Biosafety in Microbiological and Biomedical Laboratories

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Summary - Pages 13-49 (Details about Biosafety Level 1 & 2)

Section 1

Introduction

Over the past two decades, Biosafety in Microbiological and Biomedical Laboratories (BMBL) has become the code of practice for biosafety—the discipline addressing the safe handling and containment of infectious microorganisms and hazardous biological materials. The principles of biosafety introduced in 1984 in the first edition of BMBL1 and carried through in this fifth edition remain steadfast. These principles are containment and risk assessment. The fundamentals of containment include the microbiological practices, safety equipment, and facility safeguards that protect laboratory workers, the environment, and the public from exposure to infectious microorganisms that are handled and stored in the laboratory. Risk assessment is the process that enables the appropriate selection of microbiological practices, safety equipment, and facility safeguards that can prevent laboratory-associated infections (LAI). The purpose of periodic updates of BMBL is to refine guidance based on new knowledge and experiences and to address contemporary issues that present new risks that confront laboratory workers and the public health. In this way the code of practice will continue to serve the microbiological and biomedical community as a relevant and valuable authoritative reference.

Individual workers who handle pathogenic microorganisms must understand the containment conditions under which infectious agents can be safely manipulated and secured. Application of this knowledge and the use of appropriate techniques and equipment will enable the microbiological and biomedical community to prevent personal, laboratory and environmental exposure to potentially infectious agents or biohazards.

THE OCCURRENCE OF LABORATORY-ASSOCIATED INFECTIONS

Published reports of LAIs first appeared around the start of the twentieth century. By 1978, four studies by Pike and Sulkin collectively identified 4,079 LAIs resulting in 168 deaths occurring between 1930 and 1978. These studies found that the ten most common causative agents of overt infections among workers were Brucella sp., Coxiella burnetii, hepatitis B virus (HBV), Salmonella typhi, Francisella tularensis, Mycobacterium tuberculosis, Blastomyces dermatitidis, Venezuelan equine encephalitis virus, Chlamydia psittaci, and Coccidioides immitis. The authors acknowledged that the 4,079 cases did not represent all LAIs that occurred during this period since many laboratories chose not to report overt cases or conduct surveillance programs to identify sub-clinical or asymptomatic infections.

Publication of the occurrence of LAIs provides an invaluable resource for the microbiological and biomedical community. For example, one report of occupational exposures associated with Brucella melitensis, an organism capable of transmission by the aerosol route, **described how a staff member in a clinical microbiology laboratory accidentally sub-cultured B. melitensis on the open bench.** This error and breech in containment practices resulted in **eight** laboratory-associated infections (LAIs) with B. melitensis among 26 laboratory members, an attack rate of 31%. **Reports of LAIs can serve as lessons in the importance of maintaining safe conditions in biological research.**

EVOLUTION OF NATIONAL BIOSAFETY GUIDELINES

National biosafety guidelines evolved from the efforts of the microbiological and biomedical community to promote the use of safe microbiological practices, safety equipment and facility safeguards that will reduce LAIs and protect the public health and environment. **The historical accounts of LAIs raised awareness about the hazards of infectious microorganisms and the health risks to laboratory workers who handle them.**

RISK CRITERIA FOR ESTABLISHING ASCENDING LEVELS OF CONTAINMENT The primary risk criteria used to define the four ascending levels of containment, referred to as biosafety levels 1 through 4, are infectivity, severity of disease, transmissibility, and the nature of the work being conducted.

Biosafety level 1 (BSL-1) is the basic level of protection and is appropriate for agents that are not known to cause disease in normal, healthy humans.

Biosafety level 2 (BSL-2) is appropriate for handling moderate-risk agents that cause human disease of varying severity by ingestion or through percutaneous or mucous membrane exposure.

Agents requiring the following Biosafety levels 3 and 4 are not permitted at St. Norbert College.
Biosafety level 3 (BSL-3) is appropriate for agents with a known potential for aerosol transmission, for agents that may cause serious and potentially lethal infections and that are indigenous or exotic in origin. Exotic agents that pose a high individual risk of life-threatening disease by infectious aerosols and for which no treatment is available are restricted to high containment laboratories that meet biosafety level 4 (BSL-4) standards.

