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**Foreword**

This manual has been prepared as a guideline for biological research at Mississippi State University. In this manual, we have provided a core set of biosafety practices and procedures for the safe handling of known biohazards and potentially infectious materials. Labs that work with microorganisms, recombinant DNA (rDNA) technologies, lab animals, toxins of biologic origin, venom, human body fluids (blood, urine, tissue, etc.) or bloodborne pathogens are special and often require unique work environments. These labs must be managed to reduce the potential for personnel exposure and environmental release. Waste generated from these activities must also be uniquely managed.

The manual focuses on Biosafety Levels 1 and 2. No work with Biosafety Level 3 or 4 agents may be conducted at the University. We currently do not have the facilities for this type of research.

The Office for Regulatory Compliance (ORC) Biosafety Program and the requirements for MSU researchers are outlined in the manual. The manual will provide information that will protect members of the University, the community and the environment from biohazardous materials and to establish the process for compliance with federal and state guidelines. Registration and training information are provided along with details on work practices, safety equipment and facility design. It is the responsibility of the Principal Investigator (PI) or Supervisor to ensure that his/her laboratory is in compliance. That responsibility includes identification of the risk or hazards associated with their research and the application of the appropriate safety procedures. Please read the section on responsibilities for additional information.

In the past, the University has distributed copies of the Centers for Disease Control/National Institutes of Health *Biosafety in Microbiological and Biomedical Laboratories* (BMBL). The text has served as a functional biosafety manual for the University. This document and other pertinent biosafety training information and training materials are now available on the ORC Web site. Hard copies of the 5th edition of the BMBL are no longer provided by the federal government. Access is through the CDC web site.

We urge you to use the manual as a guide to reach compliance within your laboratory. Consult the sections relevant to your research and apply the appropriate safety procedures. The Biological Safety Officer is available for consultation if you have any questions or concerns with any aspect of the Biosafety Program at the University. If you are unsure of a requirement or biosafety practice, please contact the Biological Safety Office at 662-325-3294 for assistance. We also would appreciate any feedback or comments that you may have with the use of this manual and will incorporate any suggestions in future versions.
1. Regulatory Oversight

What Kinds of Research Require Oversight?

Notification through either an IBC application or Research Review for Biosafety form is required for the following:

1. any research with human, animal or plant pathogens
2. research using recombinant DNA molecules in/between prokaryotic and eukaryotic organisms
3. research with toxins, allergens of biologic origin
4. research with any human or nonhuman primate specimen including blood, other potentially infectious material and/or cell culture lines
5. research with prions
6. research with any other biohazardous material

Definition of a Biohazardous Material: Any biologic agent (parasite, virus, bacterium, fungus, prion, rickettsia, toxin, allergen, venom etc.) that can present a risk or potential risk to the health of humans, animals, plants or the environment.

All forms can be found on the Regulatory Compliance website or can be obtained from the Biosafety Officer (BSO). Each approved research application requires the submission of an annual update form or whenever a change to personnel, location, organism, procedures or animals occurs. Any laboratory or animal facility certified at biosafety level 2 requires an annual inspection. BSL-2 certification is project specific given that personnel, procedures, and the biohazardous material can vary.

IBC Application vs. the Research Review for Biosafety (RRB)

If a Principal Investigator is unsure whether his/her research will require an IBC application with full committee review, a RRB can be submitted for preliminary review. The RRB will be reviewed by the BSO and IBC Chair. If the research warrants an IBC application, the PI will be informed by the BSO. If not, then the RRB will serve as registration with the IBC for exempt work or the PI will be informed that the research does not require any IBC oversight.

Responsibilities

Department Head: The Department Head bears overall responsibility for the implementation and maintenance of safe practices and procedures in the department. The Chairperson, especially in the case of large departments, may share this responsibility with a departmental biological safety contact.

Principal Investigator: The principal investigator has the responsibility and authority for assessing risks, establishing policies and procedures, training personnel and maintaining the facility and equipment. The principal investigator is responsible for:

   a. Performing appropriate risk assessment of research projects. The level of detail should be dependent on the hazard associated with the organism under study, the planned procedures and the suitability of the facility. Each evaluation should be completed before work is undertaken and the project should be reassessed periodically as new data is obtained. The assessment should include an analysis of the risks posed by the particular organism under investigation and of any specific research methods that may affect that risk (e.g., procedures requiring highly concentrated amounts of virus or inoculation of laboratory animals). No human, plant or animal pathogen should be studied without prior written approval of the Institutional Biological Safety Committee. The procedures for handling unclassified agents must also be reviewed by the Institutional Biological Safety Committee and the Institutional Animal Care and Use Committee (IACUC) if work with animals is anticipated. The agents must be registered and information about these agents must be provided to the Biosafety Office.
b. The application of appropriate safety practices and procedures within their laboratories and instructing students and staff of potential hazards.

c. Approving research personnel to work in the laboratory and documenting that personnel are competent to conduct the work.

d. Developing policies governing the operation of the laboratory and implementing proper procedures.

e. Maintaining a liaison with the Biological Safety Office.

f. Submitting an IBC application for research work involving non-exempt recombinant DNA to the IBC. The principal investigator must complete the application. The application must have details of the nature of the proposed experiments and an assessment of the levels of physical and biological containment required for them as established by the NIH Guidelines.

Research Personnel: Research personnel are responsible for:

a. Completion of all requirements for approval to work in the laboratory and ensure that all work is conducted in compliance with MSU, NIH, CDC, OSHA and other applicable guidelines.

b. Learn the operating procedures for the laboratory, the potential hazards of the material in use and emergency procedures. Help maintain the facility in good working condition.

c. Report to the Principal Investigator any medical restrictions, reportable illnesses, and any event that may be an exposure or result in the creation of a potential hazard. Report all irregular conditions.

d. If inexperienced in handling human pathogens or tissue cultures, receive training and demonstrate proficiency in standard microbiological practices for the Principal Investigator.

e. Complete any medical surveillance requirements.

f. Perform assigned responsibilities. The operation of the facility is the responsibility of the users; therefore a number of tasks should be assigned. These tasks are as follows:

1. Training
2. Autoclaves and waste
3. Freezers
4. Cleaning
5. Vacuum trap and filter maintenance
6. Maintenance of supplies, including personnel protective equipment
7. Security of infectious agents

The Biological Safety Officer (BSO) and the Institutional Biosafety Committee (IBC)

It is essential that the University has a comprehensive biosafety policy, a biosafety manual, and supporting programs for their implementation. The responsibility for this rests with the Vice President of Research and Economic Development (VP-ORED), who may delegate certain duties to a biosafety officer or other appropriate personnel.

Laboratory safety is also the responsibility of all supervisors and laboratory employees, and individual workers are responsible for their own safety and that of their colleagues. Employees are expected to perform their work safely and should report any unsafe acts, conditions or incidents to their supervisor.

Biological Safety Officer

The biosafety officer ensures that biosafety policies and programs recommended by the IBC are followed consistently throughout the laboratories. The biosafety officer executes these duties on behalf of the Vice President for Research and Economic Development. The biosafety officer applies relevant national and international rules, regulations and guidelines, and assists the laboratory in developing standard operating procedures.

The activities of the biosafety officer include the following:

1. Biosafety, biosecurity and technical compliance consultations.
2. Evaluation and inspection of laboratory facilities for work with biohazardous agents.
3. Discussions of violations of biosafety protocols or procedures with the appropriate persons.
4. Verification that all staff has received appropriate biosafety training.
5. Provision of continuing education in biosafety.
6. Investigation of incidents involving the possible escape of potentially infectious or toxic material, and reporting of findings and recommendations to the VP-ORED and IBC.
7. Coordination with medical staff regarding possible laboratory-acquired infections.
8. Ensuring appropriate decontamination following spills or other incidents involving infectious material(s).
9. Ensuring proper waste management.
10. Providing appropriate information on decontamination of any apparatus prior to repair or servicing.
11. Maintaining awareness of community attitudes regarding health and environmental considerations.
12. Establishment of appropriate procedures for import/export of pathogenic material to/from the University, according to national regulations.
13. Reviewing the biosafety aspects of all plans, protocols and operating procedures for research work involving infectious agents and recombinant DNA prior to the implementation of these activities.
14. Institution of a system to deal with emergencies

**Institutional Biosafety Committee**

The purpose of the Institutional Biosafety Committee is to ensure protection of workers who generate, process, and dispose of potentially hazardous biological materials at Mississippi State University, as well as others who may become exposed to biological hazards within the university environment. This oversight is also intended to ensure the welfare and safety of the surrounding community and environment.

The IBC is a standing committee that reports to the Vice President for Research and Economic Development. It has the responsibility under the *NIH Guidelines for Research Involving Recombinant DNA Molecules* and on behalf of the University for formulating and recommending biosafety policies and establishing procedures, as well as reviewing research involving matters relating to biosafety for compliance and approving projects judged to be compliant. The University has further charged the IBC with responsibility for (a) oversight and establishment of procedures and policies regarding disposal of non-radioactive biohazardous wastes, (b) reviewing and advising with regard to situations which represent potential biological hazards, and (c) reviewing research personnel, facilities, procedures, and proposals in the area of recombinant DNA technology.

The IBC must file an annual report with NIH/OBA that includes an updated committee roster indicating the role of each committee member (e.g., chair person, contact person, non-institutional members, special experts as relevant, etc.), and biosketches (curricula vitae, résumé) for each member on the committee. The term of service for each member is 3 years.

The IBC meets monthly and encourages the participation of PIs with proposals being considered at that meeting. Support staff and members of the public are also welcome to attend. Minutes of these meetings are kept and made available for public inspection in accordance with MSU policy 06.04 Public Records Request Procedures.

**Required Training**

The Biosafety Office offers bloodborne pathogen training on a regular basis. The Occupational Safety and Health Administration (OSHA) created the Occupational Exposure to Bloodborne Pathogens Standard, 29 CFR Part 1910.1030 (Bloodborne Pathogens Standard) to minimize or eliminate exposure to infectious agents that may be present in human blood, tissues or certain body fluids (bloodborne pathogens.) The Bloodborne Pathogens Standard applies to all employers having
employees that are “occupationally exposed” to human blood or other potentially infectious materials. An employee is considered occupationally exposed if there is “reasonably anticipated skin, eye, mucous membrane, or parenteral contact with human blood or other potentially infectious materials in the performance of an employee’s duties.” Other potentially infectious materials include:

- Human cell or tissue cultures
- Organ cultures
- Any unfixed tissue or organ
- HIV- or HBV- containing culture media or skin, from a human being (living or dead)
- Human body fluids, except urine, saliva or tears unless visibly contaminated with blood
- Blood, organs or other tissues from experimental animals infected with HIV, HBV or other bloodborne pathogens

An individual is also considered occupationally exposed even if they do not have direct contact with blood or other potentially infectious material, but uses equipment that is used to process or store blood, other potentially infectious materials or bloodborne pathogens.

All occupationally exposed employees are required by OSHA to attend a Bloodborne Pathogens training session prior to beginning work and annually thereafter. There are additional requirements for research laboratories and production facilities engaged in the culture, production, concentration and manipulation of HIV and HBV.

OSHA has determined that occupational exposure to human blood, tissues and body fluids poses a significant health risk because these may contain bloodborne pathogens such as:

- Human Immunodeficiency virus (HIV)
- Babesia species
- Colorado Tick Fever
- Borrelia species viruses
- Hepatitis B virus (HBV)
- Brucella species
- Arboviruses
- Hepatitis C and D virus
- Leptospira species
- Spirillum minus
- Francisella species
- Creutzfeldt-Jakob prion
- Plasmodium species
- Streptobacillus
- Human T-lymphotropic
- Treponema species moniliformis Virus Type I
- Hemorrhagic Fever viruses
2. General Principles

Introduction
Throughout this manual, references are made to the relative hazards of infective microorganisms by risk group (Risk Groups 1, 2, 3 and 4). This risk group classification is to be used for laboratory work only. Organisms are assigned to risk groups based on the following criteria:

1. Pathogenicity of the organism.
2. Mode of transmission and host range of the organism. These may be influenced by existing levels of immunity in the local population, density and movement of the host population, presence of appropriate vectors, and standards of environmental hygiene.
3. Local availability of effective preventive measures. These may include: prophylaxis by immunization or administration of antisera (passive immunization); sanitary measures, e.g. food and water hygiene; control of animal reservoirs or arthropod vectors.
4. Local availability of effective treatment. This includes passive immunization, post-exposure vaccination and use of antimicrobials, anti-virals and chemotherapeutic agents, and should take into consideration the possibility of the emergence of drug-resistant strains.

Table 1 contains the risk groups as described by the NIH and WHO.

