as such must be used with consideration of applicable safety precautions. Refer to the VSU Radiation Safety Officer for appropriate guidance in laser safety.

Ultraviolet Light (UV radiation)

Under certain conditions of radiation intensity and exposure time UV radiation may kill certain types of microorganisms, its greatest effect is against vegetative forms. UV is not a sterilizing agent except in certain exceptional circumstances. It is used to reduce the numbers of microorganisms on surfaces and in the air. The age of the UV lamp, dust accumulations on the bulb, and other factors that impede direct contact of the UV on the microorganisms contribute to decreased efficacy. See Radiation Safety for additional information and safety requirements.

Microwave Ovens

Microwave ovens used in the laboratory may not be used to heat food unless that is the only use of that oven. When melting agar the following precautions must be taken to prevent explosions: Caps on screw-cap bottles must be completely loosened before heating the bottles in the microwave and wear appropriate personal protective equipment including laboratory coat or apron, heat resistant gloves, and face shield.

Laboratory Vacuum Lines

When laboratory vacuum is used to manipulate biohazard materials, suitable filters and traps are to be used to prevent contamination of the vacuum lines and pumps.

Repair and Maintenance of Equipment and Facilities and New Construction

1. University employees or outside vendors undertaking facility expansion, equipment repair and maintenance, and general maintenance activities should not be unnecessarily exposed to biological hazards.
2. It is expected that new construction and renovation projects involving biohazard laboratories are to be reviewed in the planning stages by the University Biosafety Officer, Physical Plant, Campus Planning, and other campus support groups.
3. Repair or routine preventive maintenance of mechanical or laboratory equipment in posted biohazard areas are not to be initiated without prior clearance from the Principal Investigator and Biosafety Officer.
4. Potentially contaminated equipment is not to be removed from biohazard laboratories for repair, servicing, cleaning or to surplus properties or repair shops or other areas until decontamination and removal of biohazard labels have been performed. The investigator or laboratory supervisor is to certify such equipment as being free of biohazard agents. Service personnel may ask laboratory personnel to sign a waiver stating that the piece of equipment has been appropriately decontaminated.
5. Biosafety level 3 agents should not be handled when service personnel are in the laboratory to minimize potential exposure to them.

X. Vertebrate Animal Biosafety Criteria - Selected Aspects.

Specific Medical Concerns for Persons Working with Laboratory Animals.

Allergy and musculoskeletal injury constitute the primary health risks to individuals using and caring for laboratory animals. Allergies are a significant problem, but can be reduced by providing appropriate protective equipment to affected personnel. Musculoskeletal injuries can be minimized by good laboratory planning, use of transport equipment such as carts, and training in lifting techniques and equipment use.

Infectious diseases may constitute a significant risk depending on the species and health status of animals involved, and the level of exposing animal care personnel. Infectious diseases to which animal care personnel may be at risk include a number of viral infections, such as rabies from random source dogs and lymphocytic choriomeningitis from hamsters and mice. In addition to infections potentially acquired from live animals, cell cultures, animal tissues and excreta can

serve as sources of zoonoses. Careful monitoring and quarantine of any animals with potential viral or bacterial infections is a crucial part of quality assurance in animal care programs.

Particular care must be taken in facilities handing nonhuman primates as these animals are most prone to carry infections which are known to cause serious disease in humans, for example, Herpesvirus simiae (Herpes B) encephalitis and tuberculosis. Routine periodic mycobacterial skin testing of humans and associated nonhuman primates is essential.

Animal bites and scratches are hazards common to animal facility personnel. All cases should be documented by filing an incident report and recording in an incident log. Tetanus immunizations should be routinely administered every ten years and at the time following a potential exposure such as an animal bite.

Working with Infectious Agents as Potential Zoonoses:

Agents which are potentially infective from animals to humans or humans to animals (zoonoses) should be handled according to principles outlined in the CDC/NIH "Biosafety in Microbiological and Biomedical Laboratories". Four general levels of applying biosafety practices are described in this publication for activities involving infectious disease work with experimental animals. These four combinations of practices, safety equipment, and facilities are designated Animal Biosafety Levels 1, 2, 3, and 4. Specific containment and management practices for each situation must be developed by the Principal Investigator with consultation from the Biosafety Officer and the designated Attending Veterinarian. When appropriate, the biosafety management practices should be incorporated into the standard operating procedures for animal care management and included in the personnel training program.

In general, most infectious disease studies with animals are handled at Biosafety level 2, with most exceptions being agents with true aerosol transmission capability (level 3) and certain blood-borne hemorrhagic fever agents (level 4). Exotic, non-indigenous foreign animal diseases (level 5) may require specialized containment and facilities to prevent animal to animal transmission and spread to naive animal populations.

Standard Practices for Animal Biosafety Level (BSL) 2 include*:

1. Access to the animal facility is restricted or limited.

2. Hand washing with soap and water is accomplished after handling cultures and animals, after gloves are removed, and before leaving the animal facility.

3. Eating, drinking, smoking, applying cosmetics, and storing food for human use are not permitted in animal rooms.

4. Persons who wear contact lenses in animal rooms should also wear goggles or a face shield.

5. All procedures are carefully performed so as to minimize the creation of aerosols.

6. Work surfaces are decontaminated after use or after any spill of viable materials.

7. Doors to animal rooms open inward, are self-closing, and are kept closed when experimental animals are present.

8. All wastes from animal rooms are appropriately decontaminated before disposal. .

9. Infected animal carcasses are incinerated after being transported from the animal room in leakproof, covered containers.