It is important to emphasize that the causative incident for most LAIs is unknown. Less obvious exposures such as the inhalation of infectious aerosols or direct contact of the broken skin or mucous membranes with droplets containing an infectious microorganism or surfaces contaminated by droplets may possibly explain the incident responsible for a number LAIs. Most manipulations of liquid suspensions of microorganisms produce aerosols and droplets. Small-particle aerosols have respirable size particles that may contain one or several microorganisms. These small particles stay airborne and easily disperse throughout the laboratory. When inhaled, the human lung will retain those particles. Larger particle droplets rapidly fall out of the air, contaminating gloves, the immediate work area, and the mucous membranes of unprotected workers. **A procedure's potential to release microorganisms into the air as aerosols and droplets is the most important operational risk factor that supports the need for containment equipment and facility safeguards.**

AGENT SUMMARY STATEMENTS
The fifth edition, as in all previous editions, includes agent summary statements that describe the hazards, recommended precautions, and levels of containment appropriate for handling specific human and zoonotic pathogens in the laboratory and in facilities that house laboratory vertebrate animals. Agent summary statements are included for agents that meet one or more of the following three criteria:
1) the agent is a **proven hazard** to laboratory personnel working with infectious materials;
2) the agent has a **high potential** for causing LAIs even though no documented cases exist;
3) the agent **causes grave disease or presents a significant public health hazard.**
Scientists, clinicians, and biosafety professionals prepared the statements by assessing the risks of handling the agents using standard protocols followed in many laboratories.

BIOSECURITY
Today, the nation is facing a new challenge in safeguarding the public health from potential domestic or international terrorism involving the use of dangerous biological agents or toxins. Existing standards and practices may require adaptation to ensure protection from such hostile actions. In addition, recent federal regulations mandate increased security within the microbiological and biomedical community in order to protect biological pathogens and toxins from theft, loss, or misuse. The fifth edition of BMBL includes an important new section on biosecurity – **the discipline addressing the security of microbiological agents and toxins and the threats posed to human and animal health, the environment, and the economy by deliberate misuse or release.** A careful review of the biosecurity concepts and guidelines introduced in this new section is essential for all laboratory workers.

USING Biosafety in Microbiological Biomedical Laboratories (BMBL)
BMBL is both a code of practice and an authoritative reference. Knowledge sufficient to work safely with hazardous microorganisms requires a careful review of the entire BMBL.

In The Future:

The laboratory-associated infections (LAIs) reported in the last 25 years demonstrate that accidents and unrecognized exposures continue to occur. The absence of clear evidence of the means of transmission in most documented laboratory-associated infections (LAIs) should motivate persons at risk **to be alert to all potential routes of exposure.** The accidental release of microbial aerosols is a probable cause of many LAI, which demonstrates the **importance of worker training and the ability to recognize potential hazards and correct unsafe habits. Attention to and proficient use of work practices, safety equipment and engineering controls are also essential.**

The expansion of biocontainment laboratories nationwide dramatically increases the need for training in microbiological practices and biosafety principles. **Understanding the principles of biosafety and adherence to the microbiological practices, containment and facility safeguards** described in Biosafety in Microbiological Biomedical Laboratories (BMBL) will contribute to a safer and healthier working environment for laboratory staff and adjacent personnel, and the community.

Section II

Biological Risk Assessment

Risk assessment is an important responsibility for directors and principal investigators of microbiological and biomedical laboratories. Institutional biosafety committees (IBC), animal care and use committees, biological safety professionals, and laboratory animal veterinarians share in this responsibility. Risk assessment is a process used to identify the hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a LAI, and the probable consequences of such an infection. The information identified by risk assessment will provide a guide for the selection of appropriate biosafety levels and microbiological practices, safety equipment, and facility safeguards that can prevent LAIs.
Laboratory directors and principal investigators should use risk assessment to alert their staffs to the hazards of working with infectious agents and to the need for developing proficiency in the use of selected safe practices and containment equipment. Successful control of hazards in the laboratory also protects persons not directly associated with the laboratory, such as other occupants of the same building, and the public. Risk assessment requires careful judgment. Adverse consequences are more likely to occur if the risks are underestimated. By contrast, imposition of safeguards more rigorous than actually needed may result in additional expense and burden for the laboratory, with little safety enhancement. Unnecessary burden may result in circumvention of required safeguards. However, where there is insufficient information to make a clear determination of risk, it is prudent to consider the need for additional safeguards until more data are available.

The primary factors to consider in risk assessment and selection of precautions fall into two broad categories: **agent hazards and laboratory procedure hazards.** In addition, the capability of the laboratory staff to control hazards must be considered. This capability will depend on the training, technical proficiency, and good habits of all members of the laboratory, and the operational integrity of containment equipment and facility safeguards.
The agent summary statements contained in BMBL identify the primary agent and procedure hazards for specific pathogens and recommend precautions for their control. The guest editors and contributors of this and previous editions of BMBL based their recommendations on an assessment of the risks associated with the handling of pathogens using generally routine generic laboratory procedures. A review of the summary statement for a specific pathogen is a helpful starting point for assessment of the risks of working with that agent and those for a similar agent.
HAZARDOUS CHARACTERISTICS OF AN AGENT The principal hazardous characteristics of an agent are: its capability to infect and cause disease in a susceptible human or animal host, its virulence as measured by the severity of disease, and the availability of preventive measures and effective treatments for the disease. The World Health Organization (WHO) has recommended an agent risk group classification for laboratory use that describes four general risk groups based on these principal characteristics and the route of transmission of the natural disease.
1 The four groups address the risk to both the laboratory worker and the community. The NIH Guidelines established a comparable classification and assigned human etiological agents into four risk groups on the basis of hazard.
2 The descriptions of the WHO and NIH risk group classifications are presented in Table 1. **They correlate with but do not equate to biosafety levels. A risk assessment will determine the degree of correlation between an agent's risk group classification and biosafety level.** See Section 3 for a further discussion of the differences and relatedness of risk groups and biosafety level.