Table 1. Classification of infective microorganisms by risk group

<table>
<thead>
<tr>
<th>RISK GROUP CLASSIFICATION</th>
<th>NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES 2002</th>
<th>WORLD HEALTH ORGANIZATION LABORATORY BIOSAFETY MANUAL 3rd EDITION 2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk Group 1</td>
<td>Agents that are not associated with disease in healthy adult humans.</td>
<td>(No or low individual and community risk) A microorganism that is unlikely to cause human or animal disease.</td>
</tr>
<tr>
<td>Risk Group 2</td>
<td>Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.</td>
<td>(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.</td>
</tr>
<tr>
<td>Risk Group 3</td>
<td>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).</td>
<td>(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.</td>
</tr>
<tr>
<td>Risk Group 4</td>
<td>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).</td>
<td>(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.</td>
</tr>
</tbody>
</table>
Laboratory facilities are designated as basic – Biosafety Level 1, basic – Biosafety Level 2, containment – Biosafety Level 3, and maximum containment – Biosafety Level 4. Biosafety level designations are based on a composite of the design features, construction, containment facilities, equipment, practices and operational procedures required for working with agents from the various risk groups. Table 2 relates but does not “equate” risk groups to the biosafety level of laboratories designed to work with organisms in each risk group.

Table 2. Relation of risk group to Biosafety levels, practices and equipment

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>Biosafety Level</th>
<th>Laboratory Type</th>
<th>Laboratory Practices</th>
<th>Safety Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Basic</td>
<td>Basic teaching</td>
<td>Standard Microbiological Practices (SMP)</td>
<td>None; open bench work</td>
</tr>
<tr>
<td>Biosafety Level 1</td>
<td>Research</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Basic</td>
<td>Diagnostic</td>
<td>SMP plus protective clothing, biohazard sign</td>
<td>Open bench plus BSC for potential aerosols</td>
</tr>
<tr>
<td>Biosafety Level 2</td>
<td>Research</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Containment</td>
<td>Special</td>
<td>Level 2 plus special clothing, controlled access, directional airflow</td>
<td>BSC and/or other primary devices for all activities</td>
</tr>
<tr>
<td>Biosafety Level 3</td>
<td>Diagnostic</td>
<td>Research</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Maximum</td>
<td>Dangerous</td>
<td>Level 3 plus airlock entry, shower exit, special waste disposal</td>
<td>Class III BSC or positive pressure suits in conjunction with Class II BSCs, double-ended autoclave, filtered air</td>
</tr>
<tr>
<td>Containment</td>
<td>Pathogens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biosafety Level 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The assignment of an agent to a biosafety level for laboratory work must be based on a risk assessment. Such an assessment will take the risk group as well as other factors into consideration in establishing the appropriate biosafety level. For example, an agent that is assigned to Risk Group 2 may generally require Biosafety Level 2 facilities, equipment, practices and procedures for safe conduct of work. However, if particular experiments require the generation of high-concentration aerosols, then Biosafety Level 3 may be more appropriate to provide the necessary degree of safety, since it ensures superior containment of aerosols in the laboratory workplace. The Biosafety level assigned for the specific work to be done is therefore driven by professional judgment based on a risk assessment (agent and procedures), rather than by automatic assignment of a laboratory biosafety level according to the particular risk group designation of the pathogenic agent to be used.
Table 3 summarizes the minimum facility requirements at the four biosafety levels.

<table>
<thead>
<tr>
<th>BIOSAFETY LEVELS</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isolation</strong>a of laboratory</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Room sealable for decontamination</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Ventilation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Inward air flow</td>
<td>No</td>
<td>Desirable</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>- Controlled ventilating system</td>
<td>No</td>
<td>Desirable</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>- HEPA-filtered air exhaust</td>
<td>No</td>
<td>No</td>
<td>Yes/Nob</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Double-door entry</strong></td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Airlock</strong></td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Airlock with shower</strong></td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Anteroom</strong></td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>----</td>
</tr>
<tr>
<td><strong>Anteroom with shower</strong></td>
<td>No</td>
<td>No</td>
<td>Yes/Noc</td>
<td>No</td>
</tr>
<tr>
<td><strong>Effluent treatment</strong></td>
<td>No</td>
<td>No</td>
<td>Yes/No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Autoclave</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- on site</td>
<td>No</td>
<td>Desirable</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>- in lab room</td>
<td>No</td>
<td>No</td>
<td>Desirable</td>
<td>Yes</td>
</tr>
<tr>
<td>- doubled-doored</td>
<td>No</td>
<td>No</td>
<td>Desirable</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Biological safety cabinets</strong></td>
<td>No</td>
<td>Desirable</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Personnel safety monitoring</strong>d</td>
<td>No</td>
<td>No</td>
<td>Desirable</td>
<td>Yes</td>
</tr>
</tbody>
</table>

a Environmental and functional isolation from general traffic
b dependent upon location of exhaust
c dependent upon agent(s) used in the laboratory
d Examples: window, closed-circuit TV, two-way communication

Thus, the assignment of a biosafety level takes into consideration the organism (or biohazardous material) used, the facilities available, the equipment and practices and procedures required to conduct work safely in the laboratory.

**Plant and Animal Pathogens: Other Hazards of Biologic Origin**

The federal government was shortsighted in designing guidelines for work with human pathogens only. Due to the potentially huge economic impact that a animal or plant epidemic could have on the U.S. economy, the scope of national biosafety standards now include the handling of animal and plant pathogens. The World Health Organization defines Risk Group 2 organisms as those that can cause disease in humans or animals but is unlikely to be a hazard to lab workers, the community, livestock or the environment. By consensus, the same definition applies to plant pathogens.

Other biologic hazards are also being incorporated into the risk assessment process such as allergens, toxins, and venoms from vertebrates and invertebrates.

**Routes of Exposure**

In order for biological agents to cause disease, they must first enter or invade the body in sufficient concentrations. Routes of entry include oral, respiratory, parenteral, mucous membrane and animal contacts (bites, scratches). Once inside the body, biohazards must meet other requirements to cause disease; they must colonize and establish in body cells, tissues and/or organs, overcome the body's natural defense mechanisms and mutate or adapt to body changes.

Other factors contribute to an individual's susceptibility to the disease process. These include age, immunological state, occupation, physical and geographic environment and predisposing conditions (such as alcoholism and other drug abuse, pregnancy and diseases such as diabetes).
It is difficult to determine a minimum infectious or toxic dose when discussing biohazards. The same dose of a pathogen may produce no disease symptoms in one individual but may cause serious or even fatal disease in another. There are microorganisms for which it is thought one organism entering the body is sufficient to invade and promote the disease process; the bacteria that causes tuberculosis is an example. For many pathogens, 10 to 100 or more organisms must enter the body to cause infection leading to disease.
Mucous Membranes:
Exposures to mucous membranes of the eyes, nose and mouth through splashes or splatters.

Ingestion:
Mouth pipetting, eating, drinking, smoking in the lab.

Inhalation:
Breathing in respirable sized aerosols (<5μm), centrifuge leaks, spills, pipetting, etc.

Percutaneous:
Through intact or non-intact skin via needlestick, puncture with contaminated sharp object, animal scratch or bite, through wounds abrasions, or eczema.

Contact (indirect transmission):
Via mucous membranes or non-intact skin from hands that have been in contact with a contaminated surface (i.e. benches, phones, computers, equipment handles) or by failure to wash hands after working.
Protection for the Routes of Exposure

<table>
<thead>
<tr>
<th>Route of Exposure</th>
<th>Activity</th>
<th>Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucous membranes</td>
<td>Splash, splatter generation</td>
<td>Face protection using masks and goggles, face shields, biological safety cabinet, protective shields, standard microbiological practices</td>
</tr>
<tr>
<td>Ingestion</td>
<td>Mouth pipetting, eating, drinking, smoking</td>
<td>Standard microbiological practices, mechanical pipettors</td>
</tr>
<tr>
<td>Inhalation</td>
<td>Aerosol generation from centrifuge leaks, spills, pipetting etc.</td>
<td>Biological safety cabinet, sealed rotors or canisters for centrifuges, safety containment equipment, HEPA-filtered respirator, standard microbiological practices</td>
</tr>
<tr>
<td>Percutaneous</td>
<td>Puncture of skin with contaminated sharp (needle, teeth, glass) or exposure of non-intact skin (wounds, eczema etc.)</td>
<td>Use plastic instead of glass, extreme precaution with sharps, use animal restraints, bite resistant gloves, sleeve covers, water-proof bandages, standard microbiological practices</td>
</tr>
<tr>
<td>Contact</td>
<td>Unwashed hands touching eyes, mucous membranes, non-intact skin</td>
<td>Decontaminate work surfaces, wash hands, avoid touching face with hands, do not apply cosmetics in laboratory</td>
</tr>
</tbody>
</table>

Standard microbiological practices are discussed in detail in Chapter 4.
3. Risk Assessment

The backbone of the practice of biosafety is risk assessment. While there are many tools available to assist in the assessment of risk for a given procedure or experiment, the most important component is professional judgment. Risk assessments should be performed by the individuals most familiar with the specific characteristics of the organisms being considered for use, the equipment and procedures to be employed, animal models that may be used, and the containment equipment and facilities available. The laboratory director or principal investigator is responsible for ensuring that adequate and timely risk assessments are performed, and for working closely with the Institutional Biosafety Committee (IBC) and biosafety personnel to ensure that appropriate equipment and facilities are available to support the work being considered. Once performed, risk assessments should be reviewed routinely and revised when necessary, taking into consideration the acquisition of new data having a bearing on the degree of risk and other relevant new information from the scientific literature. One of the most helpful tools available for performing a microbiological risk assessment is the listing of risk groups for microbiological agents. However, simple reference to the risk grouping for a particular agent is insufficient in the conduct of a risk assessment. Other factors that should be considered, as appropriate, include:

1. Pathogenicity of the agent and infectious dose
2. Potential outcome of exposure
3. Natural route of infection
4. Other routes of infection, resulting from laboratory manipulations (parenteral, airborne, ingestion)
5. Stability of the agent in the environment
6. Concentration of the agent and volume of concentrated material to be manipulated
7. Presence of a suitable host (human or animal)
8. Information available from animal studies and reports of laboratory-acquired infections or clinical reports
9. Laboratory activity planned (sonication, aerosolization, centrifugation, etc.)
10. Any genetic manipulation of the organism that may extend the host range of the agent or alter the agent’s sensitivity to known, effective treatment
11. Local availability of effective prophylaxis or therapeutic interventions.

On the basis of the information ascertained during the risk assessment, a Biosafety level can be assigned to the planned work, appropriate personal protective equipment selected, and standard operating procedures (SOPs) incorporating other safety interventions developed to ensure the safest possible conduct of the work.

At Mississippi State University, BSL-2 and ABSL-2 facilities, safety equipment and practices as defined by the BMBL are appropriate for the types of organisms studied here. These include agents of moderate potential hazard to animals or agriculture that are generally endemic, cause illness of varying degree and are typically treatable or preventable. Most research labs that work with food-borne pathogens and domestic diseases fall into this category.

Specimens for which there is limited information
The risk assessment procedure described above works well when there is adequate information available. However, there are situations when the information is insufficient to perform an appropriate risk assessment, for example, with clinical specimens or epidemiological samples collected in the field. In these cases, it is prudent to take a cautious approach to specimen manipulation.

1. Standard precautions should always be followed, and barrier protections applied (gloves, gowns, eye protection), whenever samples are obtained from patients or unknown animals.
2. Basic containment – Biosafety Level 2 practices and procedures should be the minimum requirement for handling specimens.
3. Transport of specimens should follow national and/or international rules and regulations.
Some information may be available to assist in determining the risk of handling these specimens:

1. Medical data
2. Epidemiological data (morbidity and mortality data, suspected route of transmission, other outbreak investigation data)
3. Information on the geographical origin of the specimen.

In the case of outbreaks of disease of unknown etiology, appropriate ad hoc guidelines may be generated and posted by national competent authorities and/or WHO on the World Wide Web (as was the case during the 2003 emergence of the severe acute respiratory syndrome (SARS)) to indicate how specimens should be consigned for shipment and the biosafety level at which they should be analyzed.

Risk assessment and genetically modified microorganisms
A detailed discussion of risk assessment and genetically modified organisms (GMOs) is provided in Chapter 15.

Risk assessment for agricultural research
Risk assessment for agriculture (plant and animal) infectious disease research has different criteria than that for human and/or zoonotic infectious disease research. The rationale for risk assessment in agriculture has to be based not on the public health standards but on the potential economic impact from diseased plants/animals and the trade implications of disease.

Animals
Some of the factors in the assessment of animal pathogens that should be considered include:

1. Is the agent indigenous or foreign to the region?
2. What is known about the morbidity and mortality caused by the agent?
3. Are there available prophylaxes, treatments or vaccines available?
4. What are the shedding patterns of the agent in relevant species?
5. Are there active control or eradication programs for the disease?
6. What is known about the environmental stability of the agent?
7. What will be the quantity and concentration of the agent?
8. How will the agent be used in animals?
9. What is the host range of the agent, and is there ongoing surveillance testing?
10. What are the biological factors of the agent?
11. Who will be working with the agent – level of experience and knowledge?
12. What practices/procedures will be used?
13. What is the experimental design and what protocols will be used?
14. What SOPs are available?
15. Is the facility design and management acceptable?

