

MEDICAL UNIVERSITY OF SOUTH CAROLINA (MUSC)
BIOLOGICAL SAFETY POLICY

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FOREWORD

The Biological Safety Policy is derived from the Public Health Service Centers for Disease Control and Prevention and the National Institute of Health Publication Biosafety in Microbiological and Biomedical Laboratories Ed.4, which describes combinations of standard and special microbiological practices, safety equipment and facilities that constitute biosafety levels 1-4, which are recommended for working with a variety of infectious agents in various laboratory settings.

The applications of these recommendations to a particular laboratory operation should be used on a risk assessment of the special agents and activities rather than as a universal and generic code applicable to all situations.

The current edition, edition 5, was published in May 2007. Biosafety in Microbiological and Biomedical Laboratories (BMBL) may be accessed online at <http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm>.

INSTITUTIONAL BIOSAFETY COMMITTEE

The Institutional Biosafety Committee (IBC) has been established to conduct initial and continuing review of all research proposals and projects involving recombinant DNA as outlined in the National Institutes of Health Guidelines for Recombinant DNA Research (April 2002) http://www4.od.nih.gov/oba/rac/guidelines_02/NIH_Gdlines_2002prn.pdf The IBC also reviews work with infectious agents and biotoxins.

The IBC shall have at least five (5) members so selected that they collectively have experience and expertise in recombinant DNA technology and microorganisms, and any potential risk to public health or the environment from hazardous materials. At least two (2) members shall not be affiliated with the institution (apart from their membership on the IBC) and shall represent the interest of the surrounding Charleston community with respect to health and protection of the environment. All appointments shall be made by the Vice President for Research as recommended by the IBC Chairperson/Vice Chairperson. This committee reports to the Vice President for Research.

PRINCIPLES OF BIOSAFETY

The term “containment” is used in describing safe methods for managing infectious agents in the laboratory equipment where they are being handled or maintained. Primary containment, the protection of personnel and the immediate laboratory equipment from exposure to infectious agents, is provided by microbiological technique and the use of appropriate safety equipment. The use of vaccines may provide an increased level of personal protection. Secondary containment, the protection of the environment external to the laboratory from exposure to infectious materials, is provided by a combination of facility design and operational practices. The purpose of containment is to reduce exposure of laboratory workers and other persons, and to prevent escape into the outside

environment of potentially hazardous agents. Three elements of containment include laboratory practice and technique, safety equipment and facility design.

The most important element of containment is strict adherence to standard microbiological practice techniques. Persons working with infectious agents or infected materials must be aware of potential hazards and must be trained and proficient in the practices and techniques required for safety handling such material. Each principal investigator is responsible for providing or arranging for appropriate training of personnel.

When standard laboratory practices are not sufficient to control the hazard associated with a particular agent or laboratory procedure, additional measures may be needed.

The person in charge of the laboratory is responsible for selecting additional safety practices, which must be in keeping with the hazards arising from the agent or the procedure.

Each principal investigator must develop a biosafety investigator plan which identifies the hazards that will or may be encountered and which specify practices and procedures designed to minimize or eliminate risks. Personnel should be advised of special hazards and should be required to read and to follow the required practices and procedures. They must sign an acknowledgement sheet indicating that they have been informed and understand the hazards, have read the safety protocol and will follow it. The acknowledgement form will be maintained in their departmental personnel records. A scientist trained and knowledgeable in appropriate laboratory techniques, safety procedures and hazards associated with handling infectious agents must direct laboratory activities.

Laboratory personnel, safety practices and techniques must be supplemented by appropriate facility design and engineering features, safety equipment and management practices. Safety equipment includes biological safety cabinets and a variety of enclosure containments. The biological safety cabinet is the principal device used to provide containment of infectious aerosols generated by

many microbiological procedures. There are three types of biological safety cabinets used in microbiological laboratories. Open fronted Class I and Class II biological safety cabinets are partial containment cabinets, which offer significant levels of protection to laboratory personnel and to the environment when, used with good microbiological techniques. The gas-tight Class III biological safety cabinet provides the highest attainable level of protection to personnel and the environment.

An example of an enclosed container is the safety centrifuge cup, which is designed to prevent aerosols from being released during centrifugation.