TABLE 1 CLASSIFICATION OF INFECTIOUS MICROORGANISMS BY RISK GROUP
RISK GROUP
CLASSIFICATION
NIH GUIDELINES FOR RESEARCH
INVOLVING RECOMBINANT DNA
MOLECULES 20022
WORLD HEALTH ORGANIZATION
LABORATORY BIOSAFETY MANUAL
3RD EDITION 2004*

Risk Group 1
Agents that are not associated with disease in healthy adult humans.
(No or low individual and community risk)
A microorganism that is unlikely to cause human or animal disease.

Risk Group 2
Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available. (Moderate individual risk; low community risk) A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.

Risk Group 3
Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).
(High individual risk; low community risk) A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.

Risk Group 4
Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).
(High individual and community risk) A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

Other hazardous characteristics of an agent include probable routes of transmission of laboratory infection, infective dose, stability in the environment, host range, and its endemic nature.
The predominant probable routes of transmission in the laboratory are: 1) direct skin, eye or mucosal membrane exposure to an agent; 2) parenteral inoculation by a syringe needle or other contaminated sharp, or by bites from infected animals and arthropod vectors; 3) ingestion of liquid suspension of an infectious agent, or by contaminated hand to mouth exposure; and 4) inhalation of infectious aerosols. An awareness of the routes of transmission for the natural human disease is helpful in identifying probable routes of transmission in the laboratory and the potential for any risk to the public health. For example, transmission of infectious agents can occur by direct contact with discharges from respiratory mucous membranes of infected persons, which would be a clear indication that a laboratory worker is at risk of infection from mucosal membrane exposure to droplets generated while handling that agent.

An agent capable of transmitting disease through respiratory exposure to infectious aerosols is a serious laboratory hazard, both for the person handling the agent and for other laboratory occupants. This hazard requires special caution because infectious aerosols may not be a recognized route of transmission for the natural disease. Infective dose and agent stability are particularly important in establishing the risk of airborne transmission of disease. For example, the reports of multiple infections in laboratories associated with the use of Coxiella burnetii are explained by its low inhalation infective dose, which is estimated to be ten inhaled infectious particles, and its resistance to environmental stresses that enables the agent to survive outside of a living host or culture media long enough to become an aerosol

hazard. When work involves the use of laboratory animals, the hazardous characteristics of zoonotic agents require careful consideration in risk assessment. Evidence that experimental animals can shed zoonotic agents and other infectious agents under study in saliva, urine, or feces is an important indicator of hazard. The death of a primate center laboratory worker from Cercopithecine herpesvirus 1 (CHV-1, also known as monkey B virus) infection following an ocular splash exposure to biologic material from a rhesus macaque emphasizes the seriousness of this hazard. Lack of awareness for this potential hazard can make laboratory staff vulnerable to an unexpected outbreak involving multiple infections. Experiments that demonstrate transmission of disease from an infected animal to a normal animal housed in the same cage are reliable indicators of hazard. Experiments that do not demonstrate transmission, however, do not rule out hazard. For example, experimental animals infected with Francisella tularensis, Coxiella burnetii, Coccidioides immitis, or Chlamydia psittaci, agents that have caused many LAIs, rarely infect cagemates.

The origin of the agent is also important in risk assessment. Non-indigenous agents are of special concern because of their potential to introduce risk of transmission, or spread of human and animal or infectious diseases from foreign countries into the United States. Importation of etiological agents of human disease requires a permit from the CDC. Importation of many etiological agents of livestock, poultry and other animal diseases requires a permit from the USDA's Animal and Plant Health Inspection Service (APHIS). For additional details see Appendix F.

Genetically-modified agent hazards. The identification and assessment of hazardous characteristics of genetically modified agents involve consideration of the same factors used in risk assessment of the wild-type organism. It is particularly important to address the possibility that the genetic modification could increase an agent's pathogenicity or affect its susceptibility to antibiotics or other effective treatments. The risk assessment can be difficult or incomplete, because important information may not be available for a newly engineered agent. Several investigators have reported that they observed unanticipated enhanced virulence in recent studies with engineered agents. These observations give reason to remain alert to the possibility that experimental alteration of virulence genes may lead to increased risk. It also suggests that risk assessment is a continuing process that requires updating as research progresses.