Risk management strategies for work with agricultural pathogens must focus on biocontainment and environmental protection in addition to worker protection. For most agriculture pathogens, the U.S. CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* can be used to establish standards for an appropriate level of biocontainment. However, infectious disease research with certain high consequence animal pathogens and loose-housed large animals requires additional enhancements since the facility barriers (which are usually secondary barriers) now become primary barriers. The U.S. standard developed by the USDA calls this BSL-3 Ag which utilizes the containment features of a standard ABSL-3 as defined by the BMBL and adds most of the enhancements assigned to ABSL-4.

Plants
Some of the factors in the assessment of plants/plant pathogens or pests that should be considered include:
For Plant Pathogens/Pests (bacteria, viruses, fungi, arthropods, nematodes, etc.)

What is the nature of the agent?

1. Is the organism pathogenic for plants, animals, humans and how virulent (aggressive) is it?
2. Is it indigenous or exotic to the United States/ Mississippi?
3. What is the route of transmission? air-borne, water-borne, soil-borne, direct contact etc.
4. What is the host range? a single species of plant or many
5. Are local hosts present?
6. How communicable is it? How easily is it transmitted from plant to plant?
7. What is the infectious dose?
8. How stable is it in the environment?
9. What is the capacity to control or eradicate the organism if it escapes?
10. What is the history of the organism in other new environments?
11. Is there risk information already available for the organism?
12. What are the location and proximity of suitable hosts and the time of year of the proposed work?
13. What is the potential rate of local and long distance spread?
14. Are there any vectors present (e.g. arthropods, fungi, nematodes)?
15. Are there vectors present in or near the containment facility?
16. How persistent is the organism in the environment and what is its potential for overwintering?
17. What are the environmental requirements for establishment and spread?
18. Will it be cultured? If so, how large of a volume and at what concentration?
19. Will it be restricted to the lab or disseminated in a greenhouse or field plot?
20. What measures will be used to avoid dissemination of the organism (recombinant or wild type) beyond the laboratory or greenhouse?
21. What is the economic and environmental significance of potential pest organisms and their host plants?
22. Are there any biosecurity related risks (e.g. potential for theft and misuse)?

For a Plant:

What is the nature of the plant?

1. Is it indigenous or exotic?
2. Is it a noxious weed?
3. Is it parasitic on other plants?
4. What impact will an accidental release have on agricultural production, forestry or natural environments?
5. Can the plant interbreed with local noxious weeds?

For Transgenic Plants/Recombinant Organisms:

A transgenic plant/organism contains a gene or genes which have been artificially inserted instead of through natural acquisition such as pollination in the case of plants or conjugation in the case of bacteria. The inserted gene sequence (known as the transgene) may come from another unrelated plant, or from a completely different species: transgenic Bt corn, for example, which produces its own insecticide, contains a gene from a bacterium. Plants containing transgenes are often called genetically modified or GM crops, although in reality all crops have been genetically modified from their original wild state by domestication, selection and controlled breeding over long periods of time. On this web site we will use the term transgenic to describe a plant which has transgenes inserted.

1. Describe the genes that will be introduced into the plant/microorganism. What is the source and nature of the DNA?
2. Is it a complete genome or a fragment of DNA?
3. What is the vector that will be used to transport the DNA?
4. What is the nature of the expressed protein? Is it a vertebrate toxin or allergen? Is it toxic to other organisms in the local environment?
5. How will the transgenic plants/recombinant microorganism be contained?
6. Will the resulting altered plant be considered a noxious weed?
7. Will the altered plant be able to interbreed with noxious weeds?
8. What impact will an accidental release have on agricultural production, forestry or natural environments?
9. What is the local environment? What is the nature and importance of nearby crops? Are sexually compatible wild or weedy species in the vicinity?
10. What are the experimental procedures for transferring: to and from greenhouse etc.
11. What are the recipient organism and its mode of dissemination?

**Containment**

Plants and plant pests rarely infect or infest healthy humans and therefore pose little direct risk to lab personnel. Some, however, can pose a significant threat to agricultural production, forests or natural ecosystems. As a result it is important that personnel working with plants and plant pests or facilities housing these organisms take steps to prevent the accidental escape of potentially damaging plants or plant pests into the environment. The containment requirements for a particular plant or plant pest are often project-specific and are determined after assessing the risk factors associated with the biology of the plant/plant pest and the impact an escape might have. The risk model for human and plant pathogens in Table 1 demonstrates the general principle of requiring increased levels of containment with increasing risk of escape/establishment and the impacts on the economy, environment, agriculture, forests and trade in the event of an escape.

The U.S. government and other entities have published sets of guidelines for containment of plants and plant pests. The most common guidelines cover the following:

1. noxious weeds and parasitic plants
2. nonindigenous, phytophagous arthropods and their parasitoids and predators
3. plant pathogenic nematodes
4. bacterial plant pathogens
5. fungal plant pathogens
6. viral plant pathogens and their vectors

Two documents specifically address work with plants: Appendix P of the *NIH Guidelines for Research Involving Recombinant DNA Molecules* and *A Practical Guide to Containment: Greenhouse Research with Transgenic Plants and Microbes*. *A Practical Guide to Containment* can be found at the Information Systems for Biotechnology website hosted by Virginia Tech University.
4. Basic Laboratories
Biosafety Levels 1 and 2

The guidelines for basic laboratories – Biosafety Levels 1 and 2 presented here are comprehensive and detailed, as they are fundamental to laboratories of all Biosafety levels. Mississippi State University has no Biosafety Level 3 or 4 labs and hence will not be discussed in this manual.

Standard Microbiological Practices
The following standards are a listing of the most essential laboratory practices and procedures that are basic to standard microbiological practices (SMP). This standard may be used to develop written practices and procedures for safe laboratory operations in individual laboratories.

Each laboratory should adopt a safety or operations manual that identifies known and potential hazards, and specifies practices and procedures to eliminate or minimize such hazards. SMP are fundamental to laboratory safety. Specialized laboratory equipment is a supplement to but can never replace appropriate procedures. The most important concepts are listed below.

Access

1. The international biohazard warning symbol and sign (Figure 1) must be displayed on the doors of the rooms where microorganisms of Risk Group 2 or higher risk groups are handled.

Figure 1. Biohazard warning sign for laboratory doors

2. Only authorized persons should be allowed to enter the laboratory working areas.
3. Laboratory doors should be kept closed.
4. Children should not be authorized or allowed to enter laboratory working areas except under special circumstances.
5. Access to animal houses should be specially authorized.
6. No animals/plants should be admitted other than those involved in the work of the laboratory.
**Personal protection**

1. Laboratory coveralls, gowns or coats must be worn at all times for work in the laboratory.
2. Appropriate gloves must be worn for all procedures that may involve direct or accidental contact with blood, body fluids and other potentially infectious materials or infected animals. After use, gloves should be removed aseptically and hands must then be washed.
3. Personnel must wash their hands after handling infectious materials and animals, and before they leave the laboratory working areas.
4. Safety glasses, face shields (visors) or other protective devices must be worn when it is necessary to protect the eyes and face from splashes, impacting objects and sources of artificial ultraviolet radiation.
5. It is prohibited to wear protective laboratory clothing outside the laboratory, e.g. in cafeterias, coffee rooms, offices, libraries, staff rooms and toilets.
6. Open-toed footwear must not be worn in laboratories.
7. Eating, drinking, smoking, applying cosmetics and handling contact lenses is prohibited in the laboratory working areas.
8. Storing human foods or drinks anywhere in the laboratory working areas is prohibited.
9. Protective laboratory clothing that has been used in the laboratory must not be stored in the same lockers or cupboards as street clothing.

**Procedures**

1. Pipetting by mouth must be strictly forbidden.
2. Items such as pens must not be placed in the mouth. Labels must not be licked.
3. All technical procedures should be performed in a way that minimizes the formation of aerosols and droplets.
4. The use of hypodermic needles and syringes should be limited. They must not be used as substitutes for pipetting devices.
5. All spills, accidents and overt or potential exposures to infectious materials must be reported to the laboratory supervisor. A written record of such accidents and incidents should be maintained.
6. A written procedure for the clean-up of all spills must be developed and followed.
7. Contaminated liquids must be decontaminated (chemically or physically) before disposal unless picked up by Regulatory Compliance for further processing.
8. Written documents that are expected to be removed from the laboratory need to be protected from contamination while in the laboratory.

**Laboratory working areas**

1. The laboratory should be kept neat, clean and free of materials that are not pertinent to the work.
2. Work surfaces must be decontaminated after any spill of potentially dangerous material and at the end of the working day.
3. All contaminated materials, specimens and cultures must be decontaminated before disposal or cleaning for reuse.
4. Packing and transportation must follow applicable national and/or international regulations.
5. When windows can be opened, they should be fitted with insect-proof screens.
6. Biohazard labels should be affixed to any item where a biohazardous agent may be found such as refrigerators, freezers, centrifuges, incubators, waste cans and tubes.

**Biosafety management**

1. It is the responsibility of the laboratory director (the person who has immediate responsibility for the laboratory) to ensure the development and adoption of a biosafety management plan and a safety or operations manual.
2. The laboratory supervisor (reporting to the laboratory director) should ensure that regular training in laboratory safety is provided.
3. Personnel should be advised of special hazards, and required to read the safety or operations manual and follow standard practices and procedures. The laboratory supervisor should make sure that all...
personnel understand these. A copy of the safety or operations manual should be available in the laboratory.
4. There should be an insect and rodent control program.
5. Appropriate medical evaluation, surveillance and treatment should be provided for all personnel in case of need, and adequate medical records should be maintained.

**Laboratory design and facilities**

In designing a laboratory and assigning certain types of work to it, special attention should be paid to conditions that are known to pose safety problems. These include:

1. Formation of aerosols
2. Work with large volumes and/or high concentrations of microorganisms
3. Overcrowding and too much equipment
4. Infestation with rodents and arthropods
5. Unauthorized entrance
6. Workflow: use of specific samples and reagents.

Examples of laboratory designs for Biosafety Levels 1 and 2 are shown in Figures 2 and 3, respectively.

**Design features**

1. Ample space must be provided for the safe conduct of laboratory work and for cleaning and maintenance.
2. Walls, ceilings and floors should be smooth, easy to clean, impermeable to liquids and resistant to the chemicals and disinfectants normally used in the laboratory. Floors should be slip-resistant.
3. Bench tops should be impervious to water and resistant to disinfectants, acids, alkalis, organic solvents and moderate heat.
4. Illumination should be adequate for all activities. Undesirable reflections and glare should be avoided.
5. Laboratory furniture should be sturdy. Open spaces between and under benches, cabinets and equipment should be accessible for cleaning.
6. Storage space must be adequate to hold supplies for immediate use and thus prevent clutter on bench tops and in aisles. Additional long-term storage space, conveniently located outside the laboratory working areas, should also be provided.
7. Space and facilities should be provided for the safe handling and storage of solvents, radioactive materials, and compressed and liquefied gases.
8. Facilities for storing outer garments and personal items should be provided outside the laboratory working areas.
9. Facilities for eating and drinking and for rest should be provided outside the laboratory working areas.
10. Hand-washing sinks should be provided in each laboratory room, preferably near the exit door.
11. Doors should have vision panels, appropriate fire ratings, and preferably be self-closing.
12. At Biosafety Level 2, an autoclave or other means of decontamination should be available in appropriate proximity to the laboratory.
13. Safety systems should cover fire, electrical emergencies, emergency shower and eye wash facilities.
14. First-aid areas or rooms suitably equipped and readily accessible should be available.
15. In the planning of new facilities, consideration should be given to the provision of mechanical ventilation systems that provide an inward flow of air without recirculation. If there is no mechanical ventilation, windows should be able to be opened and should be fitted with insect-proof screens.
16. A dependable supply of good quality water is essential. There should be no cross-connections between sources of laboratory and drinking-water supplies. An anti-backflow device should be fitted to protect the public water system.
17. There should be a reliable and adequate electricity supply and emergency lighting to permit safe exit. A stand-by generator is desirable for the support of essential equipment, such as incubators, biological safety cabinets, freezers, etc., and for the ventilation of animal cages.
18. There should be a reliable and adequate supply of gas. Good maintenance of the installation is mandatory.
19. Laboratories and animal houses are occasionally the targets of vandals. Physical and fire security must be considered. Strong doors, screened windows and restricted issue of keys are required. Other measures should be considered and applied, as appropriate, to augment security.

**Laboratory equipment**
Together with good procedures and practices, the use of safety equipment will help to reduce risks when dealing with biosafety hazards. This section deals with basic principles related to equipment suitable for laboratories of all biosafety levels.

The laboratory director should, after consultation with the biosafety officer and IBC, ensure that adequate equipment is provided and that it is used properly. Equipment should be selected to take account of certain general principles, i.e. it should be:

1. Designed to prevent or limit contact between the operator and the infectious material.
2. Constructed of materials that are impermeable to liquids, resistant to corrosion and meet structural requirements.
3. Fabricated to be free of burrs, sharp edges and unguarded moving parts.
4. Designed, constructed and installed to facilitate simple operation and provide for ease of maintenance, cleaning, decontamination and certification testing; glassware and other breakable materials should be avoided, whenever possible.