Safety equipment also includes items for personal protection such as gloves, coats, gowns, shoe covers, boots, respirators, face shields and safety glasses. These personal protective devices are often used in combination with biological safety cabinets and other devices, which contain the agents, animals or materials being worked with. In some situation in which impractical to work in biological safety cabinets, personal protective devices may form the primary barrier between personnel and the infectious materials. Examples of such activities include certain animal studies, animal necropsy, production activities and activities relating to maintenance, service or support of the laboratory facility.

The design of the facility is important in order to protect persons working in and outside the laboratory, as well as the adjoining community from infectious agents, which may be accidentally released. Laboratory management is responsible for providing facilities commensurate with the laboratory's function. Three facility designs are described below, in ascending order by level of containment:

1. **The Basic Laboratory.** This laboratory provides general space in which work is done with viable agents, which are not associated with disease in healthy adults. Basic laboratories include those facilities described in the following pages as Biosafety Level 1 and 2 facilities. This laboratory is also appropriate for work with infectious agents or potentially infectious

materials when the hazard levels are low and laboratory personnel can be adequately protected by standard laboratory practice. While work is commonly conducted on the open bench, certain operations are confined to biological safety cabinets. Conventional laboratory designs are adequate.

2. Areas known to be sources of general contamination, such as animal rooms and waste staging areas, should not be adjacent to patient care activities. Public areas and general offices to which nonlaboratory staff require frequent access should be separated from spaces which primarily support laboratory functions.
3. **The Containment Laboratory.** This laboratory has special engineering features, which make it possible for laboratory workers to handle hazardous material without endangering themselves, the community or the environment. The containment laboratory is described in the following pages as a Biosafety Level 3 facility. The unique features, which distinguish this laboratory from the basic laboratory, are the provisions for access control and a specialized ventilation system. The containment laboratory may be an entire building or a single module or a complex of modules within a building. In all cases, the laboratory is separated by a controlled access zone from areas open to the public.
4. **The Maximum Containment Laboratory.** This laboratory has special engineering and containment features that allow activities involving infectious agents that are extremely hazardous to the laboratory worker or that may cause serious epidemic disease to be conducted safely. The maximum containment laboratory is described on the following pages as Biosafety Level 4 facility. Although the maximum containment laboratory is generally a separate building, it can be constructed as an isolated area within a building. The laboratory's distinguishing characteristic is that it has secondary barriers to prevent hazardous materials from escaping into the environment. Such barriers include sealed

opening into the laboratory, airlock or liquid disinfectant barriers, a clothing-change and shower room contiguous to the laboratory, a double door autoclave, a biowaste treatment system, a separate ventilation system and a treatment system to decontaminate exhaust air. Four biosafety levels are described which consist of combinations of laboratory practices and techniques, safety equipment and laboratory facilities appropriate for the operations performed and the hazard posed by the infectious agents and for the laboratory function or activity. Biosafety Level 1 practices, safety equipment and facilities are appropriate for undergraduate and secondary educational training and teaching laboratories and for other facilities in which work is done with defined and characterized strains of viable microorganisms not known to cause disease in healthy human adults. Bacillus subtilis, Naegleria gruberi and infectious canine hepatitis virus are representative of those microorganisms meeting these criteria. Many agents not ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and in immunodeficient or immunosuppressed individuals. Vaccine strains which have undergone multiple in vivo passages should not be considered avirulent simply because they are vaccine strains.

Biosafety Level 2 practice, equipment and facilities are applicable to clinical, diagnostic, teaching and other facilities in which work is done with the broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing aerosols is low. Hepatitis B virus, the Salmonellae and Toxoplasma are representative of microorganisms assigned to this containment level. Primary hazards to personnel working with these agents may include accidental autoinoculation, ingestion and skin mucous membrane exposure to infectious materials. Procedures with high aerosol

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

Biosafety Level 4 practices safety equipment and facilities are applicable to work with dangerous and exotic agents, which pose a high individual risk of life-threatening disease. All manipulations of potentially infectious diagnostic materials, isolates and naturally or experimentally infected animals pose a high risk of exposure and infection to laboratory personnel. Lassa fever virus is representative of the microorganisms assigned to Level 4.