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

Special Practices for Animal BSL 2 may include:

1. Access to animal rooms is limited to personnel who have been advised of potential hazards, meet specific requirements (i.e., immunization, TB tests), and who need to enter the room for program or service work during conduct of the containment phase of operations. In general, persons who are at increased risk of acquiring infection, or for whom infection might be unusually hazardous, are not allowed into the animal room.

2. Under special entry provisions, a hazard warning sign, incorporating the universal biohazard symbol, is posted on the assess door to the animal room. The sign should identify the agent(s) in use, names and phone numbers of responsible persons, and special room entry requirements.

3. When appropriate, baseline serum samples from animal care, and other at-risk personnel are collected and stored.

4. Sharp items and accessories, such as needles, syringes, scalpels, glass slides, pipettes, and capillary tubes, are all handled with a high degree of precaution. Such sharp instruments are restricted in the animal facility for use only when there is no alternative, such as for parenteral injection, blood collection, or aspiration of fluids from diaphragm bottles. Plasticware should be used instead of glass whenever possible. The standard operating procedures for sharps should address proper disposal, not recapping of needles, and safe clean up of broken glassware.

5. Cages are properly decontaminated, if appropriate by autoclaving, before they are cleaned and washed.

6. Equipment and work surfaces should be decontaminated on a routine basis, especially after handling the infectious materials and following overt spills or splashes.

7. Incidents involving overt exposures are reported immediately to the lab director and followed by evaluation, surveillance and treatment.

Safety Equipment for Animal Care (primary and secondary barriers)

Laboratory coats, gowns, or uniforms are worn while in the animal room. Gloves are worn when handling infected animals and when skin contact with infectious material is possible. This protective clothing is removed before leaving the animal facility. The animal facility is designed and constructed to facilitate cleaning and housekeeping. Supplies and equipment are stored outside of the animal room area whenever possible. A handwashing sink is available in the room where the infected animals are housed.

Exhaust air is discharged to the outside without being recirculated to other rooms, and it is recommended that the direction of air flow is toward the inside of the room. An autoclave for decontaminating waste should be available in the building with the animal facility.

Biological safety cabinets, other physical containment devices, and/or protective equipment i.e., respirators, face shields, are used whenever procedures with a high potential for creating aerosols are conducted. Such procedures include necropsy of infected animals, harvesting tissues or fluids from infected animals or eggs, intranasal inoculation, and manipulations of high concentrations or large volumes of infectious materials.

*Excerpted or summarized from the CDC/NIH "Biosafety in Microbiological and Biomedical Laboratories" 3rd Edition

XI. Floral Biosafety Criteria - Selected Aspects.

Biohazard Containment for Plants.

1. The principal purpose of plant containment is to avoid unintentional transmission of recombinant DNA containing plant genome, including nuclear or organelle hereditary material; the release of recombinant DNA derived organisms associated with plants; the release of non-indigenous species; or the release of plant pathogens/pests associated with research at Valdosta State University Facilities.

2. The containment principles used in this section of the biosafety manual are based on the recognition that the organisms to which they apply pose no health threat to human or higher animals unless deliberately modified to do so, and that the intent of containment is to minimize the possibility of unanticipated deleterious effects on organisms and ecosystems outside the experimental facility.

3. The intentional release of genetically engineered organisms and products which are or are believed to be plant pests is regulated under CFR Parts 330 and 340 by the Animal and Plant

Health Inspection Service, United States Department of Agriculture. Biological pesticides and certain field trials are regulated by the United States Environmental Protection Agency. In each case of proposed intentional release, the investigator(s) shall submit appropriate information on anticipated environmental impacts (as submitted to USDA, EPA, or other Federal or State regulatory Agency) for review by the VSU Biosafety Committee.

4. It is the responsibility of the Investigator to obtain any necessary permits for transport and or work with regulated organisms/products. A copy of the permit is to be provided to the office of biosafety.

5. Laboratory experiments with biohazardous plant materials are to be conducted at Biosafety Level 2 (BSL-2) which is a good basic research laboratory and provides the investigator with greater flexibility. BSL-2 is described elsewhere in this manual. Greenhouse containment practices involve a combination of biological and physical protocols.

Greenhouse Biological Containment Practices

Biological containment practices are intended to be used in association with facility design and facility/experimental operational procedures. Effective dissemination of plants by pollen or seed can be prevented by one or more of the following:

1. Preventing insect mediated pollination by appropriate insect control measures within the greenhouse.
2. Covering reproductive structures to prevent pollen dissemination at flowering and seed dissemination at maturity.
3. Removing reproductive structures, employing male sterile strains, or terminating the experiment and harvesting the plant material prior to the reproductive stage.
4. Ensuring that the experimental plants flower at a time of year when non cross-fertile plant is flowering within the normal pollen dispersal range of the experimental plant.
5. Ensuring that no cross-fertile plant is growing within the experimental plant's known pollen dispersal range.

Facilities and definitions.

1. ‘Greenhouse' refers to a permanent structure with walls, roof, and floor designed and utilized principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.
2. ‘Greenhouse facility' includes the actual greenhouse rooms or compartments for growing plants plus all immediately contiguous hallways and headhouse areas and is considered part of the confinement area.

XII. Bloodborne Pathogens; Universal Blood and Body Fluid Precautions

The following are the key elements, which can be used at Valdosta State University to control occupational exposures to bloodborne pathogens. All blood and body fluids must be considered as potentially infectious and personnel are to use appropriate protective measures to prevent exposure.