Equipment sent out for repair or disposal must be decontaminated before leaving the laboratory. Affix a tag to the piece indicating when it was decontaminated, what disinfectant was used and who performed the decontamination. Thorough decontamination of inaccessible areas on highly technical or sensitive equipment may not be possible.
Figure 2. A typical Biosafety Level 1 laboratory

CUH2A, Princeton, NJ, USA
Figure 3. **A typical Biosafety Level 2 laboratory**

Procedures likely to generate aerosols are performed within a biological safety cabinet. Doors are kept closed and are posted with appropriate hazard signs. Potentially contaminated wastes are separated from the general waste stream.

CUH2A, Princeton, NJ, USA
Essential Biosafety Equipment

1. Pipetting aids – to avoid mouth pipetting. Many different designs are available.
2. Biological safety cabinets, to be used whenever:
   — infectious materials are handled; such materials may be centrifuged in the open laboratory if sealed centrifuge safety cups are used and if they are loaded and unloaded in a biological safety cabinet
   — there is an increased risk of airborne infection
   — procedures with a high potential for producing aerosols are used; these may include centrifugation, grinding, blending, vigorous shaking or mixing, sonic disruption, opening of containers of infectious materials whose internal pressure may be different from the ambient pressure, intranasal inoculation of animals, and harvesting of infectious tissues from animals and eggs.
3. Plastic disposable transfer loops. Alternatively, electric transfer loop incinerators may be used inside the biological safety cabinet to reduce aerosol production.
4. Screw-capped tubes and bottles.
5. Autoclaves or other appropriate means to decontaminate infectious materials.
6. Plastic disposable Pasteur pipettes, whenever available, to avoid glass.
7. Equipment such as autoclaves and biological safety cabinets must be validated with appropriate methods before use. Recertification should take place at regular intervals, according to the manufacturer’s instructions.

Health and Medical Surveillance

The employing authority, through the laboratory director, is responsible for ensuring that there is adequate surveillance of the health of laboratory personnel. The objective of such surveillance is to monitor for occupationally acquired diseases. Appropriate activities to achieve these objectives are:

1. Provision of active or passive immunization where indicated.
3. Exclusion of highly susceptible individuals (e.g. pregnant women or immunocompromised individuals) from highly hazardous laboratory work.
4. Provision for effective personal protective equipment and procedures.

Guidelines for the surveillance of laboratory workers handling microorganisms at Biosafety Level 1

Historical evidence indicates that the microorganisms handled at this level are unlikely to cause human disease or animal disease of veterinary importance. Prompt reporting of illnesses or laboratory accidents is desirable and all staff members should be made aware of the importance of maintaining SMP.

Guidelines for the surveillance of laboratory workers handling microorganisms at Biosafety Level 2

1. Records of illness and absence should be kept by the laboratory management.
2. Women of childbearing age should be made aware of the risk to an unborn child of occupational exposure to certain microorganisms, e.g. rubella virus, Listeria monocytogenes, Toxoplasma gondii. The precise steps taken to protect the fetus will vary, depending on the microorganisms to which the woman may be exposed.

Training

Human error and poor technique can compromise the best of safeguards to protect the laboratory worker. Thus, a safety-conscious staff, well informed about the recognition and control of laboratory hazards, is key to the prevention of laboratory acquired infections, incidents and accidents. For this reason, continuous training in safety measures is essential. An effective safety program begins with the laboratory managers, who should ensure that safe laboratory practices and procedures are integrated into the basic training of employees. Training in safety measures should be an integral part of new employees’ introduction to the laboratory. Employees should be introduced to the standards of practice and to local guidelines, including the safety or operations manual. Measures to assure that employees have read and understood the guidelines, such as signature pages, should be adopted. Laboratory supervisors play
the key role in training their immediate staff in good laboratory techniques. The biosafety officer can assist in training and with the development of training aids and documentation.

Staff training should always include information on safe methods for highly hazardous procedures that are commonly encountered by all laboratory personnel and which involve:

1. Inhalation risks (i.e. aerosol production) when using loops, streaking agar plates, pipetting, making smears, opening cultures, taking blood/serum samples, centrifuging, etc.
2. Ingestion risks when handling specimens, smears and cultures
3. Risks of percutaneous exposures when using syringes and needles
4. Bites and scratches when handling animals
5. Handling of blood and other potentially hazardous pathological materials
6. Decontamination and disposal of infectious material.

Waste Handling
Waste is anything that is to be discarded. In laboratories, decontamination of wastes and their ultimate disposal are closely interrelated. In terms of daily use, few if any contaminated materials will require actual removal from the laboratory or destruction. Most glassware, instruments and laboratory clothing will be reused or recycled. Ideally all infectious materials should be decontaminated, autoclaved or incinerated within the laboratory.

The principal questions to be asked before discharge of any objects or materials from laboratories that deal with potentially infectious microorganisms or animal tissues are:

1. Have the objects or materials been effectively decontaminated or disinfected by an approved procedure?
2. If not, have they been packaged in an approved manner for immediate on-site incineration or transfer to another facility with incineration capacity?
3. Does the disposal of the decontaminated objects or materials involve any additional potential hazards, biological or otherwise, to those who carry out the immediate disposal procedures or who might come into contact with discarded items outside the facility?

Decontamination
Steam autoclaving is the preferred method for all decontamination processes. Biohazardous materials for decontamination and disposal should be placed in containers, e.g. autoclavable red plastic bags or clear bags with red or red-orange print.

Handling and disposal procedures for contaminated materials and wastes
An identification and separation system for infectious materials and their containers should be adopted. Federal, state and local regulations must be followed. Categories should include:

1. Non-contaminated (non-infectious) waste that can be reused or recycled or disposed of as general, “household” waste.
2. Contaminated (infectious) “sharps” – hypodermic needles, scalpels, knives and broken glass; these should always be collected in puncture-proof containers fitted with covers and treated as infectious.
3. Contaminated material for decontamination by autoclaving and thereafter washing and reuse or recycling.
4. Contaminated material for autoclaving and disposal.
5. Contaminated material for direct incineration e.g. animal body parts or carcasses.

Disposal Procedure for Sharps
After use, hypodermic needles should not be recapped, clipped or removed from disposable syringes. The complete assembly should be placed in a sharps disposal container. Sharps disposal containers must be puncture-proof/-resistant and must not be filled to capacity. When two-thirds full they should be closed and processed. Sharps contaminated with biohazardous waste must go into a RED container.
Sharps that ARE NOT contaminated with biohazardous waste can go into a NON-RED puncture-proof-/resistant container and labeled as "Non-infectious".

All sharps contaminated with biohazardous waste can be autoclaved on site then labeled as “TREATED” and placed into the large cardboard broken glass container. Alternatively, the untreated container can be picked up by Regulatory Compliance as hazardous waste.

Contact the Regulatory Compliance Office for sharps contaminated with either radioactive substances or hazardous chemicals/drugs. A suitable container and instructions will be supplied at that time.

Contaminated materials for autoclaving and reuse
No pre-cleaning should be attempted of any contaminated (potentially infectious) materials to be autoclaved and reused. Any necessary cleaning or repair must be done only after autoclaving or disinfection.

Contaminated materials for disposal
Apart from sharps, which are dealt with above, all contaminated (potentially infectious) materials should be autoclaved in leak-proof containers, e.g. autoclavable, color-coded plastic bags, before disposal. After autoclaving, the material is labeled as "TREATED", placed into black trash bags and taken to the nearest building dumpster. Do not place the bag into area trash cans. Janitorial staff should not handle these bags.

If discard containers, pans or jars are used, they should be unbreakable (e.g. plastic), decontaminated and washed before reuse.

**Chemical, Fire, Electrical, Radiation and Equipment Safety**
A breakdown in the containment of pathogenic organisms may be the indirect result of chemical, fire, electrical or radiation accidents. It is therefore essential to maintain high standards of safety in these areas in any microbiological or biomedical laboratory.
5. Laboratory Animal Facilities

Those who use animals for experimental and diagnostic purposes have a moral obligation to take every care to avoid causing them unnecessary pain or suffering. The animals must be provided with comfortable, hygienic housing and adequate wholesome food and water. At the end of the experiment they must be dealt with in a humane manner.

For security reasons, the animal facility should ideally be an independent, detached unit. If it adjoins a laboratory, the design should provide for its isolation from the public parts of the laboratory and should allow for easy decontamination and deinfestation.

Table 4. Animal facility containment levels: summary of practices and safety equipment

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<thead>
<tr>
<th>RISK GROUP</th>
<th>CONTAINMENT LEVEL</th>
<th>LAB PRACTICES AND SAFETY EQUIPMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ABSL-1</td>
<td>Limited access, protective clothing and gloves.</td>
</tr>
<tr>
<td>2</td>
<td>ABSL-2</td>
<td>ABSL-1 practices plus: hazard warning signs. Class I or II BSCs for activities that produce aerosols. Decontamination of waste and cages before washing.</td>
</tr>
<tr>
<td>3</td>
<td>ABSL-3</td>
<td>ABSL-2 practices plus: controlled access. BSCs and special protective equipment for all activities.</td>
</tr>
<tr>
<td>4</td>
<td>ABSL-4</td>
<td>ABSL-3 plus: strictly limited access. Clothing change before entering. Class III BSCs or positive pressure suits. Shower on exit. Decontamination of all wastes before removal from facility.</td>
</tr>
</tbody>
</table>

ABSL – animal facility Biosafety level; BSCs – biological safety cabinets

Animal facilities, like laboratories, may be designated according to a risk assessment and the risk group of the microorganisms under investigation, as Animal facility Biosafety Level 1, 2, 3 or 4.

With respect to agents to be used in the animal laboratory, factors for consideration include:

1. The normal route of transmission
2. The volumes and concentrations to be used
3. The route of inoculation
4. Whether and by what route these agents may be excreted.

With respect to animals to be used in the animal laboratory, factors for consideration include:

1. The nature of the animals, i.e. their aggressiveness and tendency to bite and scratch
2. Their natural ecto- and endoparasites
3. The zoonotic diseases to which they are susceptible
4. The possible dissemination of allergens.
As with laboratories, the requirements for design features, equipment and precautions increase in stringency according to the animal biosafety level. These are described below and summarized in Table 4. These guidelines are additive, so that each higher level incorporates the standards of the lower levels.

Animal facility – Biosafety Level 1
This is suitable for the maintenance of most stock animals after quarantine (except nonhuman primates) and for animals that are deliberately inoculated with agents in Risk Group 1. SMPs are required. The animal facility director must establish policies, procedures and protocols for all operations, and for access to the vivarium. An appropriate medical surveillance program for the staff must be instituted. A safety or operations manual must be prepared and adopted.

Animal facility – Biosafety Level 2
This is suitable for work with animals that are deliberately inoculated with microorganisms in Risk Group 2. The following safety precautions apply:

1. All the requirements for animal facilities – Biosafety Level 1 must be met.
2. Biohazard warning signs (see Figure 1) should be posted on doors and other appropriate places.
3. The facility must be designed for easy cleaning and housekeeping.
4. Doors must open inwards and be self-closing.
5. Heating, ventilation and lighting must be adequate.
6. If mechanical ventilation is provided, the airflow must be inwards. Exhaust air is discharged to the outside and should not be recirculated to any part of the building.
7. Access must be restricted to authorized persons.
8. No animals should be admitted other than those for experimental use.
9. There should be an arthropod and rodent control program.
10. Windows, if present, must be secure, resistant to breakage and, if able to be opened, must be fitted with arthropod-proof screens.
11. After use, work surfaces must be decontaminated with effective disinfectants.
12. Biological safety cabinets (Classes I or II) or isolator cages with dedicated air supplies and HEPA-filtered exhaust air must be provided for work that may involve the generation of aerosols.
13. An autoclave must be available on site or in appropriate proximity to the animal facility.
14. Animal bedding materials must be removed in a manner that minimizes the generation of aerosols and dust.
15. All waste materials and bedding must be decontaminated before disposal.
16. Use of sharp instruments should be restricted whenever possible. Sharps should always be collected in puncture-proof/-resistant containers fitted with covers and treated as infectious.
17. Material for autoclaving or incineration must be transported safely, in closed containers.
18. Animal cages must be decontaminated after use.
19. Incineration of animal carcasses is recommended, however, consult with the University Laboratory Veterinarian for the current disposal policy.
20. Protective clothing and equipment must be worn in the facility, and removed on leaving.
21. Hand-washing facilities must be provided. Staff must wash their hands before leaving the animal facility.
22. All injuries, however minor, must be treated appropriately, reported and recorded.
23. Eating, drinking, smoking and application of cosmetics must be forbidden in the facility.
24. All personnel must receive appropriate training.