Four biosafety levels are also described for activities involving infectious disease activities with experimental mammals. These four combinations of practices, safety equipment and facilities are designated Animal Biosafety Levels 1, 2, 3, and 4 and provide increasing levels of protection to personnel and the environment.

The principal investigator is directly and primarily responsible for the safe operation of the laboratory. His/her knowledge and judgment are critical in assessing risks and appropriately applying these recommendations. The recommended biosafety level represents those conditions under which the agent can ordinarily be safely handled. Special characteristics of the agents used, the training and experience of personnel and nature or function of the laboratory may further influence the principle investigator in applying these recommendations.

Work with known agents should be conducted at the biosafety level recommended in the section dealing with recommended biosafety levels for infectious agents and infected animals unless specific information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns and other factors are significantly altered to require more stringent or allow less stringent practices to be used. The IBC will make the final decision regarding the biosafety level to be used after review of the IBC registration application(s)

Clinical laboratories, and especially those in health care facilities, receive clinical specimens with request for a variety of diagnostic and clinical support services. Typically, clinical laboratories receive specimens without pertinent information such as patient history or clinical finding which may be suggestive of an infectious etiology. Furthermore, such specimens are often submitted with a broad request for microbiological examination for multiple (e.g., sputum samples submitted for “routine,” acid-fast, and fungal cultures).

It is the responsibility of the laboratory supervisor to establish standard procedures in the laboratory which realistically address the issue of the infective hazard of clinical specimens. Except in extraordinary circumstances (e.g., suspected hemorrhagic fever), the initial processing of clinical specimens and identification of isolates can be and are safely conducted using a combination of practices, facilities and safety equipment described as Biosafety Level 2. Biological safety cabinets (Class I or II) should be used for initial processing of clinical specimens when the nature of the test requested or other information is suggestive that an agent readily transmissible by infectious aerosols is likely to be present. Class II biological safety cabinets are also used to protect the integrity of the specimens or cultures by preventing contamination from the laboratory environment. Segregating clinical laboratory functions and limiting or restricting access to laboratory areas are the responsibility of the laboratory supervisor.

The importation of infectious substances and vectors of human diseases is subject to the requirements of the Public Health Service Foreign Quarantine regulations. Companion regulations of the Public Health Service and the Department of Transportation specify packaging, labeling and shipping requirements for infectious substances and certain diagnostic specimens shipped in interstate commerce. (http://www.access.gpo.gov/nara/cfr/waisidx_04/49cfrv2_04.html) The U.S. Department of Agriculture regulates the importation and interstate shipment of animal pathogens and prohibits the importation, possession or use of certain exotic animal disease agents, which pose a serious disease threat to domestic livestock and poultry.

The MUSC Office of Research and Sponsored Programs handles Material Transfer Agreements (MTAs) used for the transfer of biological material. Go to <http://research.musc.edu/orsp/forms.htm>.

RECOMMENDED BIOSAFETY LEVELS FOR INFECTIOUS AGENTS AND INFECTED ANIMALS

Selection of an appropriate biosafety level for work with a particular agent or animal study depends upon a number of factors. Some of the most important are: the virulence, pathogenicity, biological stability; the procedure and manipulations involving the agent; the quantity and concentration of the agent; the endemicity of the agent; and the availability of effective vaccines or therapeutic measures.

Agent summary statements are available in the CDC and NIH publication, Biosafety in Microbiology and Biomedical Laboratories, 4th Edition. Additional information including coverage of some other agents may be found in Health Canada's Material Safety Data Sheets located at <http://www.phac-aspc.gc.ca/msds-ftss/index.html> Investigators should refer to this publication when selecting appropriate biosafety levels. Specific information on laboratory hazards associated with a particular agent and recommendations regarding practical safeguards that can significantly reduce the risk of laboratory-associated diseases are included. Agent summary statements are

presented for agents which meet one or more of the following criteria: the agent is a proven hazard to laboratory personnel working with infectious materials (e.g., hepatitis B virus, tubercle bacilli); the potential for laboratory associated infection is high even in the absence of previously documented laboratory associated infections (e.g., exotic arboviruses); or, the consequences of infection are grave (e.g., Creutzfeldt-Jacob disease, botulism).