The NIH Guidelines are the key reference in assessing risk and establishing an appropriate biosafety level for work involving recombinant DNA molecules. The purpose of the NIH Guidelines is to promote the safe conduct of research involving recombinant DNA. The guidelines specify appropriate practices and procedures for research involving constructing and handling both recombinant DNA molecules and organisms and viruses that contain recombinant DNA. They define recombinant DNA as a molecule constructed outside of a living cell with the capability to replicate in a living cell. The NIH Guidelines explicitly address experiments that involve introduction of recombinant DNA into Risk Groups 2, 3, and 4 agents, and experiments in which the DNA from Risk Groups 2, 3, and 4 agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems. Compliance with the NIH Guidelines is mandatory for investigators conducting recombinant DNA research funded by the NIH or performed at, or sponsored by, any public or private entity that receives any NIH funding for recombinant DNA research. Many other institutions have adopted these guidelines as the best current practice.

The NIH Guidelines were first published in 1976 and are revised on an ongoing basis in response to scientific and policy developments. They outline the roles and responsibilities of various entities affiliated with recombinant DNA research, including institutions, investigators, and the NIH. Recombinant DNA research subject to the NIH Guidelines may require: 1) approval by the NIH Director, review by the NIH Recombinant DNA Advisory Committee (RAC), and approval by the IBC; or 2) review by the NIH Office of Biotechnology Activities (OBA) and approval by the IBC; or 3) review by the RAC and approvals by the IBC and Institutional Review Board; or 4) approval by the IBC prior to initiation of the research; or 5) notification of the IBC simultaneous with initiation of the work. It is important to note that review by an IBC is required for all non-exempt experiments as defined by the NIH Guidelines.

Cell Cultures

Workers who handle or manipulate human or animal cells and tissues are at risk for possible exposure to potentially infectious latent and adventitious agents that may be present in those cells and tissues. This risk is well understood and illustrated by the reactivation of herpes viruses from latency, the inadvertent transmission of disease to organ recipients, and the persistence of human immunodeficiency virus (HIV), HBV, and hepatitis C virus (HCV) within infected individuals in the U.S. population. There also is evidence of accidental transplantation of human tumor cells to healthy recipients which indicates that these cells are potentially hazardous to laboratory workers who handle them. In addition, human and animal cell lines that are not well characterized or are obtained from secondary sources may introduce an

infectious hazard to the laboratory. For example, the handling of nude mice inoculated with a tumor cell line unknowingly infected with lymphocytic choriomeningitis virus resulted in multiple LAIs. The potential for human cell lines to harbor a bloodborne pathogen led the Occupational Health and Safety Administration (OSHA) to interpret that the occupational exposure to bloodborne pathogens final rule would include human cell lines.

HAZARDOUS CHARACTERISTICS OF LABORATORY PROCEDURES

Investigations of LAIs have identified five principal routes of laboratory transmission. These are parenteral inoculations with syringe needles or other contaminated sharps, spills and splashes onto skin and mucous membranes, ingestion through mouth pipetting, animal bites and scratches, and inhalation exposures to infectious aerosols. The first four routes of laboratory transmission are easy to detect, but account for less than 20 percent of all reported LAIs. Most reports of such infections do not include information sufficient to identify the route of transmission of infection. Work has shown that the probable sources of infection—animal or ectoparasite, clinical specimen, agent, and aerosol—are apparent in approximately 50 percent of cases.

Aerosols are a serious hazard because they are ubiquitous in laboratory procedures, are usually undetected, and are extremely pervasive, placing the laboratory worker carrying out the procedure and other persons in the laboratory at risk of infection. There is general agreement among biosafety professionals, laboratory directors and principal investigators who have investigated LAIs that an aerosol generated by procedures and operations is the probable source of many LAIs, particularly in cases involving workers whose only known risk factor was that they worked with an agent or in an area where that work was done.