Invertebrates
As with vertebrates, the animal facility biosafety level will be determined by the risk groups of the agents under investigation or when otherwise indicated by a risk assessment. The following additional precautions are necessary with certain arthropods, particularly with flying insects:

1. Separate rooms should be provided for infected and noninfected invertebrates.
2. The rooms should be capable of being sealed for fumigation.
3. Insecticide sprays should be readily available.
4. “Chilling” facilities should be provided to reduce, where necessary, the activity of invertebrates.
5. Access should be through an anteroom containing insect traps and with arthropod-proof screens on the doors.
6. All exhaust ventilation ducts and windows that can be opened should be fitted with arthropod-proof screens.
7. Waste traps on sinks and sluices should not be allowed to dry out.
8. All waste should be decontaminated by autoclaving, as some invertebrates are not killed by all disinfectants.
9. A check should be kept on the numbers of larval and adult forms of flying, crawling and jumping arthropods.
10. Containers for ticks and mites should stand in trays of oil.
11. Infected or potentially infected flying insects must be contained in double-netted cages.
12. Infected or potentially infected arthropods must be handled in biological safety cabinets or isolators.
13. Infected or potentially infected arthropods may be manipulated on cooling trays.
6. Guidelines for Laboratory/Facility Commissioning

Laboratory/facility commissioning may be defined as the systematic review and documentation process signifying that specified laboratory structural components, systems and/or system components have been installed, inspected, functionally tested and verified to meet national standards. The building design and function criteria establish these requirements. In other words, laboratories designated as Biosafety Levels 1–4 will have different and increasingly complex commissioning requirements. Geographical and climatic conditions, such as geological fault lines or extreme heat, cold or humidity may also affect the laboratory design and therefore the commissioning requirements. Upon the completion of the commissioning process, the pertinent structural components and support systems will have been subjected to the various operating conditions and failure modes that can be reasonably expected, and will have been approved.

The commissioning process and acceptance criteria should be established early, preferably during the programming phase of the construction or renovation project. By acknowledging the commissioning process early in the project, architects, engineers, safety and health personnel and ultimately the laboratory occupants understand the performance requirements of the specific laboratory and set uniform expectations for laboratory and/or facility performance. The commissioning process provides the institution and the surrounding community with a greater degree of confidence that the structural, electrical, mechanical and plumbing systems, containment and decontamination systems, and security and alarm systems will operate as designed, to assure containment of any potentially dangerous microorganisms being worked with in a particular laboratory or animal facility.

Commissioning activities generally begin during the programming phase of the project and proceed through the construction and subsequent warranty period for the laboratory/facility. Warranty periods should generally extend for one year following occupancy. It is recommended that a commissioning agent who is independent of the architectural, engineering and construction firms involved in the design and construction is retained. The commissioning agent serves as an advocate for the institution constructing or renovating the laboratory and should be considered as a member of the design team; involvement of the agent in the early programming phase of the project is essential. In some cases, the institution may act as its own commissioning agent. In the case of more complex laboratory facilities (Biosafety Levels 3 or 4), the institution may wish to retain an outside commissioning agent who has demonstrated experience and success in the commissioning of complex biosafety laboratory and animal facilities. When an independent commissioning agent is used, the institution should still be a member of the commissioning team. It is recommended that, in addition to the commissioning agent, the institution's Safety Officers and representatives from Facilities Maintenance become part of the team.

The following is a list of laboratory systems and components that may be included in a commissioning plan for functional testing, depending on the containment level of the facility being renovated or constructed. The list is not exhaustive. Obviously, the actual commissioning plan will reflect the complexity of the laboratory being planned.

1. Building automation systems including links to remote monitoring and control sites
2. Electronic surveillance and detection systems
3. Electronic security locks and proximity device readers
4. Heating, ventilation (supply and exhaust) and air-conditioning (HVAC) systems
5. High-efficiency particulate air (HEPA) filtration systems
6. HEPA decontamination systems
7. HVAC and exhaust air system controls and control interlocks
8. Airtight isolation dampers
9. Laboratory refrigeration systems
10. Boilers and steam systems
11. Fire detection, suppression and alarm systems
12. Domestic water backflow prevention devices
13. Processed water systems (i.e. reverse osmosis, distilled water)
14. Liquid effluent treatment and neutralization systems
15. Plumbing drain primer systems
16. Chemical decontaminant systems
17. Medical laboratory gas systems
18. Breathing air systems
19. Service and instrument air systems
20. Cascading pressure differential verification of laboratories and support areas
21. Local area network (LAN) and computer data systems
22. Normal power systems
23. Emergency power systems
24. Uninterruptible power systems
25. Emergency lighting systems
26. Lighting fixture penetration seals
27. Electrical and mechanical penetration seals
28. Telephone systems
29. Airlock door control interlocks
30. Airtight door seals
31. Window and vision-panel penetration seals
32. Barrier pass-through penetration
33. Structural integrity verification: concrete floors, walls and ceilings
34. Barrier coating verification: floors, walls and ceilings
35. Biosafety Level 4 containment envelope pressurization and isolation functions
36. Biological safety cabinets
37. Autoclaves
38. Liquid nitrogen system and alarms
39. Water detection systems (e.g. in case of flooding inside containment zone)
40. Decontamination shower and chemical additive systems
41. Cage-wash and neutralization systems
42. Waste management.
7. Guidelines for Laboratory/Facility Certification

Laboratories are complex and dynamic environments. Today’s research laboratories must be able to adapt quickly to continuously increasing public health and agricultural needs and pressures. An example of this is the need for laboratories to adjust priorities to meet the challenges of emerging or re-emerging infectious diseases. In order to assure that adaptation and maintenance are undertaken promptly and in an appropriate and safe manner, all biological research and clinical laboratories should be regularly certified. Laboratory certification helps to ensure that:

1. Proper engineering controls are being used and are functioning adequately as designed.
2. Appropriate site and protocol specific administrative controls are in place.
3. Personal protective equipment is appropriate for the tasks being performed.
4. Decontamination of waste and materials has been adequately considered and proper waste management procedures are in place.
5. Proper procedures for general laboratory safety, including physical, electrical and chemical safety are in place.

Laboratory certification differs from laboratory commissioning activities in several important ways. **Laboratory certification is the systematic examination of all safety features and processes within the laboratory (engineering controls, personal protective equipment and administrative controls).** Biosafety practices and procedures are also examined. Laboratory certification is an on-going quality and safety assurance activity that should take place on a regular basis.

Research laboratory facilities may develop audit, survey or inspection tools to help ensure consistency in the certification process. These tools should be flexible enough to allow for the physical and procedural differences between laboratories necessitated by the type of work being conducted, while at the same time providing a consistent approach within the institution. Care must be taken to ensure that these tools are used only by appropriately trained personnel, and that they are not used as a substitute for a sound professional biosafety assessment. Examples of such tools are provided in Tables 5 and 6.

Findings of the audit, survey or inspection should be discussed with laboratory personnel and management. Within the laboratory, an individual should be identified and made responsible for ensuring that corrective actions are taken for all deficiencies identified during the audit process. Certification of the laboratory should not be completed, and the laboratory should not be declared functional, until deficiencies have been adequately addressed.
### Table 5. Basic Laboratory – Biosafety Level 1: laboratory safety survey

<table>
<thead>
<tr>
<th>Checked Item</th>
<th>Yes</th>
<th>No</th>
<th>N/A</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Laboratory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proper signage: UV light, laser etc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biosafety manual available</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lab equipment properly labeled (biohazard, radioactive etc.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Laboratory Design</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Designed for easy cleaning</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All shelves secured</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bench tops impervious to water/chemicals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adequate illumination provided</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adequate storage space available/used</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gas Cylinders</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All cylinders secured</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caps on reserve cylinders</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asphyxiating/hazardous gases in ventilated rooms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excess or empty cylinders present</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Chemicals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flammables stored in flammables storage cabinet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peroxide formers double-dated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(received and opened)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemicals properly segregated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hazardous chemicals stored above eye level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemicals stored on floor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical containers left open</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All solutions properly labeled</td>
<td>☐ ☐ ☐</td>
<td>Mercury thermometers in use</td>
<td>☐ ☐ ☐</td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>--------</td>
<td>-----------------------------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td><strong>Refrigerators/Freezers/Cold Rooms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food for human consumption present</td>
<td>☐ ☐ ☐</td>
<td>Flammable in explosion-proof units</td>
<td>☐ ☐ ☐</td>
<td></td>
</tr>
<tr>
<td>Labeled externally if containing carcinogens, radioactivity and/or biohazards</td>
<td>☐ ☐ ☐</td>
<td>Cold room has emergency release</td>
<td>☐ ☐ ☐</td>
<td></td>
</tr>
<tr>
<td><strong>Electrical Equipment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extension cords present</td>
<td>☐ ☐ ☐</td>
<td>Outlets earthed/grounded with proper polarity</td>
<td>☐ ☐ ☐</td>
<td></td>
</tr>
<tr>
<td>Connections by sinks, under showers etc.</td>
<td>☐ ☐ ☐</td>
<td>Equipment with frayed/damaged wiring</td>
<td>☐ ☐ ☐</td>
<td></td>
</tr>
<tr>
<td>Overloaded outlets/strips</td>
<td>☐ ☐ ☐</td>
<td>Power strips mounted off of floor</td>
<td>☐ ☐ ☐</td>
<td></td>
</tr>
<tr>
<td>Electrical outlets near water sources meet code</td>
<td>☐ ☐ ☐</td>
<td>Proper fuses in conduits</td>
<td>☐ ☐ ☐</td>
<td></td>
</tr>
<tr>
<td>Earths/grounds present on electrical cords</td>
<td>☐ ☐ ☐</td>
<td>Portable space heaters</td>
<td>☐ ☐ ☐</td>
<td></td>
</tr>
<tr>
<td><strong>Personal Protective Equipment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eyewash available in lab</td>
<td>☐ ☐ ☐</td>
<td>Safety shower available</td>
<td>☐ ☐ ☐</td>
<td></td>
</tr>
<tr>
<td>PPE available</td>
<td>☐ ☐ ☐</td>
<td>Occupants properly attired</td>
<td>☐ ☐ ☐</td>
<td></td>
</tr>
<tr>
<td>PPE not worn outside of lab</td>
<td>☐ ☐ ☐</td>
<td>PPE available for use with cryogenics</td>
<td>☐ ☐ ☐</td>
<td></td>
</tr>
</tbody>
</table>
### Waste Management

<table>
<thead>
<tr>
<th>Description</th>
<th>Yes</th>
<th>No</th>
<th>Not Applicable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evidence of improper waste disposal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wastes segregated in proper containers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical waste containers tagged, labeled, dated and closed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical waste containers handled and stored correctly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sharps containers used/disposed of properly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No trash on floor</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Occupational Health & Safety Programs Available

<table>
<thead>
<tr>
<th>Description</th>
<th>Yes</th>
<th>No</th>
<th>Not Applicable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hazard communication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory protection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hearing conservation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formaldehyde monitoring</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethylene oxide monitoring</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaesthetic gas monitoring</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### General Engineering Controls

<table>
<thead>
<tr>
<th>Description</th>
<th>Yes</th>
<th>No</th>
<th>Not Applicable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab airflow negative to hallway</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cup sinks or drains acting as vents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand washing sink available</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposed machine parts (pulleys, gears)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vacuum line has filters/traps</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Backflow hazards to water supply</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distilled water system in good condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effective pest control program</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### General Practices/Procedures

<table>
<thead>
<tr>
<th>Description</th>
<th>Yes</th>
<th>No</th>
<th>Not Applicable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food (human) stored outside of lab</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Microwave oven labeled “No Food Prep” □ □ □ □

Eating, drinking, smoking, cosmetic application occurring in lab □ □ □ □

Pressurized glass containers taped or shielded (i.e. vacuum traps) □ □ □ □

Mouth pipetting prohibited □ □ □ □

Mechanical pipetting devices used □ □ □ □

PPE stored separately from street clothing □ □ □ □

**General Lab Housekeeping**

Glass containers stored on floor □ □ □ □

Trip hazards evident □ □ □ □

Broken glass box available □ □ □ □

Spill kit available □ □ □ □

**Fire Protection**

Sprinkler heads free/unobstructed □ □ □ □

Open penetrations in walls, ceilings, floors □ □ □ □

Wiring/tubing through doors □ □ □ □

Minimum passage width of 3 feet in lab □ □ □ □

Storage observed on ductwork or light fixtures □ □ □ □

Excess combustibles stored in lab □ □ □ □

**Heated Constant Temperature Baths**

Equipped with low water level and overheat shut-off □ □ □ □

Constructed on noncombustible materials □ □ □ □

**Safety inspector signature:** □ □ □ □

**Date:** □ □ □ □
Table 6. Basic laboratory – Biosafety Level 2: laboratory safety survey.

<table>
<thead>
<tr>
<th>Location:</th>
<th>Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responsible Person:</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Checked Item</th>
<th>Yes</th>
<th>No</th>
<th>N/A</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biological Safety Cabinet (BSC)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Certification within last year</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>BSC surfaces wiped down with appropriate disinfectant</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>at beginning/end of each procedure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Front grill and exhaust unobstructed</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>Open flames used inside cabinet</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>Vacuum lines have in-line filters/disinfectant traps</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>BSC compromised by room air or location</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>BSC used when potential for aerosol</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td><strong>Laboratory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Access restricted to authorized personnel only</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>Entry limited to personnel advised of all potential hazards</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>Biohazard sign posted on lab door</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>All doors closed</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td><strong>Decontamination</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appropriate disinfectant in use</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>Spills/accidents reported to lab supervisor</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>Work surfaces deconned before/after each procedure daily and after spills</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td><strong>Handling of Contaminated Waste</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infectious waste containers properly used</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>Containers not overfilled</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>Containers labeled/closed</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
</tr>
<tr>
<td>Category</td>
<td>Details</td>
<td>Selection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>-----------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture stocks/other biohazardous waste</td>
<td>properly deconed before disposal</td>
<td>☐ ☐ ☐</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Materials deconed outside of lab transported in closed, durable, leakproof containers</td>
<td></td>
<td>☐ ☐ ☐</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Personal Protection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lab personnel informed of available vaccinations</td>
<td></td>
<td>☐ ☐ ☐</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appropriate medical services available for surveillance/treatment</td>
<td></td>
<td>☐ ☐ ☐</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gloves worn when handling infectious material or contaminated equipment</td>
<td></td>
<td>☐ ☐ ☐</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Face protection provided when working outside of BSC with infectious material</td>
<td></td>
<td>☐ ☐ ☐</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hands washed after removing gloves, after working with infectious agents, before leaving lab</td>
<td></td>
<td>☐ ☐ ☐</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Practices</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biosafety manual prepared/adopted</td>
<td></td>
<td>☐ ☐ ☐</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Personnel documentation of training</td>
<td></td>
<td>☐ ☐ ☐</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centrifuge cups/rotors opened in BSC only</td>
<td></td>
<td>☐ ☐ ☐</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Facility</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand washing sink available near lab exit</td>
<td></td>
<td>☐ ☐ ☐</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Safety inspector signature:** ___________________________  **Date:** ____________
8. Laboratory Biosecurity Concepts