Personnel Practices

Hand-washing:

1. When hands become contaminated with blood or body fluids
2. When gloves are removed
3. Before going to lunch, breaks, or home

Contaminated Needles and Other Sharps:

1. DO NOT recap, bend, or break used needles
2. Discard needles & sharps in appropriate "Sharps" containers
3. Transport reusable sharps in leak-proof puncture-resistant container
4. Use mechanical device (forceps) to place contaminated broken glass into appropriate containers for autoclaving

Personal Protective Equipment for Blood or Body Fluid Contact

1. Gloves when touching blood or body fluids, mucous membranes, or non-intake skin of patients
2. Gloves when handling items or surfaces soiled with blood or body fluids
3. Gloves when performing vascular access procedures (phlebotomy)
4. Appropriate gowns or aprons when splashes or soiling of skin or clothing with blood or body fluids is likely
5. Masks and goggles, or face shield during procedures likely to generate splashes of blood or body fluids into the mouth, nose, or eye

Environmental Controls

General Housekeeping:

1. Maintain work area in clean and sanitary condition
2. Decontaminate work surfaces after procedures and when contaminated
3. Remove any protective work surface coverings when contaminated

Blood or Body Fluid Spills:

1. Soak up spills with absorbent material (paper towels)
2. Decontaminate area with appropriate disinfectant
3. Dispose of contaminated material appropriately

Biomedical Wastes:

1. Are to be disposed of according to State of Georgia Regulations

Transport:

1. Consider all laboratory specimens of human or animal origin as potentially infectious
2. Use leak proof containers for laboratory specimens
3. Place container in a sealable secondary container for transport

Exposures to blood or body fluids via broken skin or needle sticks or mucous membrane contact:

1. Wash affected area immediately and apply first aid
2. Contact VSU Health Service as soon as possible for post exposure follow-up.
3. Report injury to Biosafety Officer.

Biohazard Warning

1. Use appropriate biohazard labels to identify contaminated materials

XIII. Experiments Prohibited at Valdosta State

1. Experiments using pathogenic organisms or DNA from pathogenic organisms classified as requiring Biosafety Level 4 are prohibited.
2. Experiments using any organism or agent that is prohibited by any federal or state agency from importation into Georgia are prohibited.
3. Experiments using agents or organisms that require containment facilities or equipment which are not available at the Valdosta State University are prohibited.

XIV. Transporting Biohazardous Materials

All guidelines of the CDC, NIH, USDA, and EPA are to be followed when biohazardous materials are transported. Individuals transferring **select agents** must adhere to additional regulations specified by the CDC/USDA.

INFORMATION

· CDC Office of Health and Safety Information System http://www.cdc.gov/od/ohs/

· USDA, APHIS http://aphisweb.aphis.usda.gov/

· Guidelines for the Shipment of Dried Blood Spot Specimens
http://www.cdc.gov/od/ohs/biosfty/driblood.htm

· Interstate Shipment of Etiologic Agents http://www.cdc.gov/od/ohs/biosfty/shipregs.htm

· Packaging and Shipping Instructions http://www.cdc.gov/od/ohs/biosfty/shipdir.htm

· USDA, APHIS Forms http://www.aphis.usda.gov/forms/index.html

XV. Spills of Biohazards Materials

1. Primary responsibility for preventing or/and containing and cleaning up laboratory spills remains with the principal investigator or laboratory supervisor. Laboratory protocols should be carefully designed to prevent biological, chemical and/or radiation spills.

2. When accidents occur that involve the mishandling or escape of biohazardous materials, the principal investigator or laboratory supervisor is to be notified immediately. Spills of high risk organisms (certain Class 2 and all Class 3) should be reported to the Biosafety Officer during normal working hours or to the Valdosta State University Public Safety Division at the emergency telephone number after normal working hours by the principal investigator or laboratory supervisor. The Public Safety Division will contact the Biosafety Officer for appropriate response. All employees and/or students have an obligation to themselves and their colleagues to report accidents immediately in order to minimize potential hazard.

3. When a biohazardous spill also involves radioactivity, cleanup procedures may have to be modified. The extent of the modification will depend on the level of radiation and the nature of the isotope involved. The Radiation Safety Officer should be called during normal working hours, or the Valdosta State University Public Safety Division should be called after normal working hours.

4. The attached guidelines are intended to assist the principal investigator, laboratory supervisor, and other responsible individuals who may be involved in the cleanup of biological spills.

Biohazardous Spills Inside Laminar Flow Biological Safety Cabinets (LFBSC)

The occurrence of a spill in a biological safety cabinet poses less of a problem than a spill in an open laboratory as long as the spilled materials is contained in the biological safety cabinet. Decontamination of the work zone can usually be effected by direct application of concentrated liquid disinfectants along with a thorough wipe down procedure. Gaseous decontamination may be required to clean up the interior sections of the cabinet.

Procedures for Decontamination of LFBSC

1. Chemical decontamination procedures should be initiated immediately while the biological safety cabinet continues to operate. Continuing the operation of the LFBSC helps to prevent the escape of contaminants from the cabinet.
2. Wearing protective gloves spray or wipe walls, work surfaces, and equipment with an appropriate decontaminating solution. A disinfectant detergent, such as Wescodyne or

Environ has the advantage of detergent action on extraneous organic substances which may interfere with the microbicidal activity of the disinfectant.