Procedures that impart energy to a microbial suspension will produce aerosols. **Procedures and equipment used routinely for handling infectious agents in laboratories, such as pipetting, blenders, non-self-contained centrifuges, sonicators and vortex mixers are proven sources of aerosols. These procedures and equipment generate respirable-size particles that remain airborne for protracted periods.** When inhaled, these particles are retained in the lungs creating an exposure hazard for the person performing the operation, coworkers in the laboratory, and a potential hazard for persons occupying adjacent spaces open to air flow from the laboratory. A number of investigators have determined the aerosol output of common laboratory procedures. In addition, investigators have proposed a model for estimating inhalation dosage from a laboratory aerosol source. Parameters that characterize aerosol hazards include an agent's inhalation infective dose, its viability in an aerosol, aerosol concentration, and particle size. **Procedures and equipment that generate respirable size particles also generate larger size droplets that can contain multiple copies of an infectious agent. The larger size droplets settle out of the air rapidly, contaminating the gloved hands and work surface and possibly the mucous membranes of the persons performing the procedure.** An evaluation of the release of both respirable particles and droplets from laboratory operations determined that the respirable component is relatively small and does not vary widely; in contrast hand and surface contamination is substantial and varies widely. The potential risk from exposure to droplet contamination requires as much attention in a risk assessment as the respirable component of aerosols.

Technique can significantly impact aerosol output and dose. The worker who is careful and proficient will minimize the generation of aerosols. A careless and hurried worker will substantially increase the aerosol hazard. For example, the hurried worker may operate a sonic homogenizer with maximum aeration whereas the careful worker will consistently operate the device to assuring minimal aeration. **Experiments show that the aerosol burden with maximal aeration is approximately 200 times greater than aerosol burden with minimal aeration.** Similar results were shown for pipetting with bubbles and with minimal bubbles. Containment and good laboratory practices also reduce this risk.

POTENTIAL HAZARDS ASSOCIATED WITH WORK PRACTICES, SAFETY EQUIPMENT AND FACILITY SAFEGUARDS

Workers are the first line of defense for protecting themselves, others in the laboratory, and the public from exposure to hazardous agents. **Protection depends on the conscientious and proficient use of good microbiological practices and the correct use of safety equipment. A risk assessment should identify any potential deficiencies in the practices of the laboratory workers. Carelessness is the most serious concern, because it can compromise any safeguards of the laboratory and increase the risk for coworkers. Training, experience, knowledge of the agent and procedure hazards, good habits, caution, attentiveness, and concern for the health of coworkers are prerequisites for a laboratory staff in order to reduce the inherent risks that attend work with hazardous agents.** Not all workers who join a laboratory staff will have these prerequisite traits even though they may possess excellent scientific credentials. **Laboratory directors or principal investigators should train and retrain new staff to the point where aseptic techniques and safety precautions**

become second nature. There may be hazards that require specialized personal protective equipment in addition to safety glasses, laboratory gowns, and gloves. For example, a procedure that presents a splash hazard may require the use of a mask and a face shield to provide adequate protection. Inadequate training in the proper use of personal protective equipment may reduce its effectiveness, provide a false sense of security, and could increase the risk to the laboratory worker. For example, a respirator may impart a risk to the wearer independent of the agents being manipulated.

Safety equipment such as Biological Safety Cabinets (BSC), centrifuge safety cups, and sealed rotors are used to provide a high degree of protection for the laboratory worker from exposure to microbial aerosols and droplets. Safety equipment that is not working properly is hazardous, especially when the user is unaware of the malfunction. The containment capability of a BSC is compromised by poor location, room air currents, decreased airflow, leaking filters, raised sashes, crowded work surfaces, and poor user technique. The safety characteristics of modern centrifuges are only effective if the equipment is operated properly. Training in the correct use of equipment, proper procedure, routine inspections and potential malfunctions, and periodic re-certification of equipment, as needed, is essential.

AN APPROACH TO ASSESS RISKS AND SELECT APPROPRIATE SAFEGUARDS

Biological risk assessment is a subjective process requiring consideration of many hazardous characteristics of agents and procedures, with judgments based often on incomplete information. There is no standard approach for conducting a biological risk assessment, but some structure can be helpful in guiding the process. This section describes a five-step approach that gives structure to the risk assessment process.

First, identify agent hazards and perform an initial assessment of risk. Consider the principal hazardous characteristics of the agent, which include its capability to infect and cause disease in a susceptible human host, severity of disease, and the availability of preventive measures and effective treatments. There are several excellent resources that provide information and guidance for making an initial risk assessment. The BMBL provides agent summary statements for some agents associated with LAIs or are of increased public concern. Agent summary statements also identify known and suspected routes of transmission of laboratory infection and, when available, information on infective dose, host range, agent stability in the environment, protective immunizations, and attenuated strains of the agent.

A thorough examination of the agent hazards is necessary when the intended use of an agent does not correspond with the general conditions described in the Summary Statement or when an agent summary statement is not available. Although a summary statement for one agent may provide helpful information for assessing the risk of a similar agent, it should not serve as the primary resource for making the risk determination for that agent. Refer to other resources for guidance in identifying the agent hazards. The Control of Communicable Diseases Manual provides information on communicable diseases including concise summaries on severity, mode of transmission, and the susceptibility and resistance of humans to disease. In addition, it is always helpful to seek guidance from colleagues with experience in handling the agent and from biological safety professionals. Often there is not sufficient information to make an appropriate assessment of risk. For example, the hazard of an unknown agent that may be present in a diagnostic specimen will be unknown until after completing agent identification and typing procedures. It would be prudent in this case to assume the specimen contains an agent presenting the hazardous classification that correlates with BSL-2 unless additional information suggests the presence of an agent of higher risk. Identification of agent hazards associated with newly emergent pathogens also requires judgments based on incomplete information.