Laboratory biosafety manuals, in the past, have focused on traditional biosafety guidance for laboratories. The manuals emphasized the use of good microbiological work practices, appropriate containment equipment, proper facility design, operation and maintenance, and administrative considerations to minimize the risk of worker injury or illness. In following these recommendations, the risk to the environment and surrounding community-at-large was also minimized. It has now become necessary to expand this traditional approach to biosafety through the introduction of laboratory biosecurity measures. Global events in the recent past have highlighted the need to protect laboratories and the materials they contain from being intentionally compromised in ways that may harm people, livestock, agriculture or the environment. Before the laboratory biosecurity needs of a facility can be defined, however, it is important to understand the distinction between “laboratory biosafety” and “laboratory biosecurity”.

“Laboratory biosafety” is the term used to describe the containment principles, technologies and practices that are implemented to prevent unintentional exposure to pathogens and toxins, or their accidental release.

“Laboratory biosecurity” refers to institutional and personal security measures designed to prevent the loss, theft, misuse, diversion or intentional release of pathogens and toxins.

Effective biosafety practices are the very foundation of laboratory biosecurity activities. Through risk assessments, performed as an integral part of an institution’s biosafety program, information is gathered regarding the type of organisms available, their physical location, the personnel who require access to them, and the identification of those responsible for them. This information can be used to assess whether an institution possesses biological materials that are attractive to those who may wish to use them improperly.

A specific laboratory biosecurity program must be prepared and implemented for each facility according to the requirements of the facility, the type of laboratory work conducted, and the local conditions. Consequently, laboratory biosecurity activities should be representative of the institution’s various needs and should include input from scientific directors, principal investigators, biosafety officers, laboratory scientific staff, maintenance staff, administrators, information technology staff, and law enforcement agencies and security staff if appropriate.

Laboratory biosecurity measures should be based on a comprehensive program of accountability for pathogens and toxins that includes an updated inventory with storage location, identification of personnel with access, description of use, documentation of internal and external transfers within and between facilities, and any inactivation and/or disposal of the materials. Likewise, an institutional laboratory biosecurity protocol should be established for identifying, reporting, investigating and remediating breaches in laboratory biosecurity, including discrepancies in inventory results. The involvement and roles and responsibilities of public health and security authorities in the event of a security infraction must be clearly defined.

Laboratory biosecurity training, distinct from laboratory biosafety training, should be provided to all personnel. Such training should help personnel understand the need for protection of such materials and the rationale for the specific biosecurity measures, and should include a review of relevant national standards and institution specific procedures. Procedures describing the security roles and responsibilities of personnel in the event of a security infraction should also be presented during training.

The professional and ethical suitability for working with dangerous pathogens of all personnel who have regular authorized access to sensitive materials is also central to effective laboratory biosecurity activities.

In summary, security precautions should become a routine part of laboratory work, just as have aseptic techniques and other safe microbiological practices. Laboratory biosecurity measures should not hinder
the efficient sharing of materials and related information necessary for research. Competent security management should not unduly interfere with the day-to-day activities of scientific personnel or be an impediment to conducting research. Legitimate access to important research materials must be preserved. Assessment of the suitability of personnel, security-specific training and rigorous adherence to pathogen protection procedures are reasonable means of enhancing laboratory biosecurity. All such efforts must be established and maintained through regular risk and threat assessments, and regular review and updating of procedures. Checks for compliance with these procedures, with clear instructions on roles, responsibilities and remedial actions, should be integral to laboratory biosecurity.
9. Biological Safety Cabinets

Biological safety cabinets (BSCs) are designed to protect the operator, the laboratory environment and work materials from exposure to infectious aerosols and splashes that may be generated when manipulating materials containing infectious agents, such as primary cultures, stocks and diagnostic specimens. Aerosol particles are created by any activity that imparts energy into a liquid or semi-liquid material, such as shaking, pouring, stirring or dropping liquid onto a surface or into another liquid. Other laboratory activities, such as streaking agar plates, inoculating cell culture flasks with a pipette, using a multichannel pipette to dispense liquid suspensions of infectious agents into microculture plates, homogenizing and vortexing infectious materials, and centrifugation of infectious liquids, or working with animals, can generate infectious aerosols. Aerosol particles of less than 5 microns in diameter and small droplets of 5–100 microns in diameter are not visible to the naked eye. The laboratory worker is generally not aware that such particles are being generated and may be inhaled or may cross-contaminate work surface materials. BSCs, when properly used, have been shown to be highly effective in reducing laboratory-acquired infections and cross-contaminations of cultures due to aerosol exposures. BSCs also protect the environment.

Over the years the basic design of BSCs has undergone several modifications. A major change was the addition of a high-efficiency particulate air (HEPA) filter to the exhaust system. The HEPA filter traps 99.97% of particles of 0.3 microns in diameter and 99.99% of particles of greater or smaller size. This enables the HEPA filter to effectively trap all known infectious agents and ensure that only microbe-free exhaust air is discharged from the cabinet. A second design modification was to direct HEPA-filtered air over the work surface, providing protection of work surface materials from contamination. This feature is often referred to as product protection. These basic design concepts have led to the evolution of three classes of BSCs. The type of protection provided by each is described in Table 7.

Note. Horizontal and vertical outflow cabinets (“clean-air work stations”) are **not** biological safety cabinets and should not be used as such.
Table 7. Selection of a biological safety cabinet (BSC), by type of protection needed

<table>
<thead>
<tr>
<th>BSC Class</th>
<th>Face Velocity (lfpm)</th>
<th>Airflow Pattern</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>75</td>
<td>In at front through HEPA to the outside or into the room through HEPA</td>
<td>Yes</td>
</tr>
<tr>
<td>II, A1</td>
<td>75</td>
<td>70% recirculated to the cabinet work area through HEPA; 30% balance can be exhausted through HEPA back into the room or to outside through a canopy unit</td>
<td>Yes (minute amounts)</td>
</tr>
<tr>
<td>II, B1</td>
<td>100</td>
<td>30% recirculated, 70% exhausted. Exhaust cabinet air must pass through a dedicated duct to the outside through a HEPA filter</td>
<td>Yes</td>
</tr>
<tr>
<td>II, B2</td>
<td>100</td>
<td>No recirculation; total exhaust to the outside through a HEPA filter</td>
<td>Yes</td>
</tr>
<tr>
<td>II, A2</td>
<td>100</td>
<td>Similar to II, A1, but has 100 lfpm intake air velocity and plenums are under negative pressure to room; exhaust air can be ducted to outside through a canopy unit</td>
<td>Yes</td>
</tr>
<tr>
<td>III</td>
<td>N/A</td>
<td>Supply air is HEPA filtered. Exhaust air passes through two HEPA filters in series and is exhausted to the outside via a hard connection</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Lfpm: Linear feet per minute
**Class I biological safety cabinet**

Figure 4 provides a schematic diagram of a Class I BSC. Room air is drawn in through the front opening at a minimum velocity of 0.38 m/s (~1.3 feet/s). It passes over the work surface and is discharged from the cabinet through the exhaust duct. The directional flow of air whisks aerosol particles that may be generated on the work surface away from the laboratory worker and into the exhaust duct. The front opening allows the operator’s arms to reach the work surface inside the cabinet while he or she observes the work surface through a glass window sash. The window sash can also be fully raised to provide access to the work surface for cleaning or other purposes.

The air from the cabinet is exhausted through a HEPA filter: (a) into the laboratory and then to the outside of the building through the building exhaust; (b) to the outside through the building exhaust; or (c) directly to the outside. The HEPA filter may be located in the exhaust plenum of the BSC or in the building exhaust. Some Class I BSCs are equipped with an integral exhaust fan, whereas others rely on the exhaust fan in the building exhaust system.

The Class I BSC was the first recognized BSC and, because of its simple design, is still in wide use throughout the world. It has the advantage of providing personnel and environmental protection and can also be used for work with radionuclides and volatile toxic chemicals. Because unsterilized room air is drawn over the work surface through the front opening, it is not considered to provide consistently reliable product protection. The Class I BSC is mainly used today to hold equipment (centrifuge, small fermenters, harvesting equipment) or for procedures that may generate aerosols (cage dumping, culture aeration or tissue homogenization).

Figure 4. Schematic diagram of a Class I biological safety cabinet.

A. front opening; B. sash; C. exhaust HEPA filter; D. exhaust plenum
Class II biological safety cabinets
As the use of cell and tissue cultures for the propagation of viruses and other purposes grew, it was no longer considered satisfactory for unsterilized room air to pass over the work surface. The Class II BSC was designed not only to provide personnel protection but also to protect work surface materials from contaminated room air. Class II BSCs, of which there are four types (A1, A2, B1 and B2), differ from Class I BSCs by allowing only air from a HEPA-filtered (sterile) supply to flow over the work surface. The Class II BSC can be used for working with infectious agents in Risk Groups 2 and 3. Class II BSCs can be used for working with infectious agents in Risk Group 4 when positive-pressure suits are used.

Class II type A1 biological safety cabinet
The Class II type A1 BSC is shown in Figure 5. An internal fan draws room air (supply air) into the cabinet through the front opening and into the front intake grill. The inflow velocity of this air should be at least 0.38 m/s at the face of the front opening. The supply air then passes through a supply HEPA filter before flowing downwards over the work surface. As the air flows downwards it “splits” about 6 –18 cm (2.4 – 7 inches) from the work surface, one half of the downwards flowing air passing through the front exhaust grill, and the other half passing through the rear exhaust grill. Any aerosol particles generated at the work surface are immediately captured in this downward airflow and passed through the front or rear exhaust grills, thereby providing the highest level of product protection. The air is then discharged through the rear plenum into the space between the supply and exhaust filters located at the top of the cabinet. Owing to the relative size of these filters, about 70% of the air recirculates through the supply HEPA filter back into the work zone; the remaining 30% passes through the exhaust filter into the room or to the outside.

Air from the Class IIA1 BSC exhaust can be recirculated to the room or discharged to the outside of the building through a thimble connection to a dedicated duct or through the building exhaust system. Recirculating the exhaust air to the room has the advantage of lowering building fuel costs because heated and/or cooled air is not being passed to the outside environment. A connection to a ducted exhaust system also allows some BSCs to be used for work with volatile radionuclides and volatile toxic chemicals.
Class II type A2 vented to the outside, B1 and B2 biological safety cabinets
Class IIA2 vented to the outside, IIB1 (Figure 6) and IIB2 BSCs are variations of the type IIA1. Each variation allows the BSC to be used for specialized purposes (see Table 7). These BSCs differ from one another in several aspects: the air intake velocity through the front opening; the amount of air recirculated over the work surface and exhausted from the cabinet; the exhaust system, which determines whether air from the cabinet is exhausted to the room, or to the outside, through a dedicated exhaust system or through the building exhaust; and the pressure arrangements (whether cabinets have biologically contaminated ducts and plenums under negative pressure, or have biological contaminated ducts and plenums surrounded by negative pressure ducts and plenums).