3. Flood tray top, drain pans, and catch basins below work surface with decontaminating solution and allow to stand for 20 minutes.
4. Drain excess decontaminating solution from tray and drain pans into cabinet base. Lift out tray and removable exhaust grille work. Clean the top and bottom (underside) surfaces using a sponge or clean cloth soaked in decontaminant solution. Following the cleaning process, replace the tray and grille work in their proper position. Place gloves and sponge or cloth in autoclave pan and autoclave these items.
5. Drain decontaminating solution from cabinet base into appropriate container and autoclave according to standard procedures.
6. If gaseous decontamination of the cabinet's interior sections is needed, call the Biosafety Officer.

Biohazardous Spills Outside Laminar Flow Biological Safety Cabinets

The protocol to be used in cleaning up of spills involving microorganisms will depend on the amount of material spilled and the degree of laboratory containment required.

If individuals believe that their outer garments have been contaminated, they should remove their clothing in the laboratory area and place them in an autoclave or a container for autoclaving. They should change into clean clothing in a non-contaminated area. All laboratory personnel should keep a complete change of clothing, including shoes at the laboratory in case of spills.

Special care in decontamination may be necessary if a spill goes under or between fixed furniture or behind base moldings (floor/wall) or if floor penetrations are involved.

Spills Based on Organism Classification

Class 2 Organisms

<u>I. Minor Spills (less than 10 ml and generating little aerosol) on equipment, laboratory benches, walls, or floors:</u>

1. Warn all personnel not essential for spill containment to stay clear of the contaminated area. This may be accomplished verbally or, when appropriate, by posting warning signs on the doors.
2. Thoroughly wash hands and other apparently contaminated areas with soap and water. Put on clean disposable gloves.
3. Cover the spill area with paper towels soaked in appropriate decontamination solution (see Attachment A).

4. Wipe up the spill with the soaked paper towels and place the used towels in an autoclave pan and autoclave.
5. Pour decontaminating solution around and on the area of the spill. Let stand for 20 minutes then wipe up with paper towels. Place gloves and paper towels in autoclave pan and autoclave.
6. Wash hands and other apparently contaminated areas again with soap and water.

II. Major Spills (more than 10 ml or with considerable aerosol):

1. Close laboratory doors and post warning signs to prevent others from entering the laboratory.
2. Wash hands and other apparently contaminated areas with soap and water.
3. Report the accident to the Supervisor and to the Biosafety Officer.
4. If personal clothing is contaminated, remove all outer clothing and place it in autoclave or container for autoclaving. Put on clean garments.
5. Leave the laboratory for 20 minutes to allow dissipation of aerosols created by the spill.
6. Upon returning to the laboratory to start decontamination, check to see if laboratory doors are closed and appropriate signs are displayed. Put on surgical gloves. Respirators or other safety equipment may be required, depending on the microorganism involved. Check with the Principal Investigator or Laboratory Supervisor or Biosafety Officer.
7. Pour a decontamination solution (See information about disinfectants) around the spill and allow this solution to flow into the spill. Paper towels soaked with decontamination solution may be used to cover the area. Do not pour decontamination solution directly onto the spill in order to avoid additional release of aerosols.
8. Let decontamination solution – microorganism mixture stand for 20 minutes or longer to allow to allow adequate contact time.
9. Using autoclave dust pan and squeegee transfer all contaminated materials to deep autoclave pan, cover with suitable cover, and autoclave according to standard directions.
10. Place dust pan and squeegee in an autoclavable bag and autoclave according to standard directions.
11. Remove gloves and other contaminated garments and place them in an autoclave container for autoclaving.
12. Thoroughly wash hands, face, and other apparently contaminated areas.

Special care in decontamination may necessary. The Principal Investigator and/or the Biosafety Officer may require the collection of sample cultures to determine that the area has been effectively decontaminated.

Checklist

BIOHAZARD SPILL PROCEDURES FOR INSIDE LAMINAR

FLOW BIOLOGICAL SAFETY CABINETS (LFBSC)

To be posted near the biosafety cabinet.

1. KEEP THE LFBSC ON.

1. PUT ON PROTECTIVE GLOVES.

1. SPRAY/WIPE WALLS, WORK SURFACES, AND EQUIPMENT WITH DECONTAMINATING SOLUTION.

1. FLOOR TRAY TOP, DRAIN PANS, AND CATCH BASINS WITH DECONTAMINATING SOLUTION.

1. ALLOW TO STAND FOR 20 MINUTES.

1. DRAIN EXCESS SOLUTION INTO CABINET BASE.

1. LIFT OUT TRAY AND REMOVABLE EXHAUST GRILLE WORK.

1. CLEAN TOP AND BOTTOM SURFACES WITH SPONGE/CLOTH SOAKED IN DECONTAMINATING SOLUTION.

1. REPLACE TRAY AND GRILLE WORK.

1. PLACE GLOVES, SPONGE, CLOTH, ETC. IN AUTOCLAVE PAN.

1. DRAIN DECONTAMINATING SOLUTION FROM CABINET BASE INTO AUTOCLAVABLE CONTAINERS.

1. AUTOCLAVE.

1. IF GASEOUS DECONTAMINATION IS NEEDED, CALL THE BIOSAFETY OFFICER.

Checklist

BIOHAZARD SPILL PROCEDURES FOR OUTSIDE LAMINAR

FLOW BIOLOGICAL SAFETY CABINETS (LFBSC)

Minor spills – Class 2 Organisms

To be posted near the biosafety cabinet.