Consult interim biosafety guidelines prepared by the CDC and the WHO for risk assessment guidance. When assessing the hazards of a newly attenuated pathogen, experimental data should support a judgment that the attenuated pathogen is less hazardous than the wild-type parent pathogen before making any reduction in the containment recommended for that pathogen. Make a preliminary determination of the biosafety level that best correlates with the initial risk assessment based on the identification and evaluation of the agent hazards. **Remember that aerosol and droplet routes of agent transmission also are important considerations in specification of safety equipment and facility design that result in a given BSL level.**

Second, identify laboratory procedure hazards. The principal laboratory procedure hazards are agent concentration, suspension volume, equipment and procedures that generate small particle aerosols and larger airborne particles (droplets), and use of sharps.

Procedures involving animals can present a number of hazards such as bites and scratches, exposure to zoonotic agents, and the handling of experimentally generated infectious aerosols. The complexity of a laboratory procedure can also present a hazard. The agent summary statement provides information on the primary laboratory hazards associated with typically routine procedures used in handling an agent. In proposed laboratory procedures where the procedure hazards differ from the general conditions of the agent summary statement or where an agent summary statement is not available, the risk assessment should identify specific hazards associated with the procedures.

Third, make a final determination of the appropriate biosafety level and select additional precautions indicated by the risk assessment. The final selection of the appropriate biosafety level and the selection of any additional laboratory precautions require a comprehensive understanding of the practices, safety equipment, and facility safeguards described in Sections 3, 4 and 5 of this publication.

There will be situations where the intended use of an agent requires greater precautions than those described in the agent's Summary Statement. These situations will require the careful selection of additional precautions. **An obvious example would be a procedure for exposing animals to experimentally generated infectious aerosols.** It is unlikely that a risk assessment would indicate a need to alter the recommended facility safeguards specified for the selected biosafety level. If this does occur, however, it is important that a biological safety professional validate this judgment independently before augmenting any facility secondary barrier.

It is also important to recognize that individuals in the laboratory may differ in their susceptibility to disease. Preexisting diseases, medications, compromised immunity, and pregnancy or breast-feeding that may increase exposure to infants to certain agents, are some of the conditions that may increase the risk of an individual for acquiring a LAI. Consultation with an occupational physician knowledgeable in infectious diseases is advisable in these circumstances.

Fourth, evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment. The protection of laboratory workers, other persons associated with the laboratory, and the public will depend ultimately on the laboratory workers themselves. In conducting a risk assessment, the laboratory director or principal investigator should ensure that laboratory workers have acquired the technical proficiency in the use of microbiological practices and safety equipment required for the safe handling of the agent, and have developed good habits that sustain excellence in the performance of those practices. An evaluation of a person's training, experience in handling infectious agents, proficiency in the use of sterile techniques and BSCs, ability to respond to emergencies, and willingness to accept responsibility for protecting one's self and others is important insurance that a laboratory worker is capable of working safely. The laboratory director or principal investigator should also ensure that the necessary safety equipment is available and operating properly. For example, a BSC that is not certified represents a potentially serious hazard to the laboratory worker using it and to others in the laboratory. The director should have all equipment deficiencies corrected before starting work with an agent.

Fifth, review the risk assessment with a biosafety professional, subject matter expert, and the IBC. A review of the risk assessment and selected safeguards by knowledgeable individuals is always beneficial and sometimes required by regulatory or funding agencies, as is the case with the NIH Guidelines. Review of potentially high risk protocols by the local IBC should become standard practice.

Adopting this step voluntarily will promote the use of safe practices in work with hazardous agents in microbiological and biomedical laboratories.

CONCLUSION

Risk assessment is the basis for the safeguards developed by the CDC, the NIH, and the microbiological and biomedical community to protect the health of laboratory workers and the public from the risks associated with the use of hazardous biological agents in laboratories. **Experience shows that these established safe practices, equipment, and facility safeguards work.**

New knowledge and experiences may justify altering these safeguards. Risk assessment, however, must be the basis for recommended change. Assessments conducted by laboratory directors and principal investigators for the use of emergent agents and the conduct of novel experiments will contribute to our understanding of the risks these endeavors may present and the means for their control. Those risk assessments will likely mirror progress in science and technology and serve as the basis for future revisions of BMBL.