Complete descriptions of the various Class IIA and IIB BSCs can be obtained from manufacturers’ brochures and the BMBL.
**Class III biological safety cabinet**

This type (Figure 7) provides the highest level of personnel protection and is used for Risk Group 4 agents. All penetrations are sealed “gas tight”. Supply air is HEPA-filtered and exhaust air passes through two HEPA filters. Airflow is maintained by a dedicated exhaust system exterior to the cabinet, which keeps the cabinet interior under negative pressure (about 124.5 Pa). Access to the work surface is by means of heavy duty rubber gloves, which are attached to ports in the cabinet. The Class III BSC should have an attached pass-through box that can be sterilized and is equipped with a HEPA-filtered exhaust. The Class III cabinet may be connected to a double-door autoclave used to decontaminate all materials entering or exiting the cabinet. Several glove boxes can be joined together to extend the work surface. Class III BSCs are suitable for work in Biosafety Level 3 and 4 laboratories.
Figure 7. Class III BSC
The Class III BSC. A. glove ports with O-ring for attaching arm-length gloves to cabinet, B. sash, C. exhaust HEPA filter, D. supply HEPA filter, E. double ended autoclave or pass-through box. Note: A chemical dunk tank may be installed which would be located beneath the work surface of the BSC with access from above. The cabinet exhaust needs to be hard connected to an independent dedicated exhaust system. The exhaust air must be double HEPA filtered or HEPA filtered and incinerated.

Biological safety cabinet air connections
A “thimble” or “canopy hood” is designed for use with the Class IIA1 and IIA2 vented to the outside. The thimble fits over the cabinet exhaust housing, sucking the cabinet exhaust air into the building exhaust ducts. A small opening, usually 2.5 cm in diameter, is maintained between the thimble and the cabinet exhaust housing. This small opening enables room air to be sucked into the building exhaust system as well. The building exhaust capacity must be sufficient to capture both room air and the cabinet exhaust. The thimble must be removable or be designed to allow for operational testing of the cabinet. Generally, the performance of a thimble-connected BSC is not affected much by fluctuations in the airflow of the building.

Class IIB1 and IIB2 BSCs are hard-ducted, i.e. firmly connected without any openings, to the building exhaust system or, preferably, to a dedicated exhaust duct system. The building exhaust system must be precisely matched to the airflow requirements specified by the manufacturer for both volume and static pressure. Certification of hard-duct connected BSCs is more time-consuming than that for BSCs that recirculate air to the room or which are thimble-connected.

Selection of a biological safety cabinet
A BSC should be selected primarily in accordance with the type of protection needed: product protection; personnel protection against Risk Group 1–4 microorganisms; personnel protection against exposure to radionuclides and volatile toxic chemicals; or a combination of these. Table 7 shows which BSCs are
recommended for each type of protection. Refer to Table 7 for those BSCs that can be used with nonvolatile and volatile or toxic chemicals.

Using biological safety cabinets in the laboratory

Location
The velocity of air flowing through the front opening into a BSC is about 0.45 m/s. At this velocity the integrity of the directional air inflow is fragile and can be easily disrupted by air currents generated by people walking close to the BSC, open windows, air supply registers, and opening and shutting doors. Ideally, BSCs should be situated in a location remote from traffic and potentially disturbing air currents. Whenever possible a 30-cm (~1 foot) clearance should be provided behind and on each side of the cabinet to allow easy access for maintenance. A clearance of 30–35 cm above the cabinet may be required to provide for accurate air velocity measurement across the exhaust filter and for exhaust filter changes.

Operators
If BSCs are not used properly, their protective benefits may be greatly diminished. Operators must be careful to maintain the integrity of the front opening air inflow when moving their arms into and out of cabinets. Arms should be moved in and out slowly, perpendicular to the front opening. Manipulations of materials within BSCs should be delayed for about 1 min after placing hands and arms inside to allow the cabinet to adjust and to “air sweep” the surface of the hands and arms. The number of movements across the front opening should also be minimized by placing all necessary items into the cabinet before beginning manipulations.

Material placement
The front intake grill of Class II BSCs must not be blocked with paper, equipment or other items. See Figure 8. Materials to be placed inside the cabinet should be surface-decontaminated with 70% alcohol. Work may be performed on disinfectant-soaked absorbent towels to capture splatters and splashes. All materials should be placed as far back in the cabinet, towards the rear edge of the work surface, as practical without blocking the rear grill. Aerosol-generating equipment (e.g. mixers, centrifuges, etc.) should be placed towards the rear of the cabinet. Bulky items, such as biohazard bags, discard pipette trays and suction collection flasks should be placed to one side of the interior of the cabinet. Active work should flow from clean to contaminated areas across the work surface.

The autoclavable biohazard collection bag and pipette collection tray should not be placed outside the cabinet. The frequent in-and-out movement needed to use these containers is disruptive to the integrity of the cabinet’s air barrier, and can compromise both personnel and product protection.
**Operation and maintenance**

Most BSCs are designed to permit operation 24 h/day, and investigators find that continuous operation helps to control the levels of dust and particulate materials in the laboratory. Class II A1 and II A2 BSCs exhausting to the room or connected by thimble connections to dedicated exhaust ducts can be turned off when not in use. Other types such as II B1 and II B2 BSCs, which have hard-duct installations, must have airflow through them at all times to help maintain room air balance. Cabinets should be turned on at least 5 min before beginning work and after completion of work to allow the cabinet to “purge”, i.e. to allow time for contaminated air to be removed from the cabinet environment. All repairs made on BSCs should be made by a qualified technician. Any malfunction in the operation of the BSC should be reported and repaired before the BSC is used again.

**Ultraviolet lights**

Ultraviolet lights are not required in BSCs. If they are used, they must be cleaned weekly to remove any dust and dirt that may block the germicidal effectiveness of the light. Ultraviolet light intensity should be checked when the cabinet is recertified to ensure that light emission is appropriate. Ultraviolet lights must be turned off while the room is occupied, to protect eyes and skin from inadvertent exposure.

**Open flames**

Open flames should be avoided in the near microbe-free environment created inside the BSC. They disrupt the airflow patterns and can be dangerous when volatile, flammable substances are also used. To sterilize bacteriological loops, microburners or electric “furnaces” are available and are preferable to open flames.

**Spills**

A copy of the laboratory’s protocol for handling spills should be posted, read and understood by everyone who uses the laboratory. When a spill of biohazardous material occurs within a BSC, clean-up should begin immediately, while the cabinet continues to operate. An effective disinfectant should be used and applied in a manner that minimizes the generation of aerosols. All materials that come into contact with the spilled agent should be disinfected and/or autoclaved.

**Certification**

The functional operation and integrity of each BSC should be certified to current performance standards at the time of installation and annually thereafter by qualified technicians, according to the manufacturer’s instructions. Evaluation of the effectiveness of cabinet containment should include tests for cabinet integrity, HEPA filter leaks, down-flow velocity profile, face velocity, negative pressure/ventilation rate, air-
flow smoke pattern, and alarms and interlocks. Optional tests for electrical leaks, lighting intensity, ultraviolet light intensity, noise level and vibration may also be conducted. Special training, skills and equipment are required to perform these tests and it is highly recommended that they are undertaken by a qualified professional.

Cleaning and disinfection
All items within BSCs, including equipment, should be surface-decontaminated and removed from the cabinet when work is completed, since residual culture media may provide an opportunity for microbial growth. The interior surfaces of BSCs should be decontaminated before and after each use. The work surfaces and interior walls should be wiped with a disinfectant that will kill any microorganisms that might be found inside the cabinet. At the end of the work day, the final surface decontamination should include a wipe-down of the work surface, the sides, back and interior of the glass. A solution of bleach or 70% alcohol should be used where effective for target organisms. A second wiping with sterile water is needed when a corrosive disinfectant, such as bleach, is used. It is recommended that the cabinet is left running. If not, it should be run for 5 min in order to purge the atmosphere inside before it is switched off.

Decontamination
BSCs must be decontaminated before filter changes and before being moved. The most common decontamination methods are by fumigation with formaldehyde gas or vaporized hydrogen peroxide. BSC decontamination should be performed by a qualified professional.

Personal protective equipment
Personal protective clothing should be worn whenever using a BSC. Laboratory coats are acceptable for work being performed at Biosafety Levels 1 and 2. Gloves should be pulled over the wrists of the gown rather than worn inside. Elasticized sleeves can be worn to protect the investigator’s wrists. Masks and safety glasses may be required for some procedures.

Alarms
BSCs can be equipped with one of two kinds of alarm. Sash alarms are found only on cabinets with sliding sashes. The alarm signifies that the operator has moved the sash to an improper position. Corrective action for this type of alarm is returning the sash to the proper position. Airflow alarms indicate a disruption in the cabinet’s normal airflow pattern. This represents an immediate danger to the operator or product. When an airflow alarm sounds, work should cease immediately and the laboratory supervisor should be notified. Manufacturers’ instruction manuals should provide further details. Training in the use of BSCs should cover this aspect.
10. Safety equipment

Since aerosols are important sources of infection, care should be taken to reduce the extent of their formation and dispersion. Hazardous aerosols can be generated by many laboratory operations such as blending, mixing, grinding, shaking, stirring, sonicating and centrifuging of infectious materials. Even when safe equipment is used, it is best to carry out these operations in an approved biological safety cabinet whenever possible. The use of safety equipment is no assurance of protection unless the operator is trained and uses proper techniques. Equipment should be tested regularly to ensure its continued safe performance. Table 8 provides a checklist of safety equipment designed to eliminate or reduce certain hazards and briefly outlines the safety features.

Pipetting aids
A pipetting aid must always be used for pipetting procedures. Mouth pipetting is strictly forbidden.

The importance of pipetting aids cannot be overemphasized. The most common hazards associated with pipetting procedures are the result of mouth suction. Oral aspiration and ingestion of hazardous materials have been responsible for many laboratory-associated infections.

Pathogens can also be transferred to the mouth if a contaminated finger is placed on the suction end of a pipette. A lesser known hazard of mouth pipetting is the inhalation of aerosols caused by suction. The cotton plug is not an efficient microbial filter at negative or positive pressure, and particles may be sucked through it. Violent suction may be applied when the plug is tightly packed, resulting in the aspiration of plug, aerosol and even liquid. The ingestion of pathogens is prevented by the use of pipetting aids.

Aerosols can also be generated when a liquid is dropped from a pipette on to a work surface, when cultures are mixed by alternate sucking and blowing, and when the last drop is blown out of a pipette. The inhalation of aerosols unavoidably generated during pipetting operations can be prevented by working in a biological safety cabinet.

Pipetting aids should be selected with care. Their design and use should not create an additional infectious hazard and they should be easy to sterilize and clean. Plugged (aerosol-resistant) pipette tips should be used when manipulating microorganisms and cell cultures.

Pipettes with cracked or chipped suction ends should not be used as they damage the seating seals of pipetting aids and so create a hazard.

Homogenizers, shakers, blenders and sonicators
Domestic (kitchen) homogenizers are not sealed and release aerosols. Only equipment designed for laboratory use should be used. Their construction minimizes or prevents such release. Stomachers, which are now available for use with large and small volumes, may also produce aerosols.

Sonicators may release aerosols. They should be operated in biological safety cabinets or covered with shields during use. The shields and outsides of sonicators should be decontaminated after use.

Disposable transfer loops
The advantage of disposable transfer loops is that they do not have to be sterilized and can therefore be used in biological safety cabinets where Bunsen burners and microincinerators would disturb the airflow. These loops should be placed in disinfectant after use and discarded as contaminated waste.

Microincinerators
Gas- and electrically-heated microincinerators have borosilicate glass or ceramic shields that minimize the spatter and dispersal of infected material when transfer loops are sterilized. However, microincinerators can disturb the airflow and should therefore be placed towards the back of the work surface in biological safety cabinets.
<table>
<thead>
<tr>
<th>EQUIPMENT</th>
<th>HAZARD CORRECTED</th>
<th>SAFETY FEATURES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological safety cabinet</td>
<td></td>
<td>Minimum inward airflow at work access opening. Adequate filtration of exhaust air. Does not provide product protection.</td>
</tr>
<tr>
<td>Class I</td>
<td>Aerosol and spatter</td>
<td>Minimum inward airflow at work access opening. Adequate filtration of exhaust air. Does provide product protection.</td>
</tr>
<tr>
<td>Class II</td>
<td>Aerosol and spatter</td>
<td>Maximum containment Provides product protection if laminar flow air is included</td>
</tr>
<tr>
<td>Class III</td>
<td>Aerosol and spatter</td>
<td>Maximum containment especially in a field setting</td>
</tr>
<tr>
<td>Negative pressure flexible-film isolator</td>
<td>Aerosol and spatter</td>
<td>Maximum containment especially in a field setting</td>
</tr>
<tr>
<td>Spatter shield</td>
<td>Spatter</td>
<td>Forms screen between operator and work</td>
</tr>
<tr>
<td>Pipetting aids</td>
<td>Hazards from pipetting by mouth, e.g. ingestion of pathogens Inhalation of aerosols produced by suction on pipette Blowing liquid out of pipette Contamination of suction end by fingers etc.</td>
<td>Ease of use Controls contamination of suction end of pipette, protecting pipetting aid, user and vacuum line Can be sterilized Controls leakage from pipette tip</td>
</tr>
<tr>
<td>Loop microincinerators</td>
<td>Spatter from transfer loops</td>
<td>Shielded in open–ended tube. Heated by gas or electricity.</td>
</tr>
<tr>
<td>Disposable loops</td>
<td></td>
<td>Disposable, no heating necessary</td>
</tr>
<tr>
<td>Leakproof vessels for collection and in-house transport of infectious materials for sterilization</td>
<td>Aerosols, spillage and leakage</td>
<td>Leakproof construction with lid or cover Durable Autoclavable</td>
</tr>
<tr>
<td>Sharps disposal containers</td>
<td>Puncture wounds</td>
<td>Autoclavable Robust, puncture-proof</td>
</tr>
<tr>
<td>Transport containers between labs, institutions</td>
<td>Release of microorganisms</td>
<td>Robust Watertight primary and secondary containers to control spills Absorbent material to contain spills</td>
</tr>
<tr>
<td>Autoclaves</td>
<td>Infectious material (made safe for disposal or reuse)</td>
<td>Approved design Effective heat sterilization</td>
</tr>
<tr>
<td>Screw-capped bottles</td>
<td>Aerosol and spillage</td>
<td>Effective containment</td>
</tr>
<tr>
<td>Vacuum line protection</td>
<td>Contamination of lab vacuum system with aerosols and overflow fluids</td>
<td>Cartridge-type filter prevents passage of aerosols (particle size 0.45 microns) Overflow flask contains appropriate disinfectant. Entire unit autoclavable</td>
</tr>
</tbody>
</table>
Personal protective equipment and clothing
Personal protective equipment and clothing may act as a barrier to minimize the risk of exposure to aerosols, splashes and accidental inoculation. The clothing and equipment selected is dependent on the nature of the work performed. Protective clothing should be worn when working in the laboratory. Before leaving the laboratory, protective clothing should be removed, and hands should be washed. Table 9 summarizes some personal protective equipment used in laboratories and the protection afforded.