1. WASH HANDS AND OTHER APPARENTLY CONTAMINATED BODY PARTS WITH SOAP AND WATER.

1. POST WARNING TO KEEP NON-ESSENTIAL PERSONNEL FROM SPILL AREA.

1. PUT ON PROTECTIVE GLOVES.

1. COVER SPILL AREA WITH PAPER TOWELS SOAKED IN DECONTAMINATING SOLUTION.

1. WIPE UP SPILL WITH SOAKED PAPER TOWELS.

1. PLACE USED TOWELS IN AUTOCLAVE PAN.

1. POUR DECONTAMINATING SOLUTION AROUND AND ON SPILL AREA.

1. LET SOLUTION STAND FOR 20 MINUTES.

1. WIPE UP WITH PAPER TOWELS.

1. PLACE PAPER TOWELS AND GLOVES IN AUTOCLAVE PAN.

1. WASH HANDS WITH SOAP AND WATER.

1. AUTOCLAVE.

Checklist

BIOHAZARD SPILL PROCEDURES FOR OUTSIDE LAMINAR

FLOW BIOLOGICAL SAFETY CABINET

Major Spills – Class 2 and 3 Organisms

To be posted near the biosafety cabinet.

1. WASH HANDS AND OTHER APPARENTLY CONTAMINATED BODY PARTS

WITH SOAP AND WATER.

1. POST WARNING SIGNS AND CLOSE LABORATORY DOOR.

1. REPORT SPILL TO SUPERVISOR AND BIOSAFETY OFFICER.

1. IF CLOTHING IS CONTAMINATED, REMOVE ALL CONTAMINATED GARMENTS.

1. PLACE CONTAMINATED CLOTHING IN AUTOCLAVE CONTAINER.

1. PUT ON CLEAN GARMENTS.

1. LEAVE LABORATORY FOR 20 MINUTES.

1. CHECK TO SEE THAT LABORATORY DOORS ARE CLOSED AND WARNING SIGNS DISPLAYED UPON RETURNING TO LAB.

1. PUT ON NEEDED SAFETY EQUIPMENT (DISPOSABLE GLOVES, RESPIRATORS, ETC.).

1. PLACE PAPER TOWELS SOAKED IN DECONTAMINATION SOLUTION OVER THE SPILL.

1. POUR DECONTAMINATION SOLUTION AROUND SPILL – ALLOW SOLUTION TO FLOW INTO SPILL. DO NOT POUR DECONTAMINATION SOLUTION INTO SPILL.

1. LET STAND FOR AT LEAST 20 MINUTES.

1. TRANSFER CONTAMINATED MATERIALS TO AUTOCLAVE CONTAINER USING AUTOCLAVABLE DUST PAN AND SQUEEGEE.

1. PLACE DUST PAN AND SQUEEGEE IN AUTOCLAVE CONTAINER.

1. REMOVE GLOVES AND OTHER CONTAMINATED GARMENTS AND PLACE IN AUTOCLAVE CONTAINER.

1. WASH FACE, HANDS, AND OTHER APPARENTLY CONTAMINATED BODY PARTS.

1. AUTOCLAVE ALL MATERIALS THAT REQUIRE AUTOCLAVING.

Liquid Disinfectants

Laboratory personnel should be familiar with the various disinfectants that will effectively kills the biohazardous agents being used. The following information is provided to assist in your selection of appropriate disinfectants.

Alcohols – Ethyl and Isopropyl are good disinfectants for the vegetative forms of bacteria and enveloped viruses.

Ethyl Alcohol

1. Use Dilution: 70-95%

2. Inactivates: vegetative bacteria and enveloped viruses, has variable results with non-enveloped

3. viruses and is ineffective with bacterial spores.

4. Other Characteristics: flammable, eye irritant, and toxic [Threshold limit value (TLV) – 1000 ppm]

Isopropyl Alcohol

1. Same as for Ethyl Alcohol except the TLV = 400 ppm.

Chlorine Compounds – The germicidal effect of chlorine compounds is dependent upon the release

of hypochlorous acid and is therefore dependent upon the available chlorine.

1. Allow a contact time of from 10 to 30 minutes.

2. Use Dilution: 500 ppm available chlorine is recommended for vegetative bacteria and most viruses.

3. Chlorine solutions that are neutral or slightly acidic and with a concentration of approximately 2500 ppm are needed for effectiveness against bacterial spores. Undiluted common household bleach (Clorox) is alkaline with a pH of 8. or greater. Household bleach typically contains 5.25% sodium hypochlorite for 52500 ppm available chlorine.

4. Other Characteristics: Chlorine compounds are corrosive to metals; leave a residue; irritate the skin, eyes, and respiratory tract, and are toxic. Chlorine compounds are also rapidly inactivated by organic matter. While chlorine compounds are not generally recommended for routine use, undiluted household bleach is frequently used with biological spills.

Iodophors – The germicidal effect of iodophors is dependent on the free iodine released from

the compound in which it is contained. Allow a contact time of 10 to 30 minutes.

1. Use Dilution: 25 to 1600 ppm of available iodine. Solutions containing 75 to 150 ppm are generally recommended.

2. Inactivates: vegetative bacteria, fungi and viruses. There is poor activity against bacterial spores.

3. Other Characteristics: Although iodophors are less harmful to man than chlorine compounds they can irritate the skin and eyes. Iodophors are corrosive (less than chlorine), they leave a residue and may stain. Iodophor stains, however, can be readily removed with solutions of sodium thiosulfate (Na2S2O3). As with the chlorine compounds, iodophors are rapidly inactivated by organic matter. One advantage is that iodophors have a built-in indicator. As long as the solution is brown or yellow it is still active.