Recommendations for the use of vaccines and toxins are included in agent summary statements when such products are available-either as licensed or Investigational New Drug (IND) products. When applicable, recommendations for the use of these products are based on current recommendations of the Public Health Service Advisory Committee on Immunization Practice and are specifically targeted to at-risk laboratory personnel and others who must work in or enter laboratory areas. These specific recommendations should in no way preclude the routine use of such products as diphtheria-tetanus toxoids, poliovirus vaccine, influenza vaccine and others because of the potential risk of community exposures irrespective of any laboratory risks. Appropriate precautions should be taken in the administration of live attenuated virus vaccines in individuals with altered immunocompetence.

Risk assessments and Biosafety Levels recommended in the agent summary statements presuppose a population of immunocompetent individuals. Those with altered immunocompetence may be at increased risk when exposed to infectious agents. Immunodeficiency may be hereditary, congenital or induced by a number of neoplastic diseases, by therapy or by radiation. The risk of becoming infected or the consequences of infection may also be influenced by such factors as age, sex, race, pregnancy, surgery (e.g., splenectomy, gastrectomy), predisposing diseases (e.g., diabetes, lupus erythematosus) or altered physiological function. These and other variables must be considered in individualizing the generic risk assessments of the agent summary statements for specific activities.

The basic biosafety level assigned to an agent is based on the activities typically associated with the growth and manipulation of quantities and concentrations of infectious agents required to accomplish identification or typing. If activities with clinical materials pose a lower risk to personnel than those activities with manipulation of cultures, a lower biosafety level is recommended. On the other hand, if the activities involve large volumes of highly concentrated preparation (“production quantities”) or manipulations which are likely to produce aerosols or which are otherwise intrinsically hazardous, additional personnel precautions and increased levels of primary and secondary containment may be indicated. “Production quantities” refers to large volumes or concentrations of infectious agents considerably in excess of those typically used for identification and typing activities. Propagation and concentration of infectious agents as occurs in large-scale fermentation’s, antigen and vaccine production, and a variety of other commercial and research activities clearly deal with significant masses of infectious agents that are reasonably considered “production quantities.” However, in terms of potentially increased risk as a function of the mass of infectious agents, it is not possible to define “production quantities” in finite volumes or concentrations for any given agent. Therefore, the principal investigator and/or Biosafety Officer must make a risk assessment of the activities conducted and selected practices, containment equipment and facilities appropriate to the risk, irrespective of the volume or concentration of agent involved.

Occasions will arise when the principal investigator and/or Biosafety Officer should select a biosafety level higher than that recommended. For example, a higher biosafety level may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing and susceptibility testing) in laboratories where facility features satisfy Biosafety Level 2 recommendations, provided the recommended “Standard Microbiological Practices,” “Special Practices,” and “Containment Equipment” for Biosafety Level 3 are rigorously

followed. The decision to adapt Biosafety Level 3 recommendations in this manner should be made only by the principal investigator and/or Biosafety Officer. This adaptation, however, is not suggested for agent production operations or activities where procedures are frequently changing. The Biosafety Office should also give special consideration to selecting appropriate safeguards for materials that may contain a suspected agent. For example, sera of human origin may contain hepatitis B virus and should be handled under conditions which reasonably preclude cutaneous, mucous membrane or parental exposure of personnel; and sputa submitted to the laboratory for assay for tubercle bacilli should be handled under conditions which reasonably preclude the generation of aerosols or which contain any aerosols that may be generated during the manipulation of clinical materials or cultures.

The principal investigator is also responsible for appropriate risk assessment of agents not included in the Agent Summary Statements and for utilization of appropriate practices, containment equipment and facilities for the agent used.

Risk Assessment

The risk assessment of laboratory activities involving the use of infectious microorganisms is ultimately a subjective process. Those risks associated with the agent, as well as with the activity to be conducted, must be considered in the assessment.

The hepatitis B virus (HBV) is an appropriate model for illustrating the risk assessment process. HBV is among the most ubiquitous of human pathogens and most prevalent of laboratory-associated infections. The agent has been demonstrated in a variety of body secretions and excretions. Blood, saliva and semen have been shown to be infectious. Natural transmission is associated with parental inoculation or with contamination of the broken skin or of mucous membranes with infectious body fluids. There is no evidence of airborne or interpersonal spread

through casual contact. Prophylactic measures include the use of a licensed vaccine in high-risk groups and the use of hepatitis B immune globulin following overt exposure.