Section III

Principles of Biosafety

A fundamental objective of any biosafety program is the containment of potentially harmful biological agents. **The term "containment" is used in describing safe methods, facilities and equipment for managing infectious materials in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.** The use of vaccines may provide an increased level of personal protection. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements.

LABORATORY PRACTICE AND TECHNIQUE

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or potentially infected materials must be aware of potential hazards, and must be trained and proficient in the practices and techniques required for handling such material safely. The director or person in charge of the laboratory is responsible for providing or arranging the appropriate training of personnel. Each laboratory should develop or adopt a biosafety or operations manual that identifies the hazards that will or may be encountered, and that specifies practices and procedures designed to minimize or eliminate exposures to these hazards. **Personnel should be advised of special hazards and should be required to read and follow the required practices and procedures. A scientist, trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling infectious agents must be responsible for the conduct of work with any infectious agents or materials.** This individual should consult with biosafety or other health and safety professionals with regard to risk assessment.

When standard laboratory practices are not sufficient to control the hazards associated with a particular agent or laboratory procedure, additional measures may be needed. The laboratory director is responsible for selecting additional safety practices, which must be in keeping with the hazards associated with the agent or procedure. Laboratory personnel, safety practices, and techniques must be supplemented by appropriate facility design and engineering features, safety equipment, and management practices.

SAFETY EQUIPMENT (PRIMARY BARRIERS AND PERSONAL PROTECTIVE EQUIPMENT)

Safety equipment includes BSCs, enclosed containers, and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The BSC is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures.

Three types of BSCs (Class I, II, III) used in microbiological laboratories are described and illustrated in Appendix A. **Open fronted Class I and Class II BSCs** are primary barriers that offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques. **The Class II biological safety cabinet also provides protection from external contamination of the materials (e.g., cell cultures, microbiological stocks) being manipulated inside the cabinet.**

The gas-tight Class III biological safety cabinet provides the highest attainable level of protection to personnel and the environment. An example of another primary barrier is the safety centrifuge cup, an enclosed container designed to prevent aerosols from being released during centrifugation. To minimize aerosol hazards, containment controls such as BSCs or centrifuge cups must be used when handling infectious agents. Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Personal protective equipment is often used in combination with BSCs and other devices that contain the agents, animals, or materials being handled. In some situations in which it is impractical to work in BSCs, personal protective equipment may form the primary barrier between personnel and the infectious materials. Examples include certain animal studies, animal necropsy, agent production activities, and activities relating to maintenance, service, or support of the laboratory facility.

FACILITY DESIGN AND CONSTRUCTION (SECONDARY BARRIERS)

The design and construction of the facility contributes to the laboratory workers' protection, provides a barrier to protect persons outside the laboratory, and protects persons or animals in the community from infectious agents that may be accidentally released from the laboratory. Laboratory directors are responsible for providing facilities commensurate with the laboratory's function and the recommended biosafety level for the agents being manipulated. The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. **For example, the**

exposure risks for most laboratory work in BSL-1 and BSL-2 facilities will be direct contact with the agents, or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave), and hand washing facilities.
When the risk of infection by exposure to an infectious aerosol is present, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features include specialized ventilation systems to ensure directional air flow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks as laboratory entrances, or separate buildings or modules to isolate the laboratory. Design engineers for laboratories may refer to specific ventilation recommendations as found in the ASHRAE Laboratory Design Guide published by the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE).

BIOSAFETY LEVELS

Four BSLs are described in Section 4, which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed, the documented or suspected routes of transmission of the infectious agents, and the laboratory function or activity. The BSLs described in this manual should be differentiated from Risk Groups, as described in the NIH Guidelines and the World Health Organization Laboratory Biosafety Manual. Risk groups are the result of a classification of microbiological agents based on their association with, and resulting severity of, disease in humans. The risk group of an agent should be one factor, to be considered in association with mode of transmission, procedural protocols, experience of staff, and other factors in determining the BSL in which the work will be conducted.
The recommended biosafety level(s) for the organisms in Section 8 (Agent Summary Statements) represent those conditions under which the agent ordinarily can be safely handled. Of course, not all of the organisms capable of causing disease are included in Section 8 and an institution must be prepared to perform risk assessments for these agents using the best available information. Detailed information regarding the conduct of biological risk assessments can be found in Section 2. The laboratory director is specifically and primarily responsible for assessing the risks and applying the appropriate biosafety levels. The institution's Biological Safety Officer (BSO) and IBC can be of great assistance in performing and reviewing the required risk assessment. At one point in time, under the NIH Guidelines, BSOs were required only when large scale research or production of organisms containing recombinant DNA molecules was performed or when work with recombinant DNA molecules was conducted at BSL-3 or above. IBCs were required only when an institution was performing non-exempt recombinant DNA experiments. Today, however, it is strongly suggested that an institution conducting research or otherwise working with pathogenic agents have a BSO and properly constituted and functioning IBC. The responsibilities of each now extend beyond those described in the NIH Guidelines and depend on the size and complexity of the program. **Generally, work with known agents should be conducted at the biosafety level recommended in Section 8.** When information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors are significantly altered, more (or less) stringent practices may be specified.