Phenolic Compounds – These are effective against vegetative bacteria (including *Mycobacterium*

tuberculosis), fungi, and enveloped viruses. Effectiveness against non-enveloped viruses is variable depending on the virus. The phenols are ineffective against bacterial spores.

1. Use Dilutions: 1.0 – 5.0% Solutions containing 0.5 – 2.0% phenol are effective against enveloped viruses.

2. Other Characteristics: Phenols are corrosive and may leave a sticky, gummy residue.

3. Phenolic compounds are irritating to the skin and eyes and are relatively toxic – Phenol

4. TLV for skin is 5 ppm.

Quaternary Ammonium Compounds – The efficacy of Quaternary Ammonium compounds still generates considerable controversy. Quats are effective in destroying ordinary vegetative bacteria and lipid containing virus but are not effective against *Pseudomonas, Proteus* and other gram-negative bacilli. Also, Quats are not effective against bacterial spores at the usual use concentrations of 1:750.

1. Use Dilutions: 0.1 to 2.0%

2. Other Characteristics: Quats are surface-active compounds which posses the useful property of lowering the surface tension of the solution.

3. Other advantages include being nontoxic, odorless, nonstaining, noncorrosive to metals and stable. If used at recommended concentrations, Quats are nonirritating.

4. Quaternary Ammonium compounds are rapidly inactivated by organic matter.

Formaldehyde Solutions – Formaldehyde in a 5-8% concentration in an effective liquid decontaminant

which inactivates vegetative bacteria, bacterial spores, lipid and nonlipid viruses and fungi.

1. Use Dilutions: 5.0-8.0%

2. Other Characteristics: The odor and irritating (skin and eyes) and toxic features (TLV = 1.0 ppm) of formaldehyde solutions reduce the desirability of this solution for general use. Formaldehyde

3. solutions are active in the presence of organic matter an do not corrode metal.

VSU BIOSAFETY FORMS

- Memorandum of Understanding and Agreement (MUA) for Recombinant DNA Experiments
- MUA for Biohazards other than Recombinant DNA Experiments
- VSU Biosafety Infectious Agent Risk Assessment
- Laboratory Equipment Approval Form
- Biohazardous Research Checklist

VALDOSTA STATE UNIVERSITY

MEMORANDUM OF UNDERSTANDING AND AGREEMENT (MUA) FOR RECOMBINANT DNA EXPERIMENTS

DATE: _____

RESEARCHER'S NAME

_____

RESEARCHER'S TITLE _____

PHONE NO: _____

DEPARTMENT _____

BUILDING & ROOM NO(s)_____

GRANTING AGENCY _____

GRANT NO. (IF APPLICABLE) _____

TITLE OF GRANT OR PROJECT:

_____
_____

_____
_____

_____
_____

A. Describe the experiment involving recombinant DNA techniques. Your description is to be sufficiently complete so as to provide committee members an understanding of what you intend to do and how you will do it. A summary or abstract of your methods and materials section will also be helpful. Please reference this discussion to appropriate NIH Guidelines and/or USDA/APHIS, and EPA regulations.

MEMORANDUM OF UNDERSTANDING AND AGREEMENT

FOR RECOMBINANT DNA EXPERIMENTS (Contiued)

B. ASSESSMENT LEVELS OF PHYSICAL AND BIOLOGICAL CONTAINMENT.

1. Describe how you intend to meet physical and biological containment requirements (reference NIH/USDA/EPA guidelines).
2. Will this project involve environmental release?
3. Describe procedures and precautions to be followed in transporting biohazardous agents between laboratories.

C. Agreements

____ I agree to accept responsibility for training of all laboratory workers involved in the project.

____ I agree to comply with all appropriate requirements pertaining to shipment and transfer of recombinant DNA materials.

____ I am familiar with and agree to abide by the provisions of the current NIH/USDA/EPA Guidelines and other specific instructions pertaining to the proposed project.

THE INFORMATION ABOVE IS ACCURATE AND COMPLETE.

_____ _____

Principal Investigator Date

_____ _____

Department Head Date

MEMORANDUM OF UNDERSTANDING AND AGREEMENT

FOR RECOMBINANT DNA EXPERIMENTS (Continued)

D. We certify that the Valdosta State University Committee (or Subcommittee) on Biosafety has reviewed the proposed project for recombinant DNA experiments reviewed the proposed project for recombinant DNA experiments for compliance with the NIH/USDA/EPA and VSU Guidelines.

E. The Valdosta State University Committee on Biosafety will monitor throughout the duration of the project the facilities, procedures, and the training and expertise of the personnel involved in the recombinant DNA activity.

F. The Valdosta State University Committee on Biosafety has determined, based on information provided by the Principal Investigator, that no special medical surveillance (other than usual University health programs) is required for the project described in this MUA.

G. SPECIAL INSTRUCTIONS:

H. _____ _____

Chairman, VSU Committee on Biosafety Date

I. _____
_____

VSU Biosafety Officer Date

RETURN THIS FORM TO THE BIOSAFETY OFFICER AND MAKE A COPY FOR YOUR FILES

VALDOSTA STATE UNIVERSITY

MEMORANDUM OF UNDERSTANDING AND

AGREEMENT (MUA) FOR BIOHAZARDS OTHER THAN RECOMBINANT DNA EXPERIMENTS

DATE: _____

RESEARCHER'S
NAME_____

RESEARCHER'S TITLE _____

PHONE NO._____

DEPARTMENT _____

BUILDING. & ROOM NO(s)_____

GRANTING AGENCY _____

GRANT NO. (IF APPLICABLE)_____

TITLE OF GRANT OR PROJECT:

_____
_____

_____
_____

_____
_____

A. Describe the experiments involving biohazard(s). Your description is to be sufficiently complete so as to provide committee members an understanding of what you intend to do and how you will do it.