The primary risk of HBV infection in laboratory personnel is associated with accidental parenteral inoculation, or exposure of broken skin or mucous membrane of the eyes, nose or mouth. These risks are typical of those described for Biosafety Level 2 agents are addressed by using the recommended standard and special microbiological practices to minimize or eliminate these overt exposures.

The described risk assessment process is also applicable to laboratory operations other than those involving the use of primary agents of human disease. Microbiological studies of animal host-specific pathogens, soil, water, food, feeds and other natural or manufactured materials, by comparison, pose substantially lower risks of laboratory infection. Microbiologists and other scientist working with such materials may, nevertheless, find the practices, containment equipment and facility recommendation described in the manual of value in developing operational standards to meet their own assessed needs.

TABLE 1. SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FOR INFECTIOUS AGENTS

Biosafety Level	Practice and Techniques	Safety Equipment	Facilities
1	Standard microbiological practices.	None: primary containment provided by adherence to standard laboratory practices during open bench operations.	Basic
2	Level 1 practices plus: Laboratory costs, decontamination of all infectious wastes; limited access; protective gloves and biohazard warning signs as indicated.	Partial containment equipment (i.e., Class I or II Biological Safety Cabinets) used to conduct mechanical manipulative procedures that have high aerosol potential that may increase the risk of exposure to personnel.	Basic
3	Level 2 practices plus: special laboratory clothing; controlled access.	Partial containment equipment used for all manipulations of infectious material.	Containment
4	Level 3 practices plus: entrance through change room where street clothes is removed and laboratory clothing is put on; shower on exit; all wastes are decontaminated on exit from the facility.	Maximum containment equipment (i.e., Class III biological safety cabinet or partial containment equipment in combination with full-body, air-supplied, positive pressure personnel suit) used for all procedures and activities.	Maximum Containment

Working with Biohazardous Materials

It is the responsibility of the principal investigator to acquaint members of his/her laboratory with risks associated with working with biohazardous materials and agents. Each principal investigator (PI) must develop a biosafety plan which identifies the hazards present in his/her laboratory and the specific practices and procedures that need be followed in order to reduce the risk of working with these biohazardous agents.

It is accepted practice to give **specific training** relevant to requirements for working in an environment with biohazardous materials that may result in deleterious effects to laboratorians, any fetus they may be carrying, or close associates such as household contacts. Laboratory personnel should be **advised of specific hazards** and be required to read and to follow the biosafety plan prepared by the PI. Special care should be taken to advise at risk populations. An at risk population includes, but is not limited to, immunocompromised individuals, those individuals who are pregnant, and individuals of child bearing age.

Specific biohazards to pregnant women and their fetuses include, but are not limited to, those agents in the *TORCH* group including **T**, *Toxoplasma gondii*, **O**, *Treponema pallidum* (syphilis), **R**, rubella, **C**, cytomegalovirus (CMV), and **H**, herpes simplex virus. However, there is also evidence that a number of other viruses including, but not limited to, adenovirus, coxsackie virus, Epstein-Barr virus, hepatitis B virus, human parvovirus, and varicella-zoster virus may result in adverse pregnancy outcomes. Further, bacterial agents of special concern are those classified as BSL3 agents and those BSL2 agents with known consequences to the fetus such as *Streptococcus agalactiae*, group B Streptococcus (GBS).

In addition to participating in training, reading and following the biosafety plan, it is also the responsibility of the laboratorian to inform their immediate supervisor of any change in their health status (such as pregnancy, taking medications resulting in reduced immunity etc.). Furthermore, the laboratorian may wish to consult with student or employee health and/or their personal physician to seek guidance with respect to how best to manage the risk. Appropriate action should be taken by the PI /supervisor to safeguard the health of the individual and, if necessary, the developing fetus. A written, confidential, signed plan outlining the management of the specific risk shall be placed in the laboratorian's personnel file acknowledging their understanding and acceptance of the management plan.