Often an increased volume or a high concentration of agent may require additional containment practices.
Biosafety Level 1 practices, safety equipment, and facility design and construction are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans. Bacillus subtilis, Naegleria gruberi, infectious canine hepatitis virus, and exempt organisms under the NIH Guidelines are representative of 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 immunodeficient or immunosuppressed individuals.
Vaccine strains that have undergone multiple in vivo passages should not be considered avirulent simply because they are vaccine strains.
BSL-1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for handwashing.

Biosafety Level 2 practices, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents

that are 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 splashes or aerosols is low. Hepatitis B virus, HIV, the salmonellae, and Toxoplasma spp. are representative of microorganisms assigned to this containment level. **BSL-2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown.** (Laboratory personnel working with human-derived materials should refer to the OSHA Bloodborne Pathogen Standard 2 for specific required precautions).

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or in devices such as a BSC or safety centrifuge cups. **Personal protective equipment should be used as appropriate, such as splash shields, face protection, gowns, and gloves. Secondary barriers such as hand washing sinks and waste decontamination facilities must be available to reduce potential environmental contamination.**

Biosafety Level 3 practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. Mycobacterium tuberculosis, St. Louis encephalitis virus, and Coxiella burnetii are representative of the microorganisms assigned to this level. **Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols.**

Agents requiring Biosafety Level 3 & 4 are not permitted at St. Norbert College

Biosafety Level 3 practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. Mycobacterium tuberculosis, St. Louis encephalitis virus, and Coxiella burnetii are representative of the microorganisms assigned to this level. **Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols.**

At BSL-3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all laboratory manipulations should be performed in a BSC or other enclosed equipment, such as a gas-tight aerosol generation chamber. Secondary barriers for this level include controlled access to the laboratory and ventilation requirements that minimize the release of infectious aerosols from the laboratory.

Biosafety Level 4 practices, safety equipment, and facility design and construction are applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Agents with a close or identical antigenic relationship to BSL-4 agents also should be handled at this level. When sufficient data are obtained, work with these agents may continue at this level or at a lower level.

Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at BSL- 4.

The primary hazards to personnel working with BSL-4 agents are respiratory exposure to infectious aerosols, mucous membrane or broken skin exposure to infectious droplets, and autoinoculation. 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, the community, and the environment. The laboratory worker's complete isolation from aerosolized infectious materials is accomplished primarily by working in a Class III BSC or in a full-body, air-supplied positive-pressure personnel suit. The BSL-4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems to prevent release of viable agents to the environment.

The laboratory director is specifically 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, procedures being conducted and the nature or function of the laboratory may further influence the director in applying these recommendations.

Section IV

Laboratory Biosafety Level Criteria

The essential elements of the four biosafety levels for activities involving infectious microorganisms and laboratory animals are summarized in Table 1 of this section and discussed in Section 2. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community. Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment.

Biosafety Level 1

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. **BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices.** Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

The following standard practices, safety equipment, and facility requirements apply to BSL-1:

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.

2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

a. **Careful management of needles and other sharps are of primary importance.** Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

c. Non disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:

a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number

of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.

10. An effective integrated pest management program is required. See Appendix G.

11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices None required.

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

1. Special containment devices or equipment, such as BSCs, are not generally required.
2. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
3. Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers should:

a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.
b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratories should have doors for access control.
2. Laboratories must have a sink for hand washing.
3. The laboratory should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

5. Laboratories windows that open to the exterior should be fitted with screens.

Biosafety Level 2 Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that

1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures;
2) access to the laboratory is restricted when work is being conducted; and
3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

The following standard and special practices, safety equipment, and facility requirements apply to BSL-2:

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.
Precautions, including those listed below, must always be taken with sharp items. These include: a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required. See Appendix G.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
3. When appropriate, a baseline serum sample should be stored.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.

5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination. a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material. b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

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

1. Properly maintained BSCs (preferably Class II), other appropriate personal protective equipment, or other physical containment devices must be used whenever:

a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.

2. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.

3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.

4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.
2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.
3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
4. Laboratory furniture must be capable of supporting anticipated loads and uses.
Spaces between benches, cabinets, and equipment should be accessible for cleaning.
a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
8. An eyewash station must be readily available.
9. There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.
11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).