(CONTINUED)

B. Assess the levels of physical containment required for the experiments.

C. Describe the facilities and specific procedures that will be used to provide the required levels of containment.

D. Describe the procedures and precautions to be followed if biohazardous organisms or agents are to be transported between laboratories.

MUA FOR BIOHAZARDS OTHER THAN RECOMBINANT DNA EXPERIMENTS (CONTIUED)

E. The undersigned agree to certify the following conditions of the proposed research:

1. The information above is accurate and complete. We agree to accept responsibility for training of all laboratory workers involved in the project. We agree to comply with the CDC/NIH/USDA/EPA requirements pertaining to shipment and transfer of hazardous biological materials. We are familiar with and agree to abide by the provisions of Valdosta State University Biosafety Manual, which outlines standards for conducting experiments with biohazardous agents.
2. We understand that only the organisms specified are covered by this MUA, and that work with other organisms or types of biohazards may require other MUAs.

_____ _____

Principal Investigator Date

_____ _____

Department Head Date

* *

F. The VSU Committee on Biosafety has determined, based on information provided by the principal investigator, that:

____ (a) No special medical surveillance (other than usual University health programs) is required for the project described in this MUA;

____ (b) The following specific medical surveillance procedures must be carried out, for the

individuals listed by name, before commencing the project described in this MUA:

G. We certify that the Valdosta State University Committee on Biosafety has reviewed the proposed project and has found it to be in compliance with the VSU Biosafety Manual, which outlines standards for conducting experiments with biohazardous agents.

_____
_____

Chairperson, VSU Committee on Biosafety DATE

_____
_____

VSU Biosafety Officer DATE

VALDOSTA STATE UNIVERSITY

BIOSAFETY INFECTIOUS AGENT RISK ASSESSMENT

Investigator Name _____

Department_____

Section I - Agent Identification:

Agent Name: (common) _____

(Scientific) _____

Nature of Agent ____bacterial ____viral ____parasitic ____fungal ____other

Supplier/Source:

OUTSIDE

Name
_____
_

Address
_____

_____

_____

Phone (_____)-_____

Other
information_____
_

DESCRIBE PRIMARY ISOLATION
_____

_____
_____

_____
_____

_____
_____

_____
_____

Section II - Biosafety level

Biosafety Level _____1 _____2 _____3 _____4

Source of determination
_____

_____
_____

_____
_____

VALDOSTA STATE UNIVERSITY

BIOSAFETY INFECTIOUS AGENT RISK ASSESSMENT (CONTINUED)

Describe Unique features of the agent (if any) -

_____

_____
_____

_____
_____

_____
_____

_____
_____

_____
_____

Section III - Health Hazard Information

_____Primary pathogen _____Opportunistic pathogen

Name of Disease/Illness
_____

Describe Signs and
Symptoms_____

_____
_____

_____
_____

Incubation
Period_____

Method of Transmission

Direct Transmission _____yes _____no _____unknown

Indirect Transmission _____yes _____no _____unknown

Airborne Transmission _____yes _____no _____unknown

Direct contact _____yes _____no _____unknown

Vector borne _____yes _____no _____unknown

Droplet _____yes _____no _____unknown

Vehicle borne _____yes _____no _____unknown

Aerosol _____yes _____no _____unknown

Others_____
_____

Transovarial _____yes _____no _____unknown

In Utero _____yes _____no _____unknown

Colostral _____yes _____no _____unknown

Carriers/hosts/host factors
_____

_____
_____

_____
_____

VALDOSTA STATE UNIVERSITY

BIOSAFETY INFECTIOUS AGENT RISK ASSESSMENT (CONTINUED)

Identify high risk groups
_____

_____
_____

Are there reported Laboratory Infections with this agent? _____yes _____no

If Yes describe and/or comment
_____

_____
_____

_____
_____

_____
_____

_____
_____

Section IV - Control/Handling/Containment Level

Vaccine available:

Standard _____yes _____no

Investigational _____yes _____no

If vaccine is available have research personnel been vaccinated?

_____yes Date vaccinated _____

_____no Why not? _____

_____

_____

Laboratory procedures used that may produce droplets or aerosols

_____yes _____no

Universal precautions used

_____yes _____no

Safety equipment - biological safety cabinet

_____yes _____no

Personnel protective equipment

Gloves _____yes _____no

Lab coat/gown/apron _____yes _____no

Respirator (HEPA filter) needed _____yes _____no

If yes,
describe_____

_____

VALDOSTA STATE UNIVERSITY

BIOSAFETY INFECTIOUS AGENT RISK ASSESSMENT (CONTINUED)

Section V - Background Information:

Oxygen requirements _____

Gram stain_____

Spore forming _____Yes _____No

Generation
time_____

Incubation
period_____

Nutrient requirements
_____

_____
_____

_____
_____

Sensitivities:

Desiccation _____yes _____no

Light _____yes _____no

Temperature _____yes _____no

Chemicals _____yes _____no

Specify_____
_____

Radiation _____yes _____no

Antibiotic _____yes _____no

Specify
_____

Environmental Factors: (extrinsic factors that affect agent and opportunity for spread)
_____

_____
_____

Biosafety Committee Assessment _____Agree _____Disagree

Modifications_____
_____

_____
_____

_____
_____

_____
_____

_____
_____

_____
_____

VALDOSTA STATE UNIVERSITY

BIOSAFETY INFECTIOUS AGENT RISK ASSESSMENT (CONTINUED)

Date_____

Principal

Investigator_____
_____

Department_____

Telephone_____

Building & Room Number where unit is to be
installed_____

Equipment List/Desired Unit

_____ Class II A Biological Safety Cabinet

_____ Class II B1 Biological Safety Cabinet

_____ Class II B2 Biological Safety Cabinet

_____ Glove Box

_____ Horizontal Flow Clean Bench

_____ Vertical Flow Clean Bench

The above unit is for use with:

_____Tissue Culture
(Identify)_____

_____Clinical Specimens
(Identify)_____

_____Pathogenic Microorganisms
(Identify)_____

CDC/Biosafety Level _____2 _____3 _____4

_____ Carcinogens
(Identify)_____

Amounts to be
used_____

_____Media Preparation

_____Sterile Apparatus Assembly

_____Flammable or/and Toxic Chemicals

_____Other
_____

Will unit be attached to building exhaust system? _____yes _____no

This Unit Approved By:

_____ _____

Chairperson, Biosafety Committee Date

_____ _____

Engineering Dept./Physical Plant Date

One of these forms must be filed with Procurement for each individual unit purchased.

VSU BIOSAFETY

LABORATORY EQUIPMENT APPROVAL FORM

Date_____

Principal
Investigator_____
_____

Department_____
Telephone_____

Building & Room Number where unit is to be
installed_____

Equipment List/Desired Unit

_____ Class II A Biological Safety Cabinet

_____ Class II B1 Biological Safety Cabinet

_____ Class II B2 Biological Safety Cabinet

_____ Glove Box

_____ Horizontal Flow Clean Bench

_____ Vertical Flow Clean Bench

The above unit is for use with:

_____Tissue Culture
(Identify)_____

_____Clinical Specimens
(Identify)_____

_____Pathogenic Microorganisms
(Identify)_____

CDC/Biosafety Level _____2 _____3 _____4

_____ Carcinogens
(Identify)_____

Amounts to be
used_____

_____Media Preparation

_____Sterile Apparatus Assembly

_____Flammable or/and Toxic Chemicals

_____Other
_____
___

Will unit be attached to building exhaust system? _____yes _____no

This Unit Approved By:

_____
_____

Chairperson, Biosafety Committee Date

_____
_____

Engineering Dept./Physical Plant Date

One of these forms must be filed with Procurement for each individual unit purchased.

VALDOSTA STATE UNIVERSITY - BIOHAZARDOUS RESEARCH CHECKLIST

DATE_____

INVESTIGATOR_____ PHONE NO._____

DEPARTMENT_____
LOCATION_____

CO-PIs_____
____

FUNDING AGENCY_____

GRANT: NEW_____
CONTINUATION/RENEWAL_____

MUA PREVIOUSLY SUBMITTED--GIVE APPROVAL DATE_____

TITLE OF PROPOSAL_____

PLEASE INDICATE WHETHER THE STUDIES IN THE ACCOMPANYING RESEARCH GRANT PROPOSAL INVOLVE ANY OF THE FOLLOWING BY COMPLETING THE APPROPRIATE SPACES BELOW. (FOR FURTHER INFORMATION, SEE THE VSU BIOSAFETY MANUAL.)

TYPE I. In vitro construction and/or propagation of recombinant DNA molecules

_____yes _____no

A. Formation of rDNAs containing genes for the biosynthesis of toxic molecules lethal for vertebrates at an LK50 of less than 100 ng per kg body weight

_____yes _____no

B. Deliberate release into the environment of any organism containing rDNA

_____yes _____no

C. Deliberate transfer of a drug-resistance trait in microorganisms not known to acquire it naturally. _____yes _____no

D. Involves plant pests _____yes _____no

E. Involves transformation of whole plants _____yes _____no

F. Involves transformation of animals _____yes _____no

TYPE II. Experiments with organisms of demonstrated human pathogenicity. Specify agent(s) in subsequent section.

A. Involves the infection of animals _____yes _____no

TYPE III. Experiments on oncogenic (cancer-causing) animal viruses. Specify viruses in subsequent section.

A. Involves the infection of animals _____yes _____no

TYPE IV. Studies involving the laboratory culture of human or other primate tissues or cells. Specify the cell line/type of tissue culture in subsequent section.

_____yes _____no

TYPE V. Experiments involving venomous vertebrates or invertebrates. Specify organisms in subsequent section.

_____yes _____no

TYPE VI. Experiments involving the movement into Georgia of:

A. Pathogens that adversely affect plants or animals

_____yes _____no

B. Any non-indigenous species of plants or animals (including offspring).

_____yes _____no

C. Any indigenous species of plants or animals infected with pathogenic organisms outside of the state

_____yes _____no

TYPE VII. Experiments involving the transfer or receipt of select agents as defined in the Code of Federal Regulations (42 CFR Part 72.6). Specify agents in subsequent section.

_____yes _____no

Type II. Specify agents:

Type III. Specify viruses:

Type IV **Specify cell lines/types of tissue cultures:**

Type V **Specify organisms:**

Type VI **Specify select agents:**

_____

_____

_____

Signature of Investigator Date Signature of Department Head

Date

_____

_____

Submit this form to the VSU Biosafety Officer