Laboratory coats, gowns, coveralls, aprons
Laboratory coats should preferably be fully buttoned. However, long-sleeved, back opening gowns or coveralls give better protection than laboratory coats and are preferred in microbiology laboratories and when working at the biological safety cabinet. Aprons may be worn over laboratory coats or gowns where necessary to give further protection against spillage of chemicals or biological materials such as blood or culture fluids. Laundering services should be provided at/near the facility.

Laboratory coats, gowns, coveralls, or aprons should not be worn outside the laboratory areas.

Goggles, safety spectacles, face shields
The choice of equipment to protect the eyes and face from splashes and impacting objects will depend on the activity performed. Prescription or plain eye glasses can be manufactured with special frames that allow lenses to be placed in frame from the front, using shatterproof material either curved or fitted with side shields (safety glasses). Safety spectacles do not provide adequate splash protection even when side shields are worn with them. Goggles for splash and impact protection should be worn over normal prescription eye glasses and contact lenses (which do not provide protection against biological or chemical hazards). Face shields (visors) are made of shatterproof plastic, fit over the face and are held in place by head straps or caps. Goggles, safety spectacles, or face shields should not be worn outside the laboratory areas.

Respirators
Respiratory protection may be used when carrying out high-hazard procedures (e.g. cleaning up a spill of infectious material). The choice of respirator will depend on the type of hazard(s). Respirators are available with interchangeable filters for protection against gases, vapors, particulates and microorganisms. It is imperative that the filter is fitted in the correct type of respirator. To achieve optimal protection, respirators should be individually fitted to the operator's face and tested. Contact the Chemical Hygiene Officer for fit testing. Fully self-contained respirators with an integral air supply provide full protection. Advice should be sought from a suitably qualified person, e.g. an industrial hygienist, for selection of the correct respirator. Surgical type masks are designed solely for patient protection and do not provide respiratory protection to workers. Some single-use disposable respirators have been designed for protection against exposures to biological agents. N95 particulate respirators are a good choice for work conducted in BSL-1 and BSL-2 labs. N95’s filter at least 95% of airborne particles that do not contain oil. No respirators should be worn outside the laboratory areas.

Gloves
Contamination of hands may occur when laboratory procedures are performed. Hands are also vulnerable to “sharps” injuries. Disposable microbiologically approved latex, vinyl or nitrile surgical-type gloves are used widely for general laboratory work, and for handling infectious agents and blood and body fluids.

Gloves should be removed and hands thoroughly washed after handling infectious materials, working in a biological safety cabinet and before leaving the laboratory. Used disposable gloves should be discarded with infected laboratory wastes.

Allergic reactions such as dermatitis and immediate hypersensitivity have been reported in laboratory and other workers wearing latex gloves, particularly those with powder. Alternatives to powdered latex gloves should be available.
Stainless steel mesh gloves should be worn when there is a potential exposure to sharp instruments e.g. during postmortem examinations. Such gloves protect against slicing motion but do not protect against puncture injury.

Gloves should not be worn outside the laboratory areas.

Table 9. **Personal protective equipment**

<table>
<thead>
<tr>
<th>EQUIPMENT</th>
<th>HAZARD CORRECTED</th>
<th>SAFETY FEATURES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab coats, gowns, coveralls</td>
<td>Contamination of clothing</td>
<td>Cover street clothing</td>
</tr>
<tr>
<td>Plastic aprons</td>
<td>Contamination of clothing</td>
<td>Waterproof</td>
</tr>
<tr>
<td>Footwear</td>
<td>Impact and splash</td>
<td>Closed-toe</td>
</tr>
<tr>
<td>Goggles</td>
<td>Impact and splash</td>
<td>Impact resistant lenses</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Side shields</td>
</tr>
<tr>
<td>Safety glasses</td>
<td>Impact</td>
<td>Impact resistant lenses</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Side shields</td>
</tr>
<tr>
<td>Face shields</td>
<td>Impact and splash</td>
<td>Shield entire face</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Easily removable in case of accident</td>
</tr>
<tr>
<td>Respirators</td>
<td>Inhalation of aerosols</td>
<td>Designs available include single-use disposable; full-face or half-face air purifying; full-face or hooded power air purifying (PAPR); and supplied air respirators</td>
</tr>
<tr>
<td>Gloves</td>
<td>Direct contact with microorganisms</td>
<td>Disposable microbiologically approved latex, vinyl or nitrile</td>
</tr>
<tr>
<td></td>
<td>Cuts</td>
<td>Hand protection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mesh</td>
</tr>
</tbody>
</table>
11. Laboratory Techniques

Human error, poor laboratory techniques and misuse of equipment cause the majority of laboratory injuries and work-related infections. This chapter provides a summary of technical methods that are designed to avoid or minimize the most commonly reported problems of this nature.

Safe handling of specimens in the laboratory
Improper collection, transport and handling of specimens in the laboratory carry a risk of infection to the personnel involved.

Specimen containers
Specimen containers may be of glass or preferably plastic. They should be robust and should not leak when the cap or stopper is correctly applied. No material should remain on the outside of the container. Containers should be correctly labeled to facilitate identification.

Transport of specimens between labs or buildings
The transport of specimens on campus should consist of a two container system: a sealed primary container and a sealed secondary container. If transporting liquid, some kind of absorbent packing such as paper towels should be inserted between the primary and secondary receptacle. To avoid accidental leakage or spillage of tubes, secondary containers, such as boxes, should be large enough for racks so that the specimen containers remain upright. A biohazard sticker should be affixed to the secondary container with the agent name and contact information of responsible person. The secondary containers may be of metal or plastic, should be autoclavable or resistant to the action of chemical disinfectants, and the seal should preferably have a gasket. Decontaminate the outside of the primary container before placing into the secondary container. Decontaminate the secondary container before leaving the laboratory.

Opening packages
Personnel who receive and unpack specimens should be aware of the potential health hazards involved and should be trained to adopt universal precautions, particularly when dealing with broken or leaking containers. Primary specimen containers should be opened in a biological safety cabinet. Disinfectants should be available.

Use of pipettes and pipetting aids

1. A pipetting aid must always be used. Pipetting by mouth is prohibited.
2. All pipettes should have cotton plugs to reduce contamination of pipetting devices.
3. Air should never be blown through a liquid containing infectious agents.
4. Infectious materials should not be mixed by alternate suction and expulsion through a pipette.
5. Liquids should not be forcibly expelled from pipettes.
6. Mark-to-mark pipettes are preferable to other types as they do not require expulsion of the last drop.
7. Contaminated pipettes should be completely submerged in a suitable disinfectant contained in an unbreakable container. They should be left in the disinfectant for the appropriate length of time before disposal.
8. A discard container for pipettes should be placed within the biological safety cabinet, not outside it.
9. Syringes fitted with hypodermic needles must not be used for pipetting.
10. Devices for opening septum-capped bottles that allow pipettes to be used and avoid the use of hypodermic needles and syringes should be used.
11. To avoid dispersion of infectious material dropped from a pipette, an absorbent material should be placed on the working surface; this should be disposed of as infectious waste after use.
12. Discharge from pipettes should be as close as possible to the fluid or agar level, or the contents should be allowed to run down the wall of the tube or bottle and not dropped from a height.
Avoiding the dispersal of infectious materials

1. In order to avoid the premature shedding of their loads, microbiological transfer loops should have a diameter of 2–3 mm and be completely closed. The shanks should be fairly short in length to minimize vibration.
2. The risk of spatter of infectious material in an open Bunsen burner flame should be avoided by using an enclosed electric microincinerator to sterilize transfer loops. Disposable transfer loops are preferable.
3. Discarded specimens and cultures for autoclaving and/or disposal should be placed in leakproof containers, e.g. laboratory discard bags. Tops should be secured (e.g. with autoclave tape) prior to disposal into waste containers.
4. Working areas must be decontaminated with a suitable disinfectant at the end of each work period.

Opening culture plates, tubes, bottles; Inoculating and harvesting cultures

It is generally better to gently swirl a liquid culture rather than vigorously shake to reduce the amount of aerosol produced. The insertion of a sterile, hot wire loop or needle into a liquid or slant culture can cause spattering and release of an aerosol. To minimize aerosol production, the loop should be allowed to cool. Disposable inoculating loops are best since any type of heating is not required. If disposable loops are not available then the use of electric or gas incinerators with the ceramic shielding is preferred over an open flame.

Streaking inoculum on rough agar can also generate an aerosol due to the vibrating loop or needle. This is rarely seen with smooth agar so it is best to discard all rough agar plates that are intended for culture isolation.

Water that accumulates around the edges of petri dish cultures usually contains viable organisms and forms a film between the rim and lid of the inverted plate. Aerosols are dispersed when this film is broken by opening the plate. The risk may be minimized by using properly dried plates but liquid still may be formed due to the incubation conditions.

Less obvious is the release of aerosols when screw-capped bottles or plugged tubes are opened. This happens when a film of contaminated liquid which may collect between the rim and the liner, is broken during removal of the closure. The practice of removing cotton plugs or other closures from flasks, bottles, centrifuge tubes, etc., immediately following shaking or centrifugation can cause environmental contamination. The technique of shaking tissue cultures with glass beads to release viruses can create a virus-laden aerosol. Removal of wet closures, which can occur if the flask or centrifuge tube is not held in an upright position, is also hazardous. In addition, when using the centrifuge, there may be a small amount of foaming, and the closures may become slightly moistened. Because of these possibilities, it is good safety practice to open all liquid cultures of infectious or hazardous material in a biological safety cabinet wearing gloves and a long-sleeved laboratory garment.

Dried, infectious culture material may also collect at or near the rim or neck of culture tubes and may be dispersed into the air when disturbed. Containers of dry powdered hazardous materials should be opened only in a biological safety cabinet.

Harvesting cultures from embryonated eggs is a hazardous procedure and leads to heavy surface contamination of the egg trays, shells, the environment, and the hands of the operator. All operations should occur in a biological safety cabinet with frequent use of a suitable disinfectant.

Use of biological safety cabinets

1. The use and limitations of biological safety cabinets should be explained to all potential users with reference to national standards and relevant literature. Written protocols or safety or operations manuals should be issued to staff. In particular, it must be made clear that the cabinet will not protect the operator from spillage, breakage or poor technique.
2. The cabinet must not be used unless it is working properly.
3. The glass viewing panel must not be opened when the cabinet is in use.
4. Apparatus and materials in the cabinet must be kept to a minimum. Air circulation at the rear plenum must not be blocked.
5. Bunsen burners must not be used in the cabinet. The heat produced will distort the airflow and may damage the filters. An electric microincinerator is permissible but sterile disposable transfer loops are better.
6. All work must be carried out in the middle or rear part of the working surface and be visible through the viewing panel.
7. Traffic behind the operator should be minimized.
8. The operator should not disturb the airflow by repeated removal and reintroduction of his or her arms.
9. Air grills must not be blocked with notes, pipette s or other materials, as this will disrupt the airflow causing potential contamination of the material and exposure of the operator.
10. The surface of the biological safety cabinet should be wiped using an appropriate disinfectant after work is completed and at the end of the day.
11. The cabinet fan should be run for at least 5 min before beginning work and after completion of work in the cabinet.
12. Paperwork should never be placed inside biological safety cabinets.

Avoiding ingestion of infectious materials and contact with skin and eyes

1. Large particles and droplets (> 5 microns in diameter) released during microbiological manipulations settle rapidly on bench surfaces and on the hands of the operator. Disposable gloves should be worn. Laboratory workers should avoid touching their mouth, eyes and face.
2. Food and drink must not be consumed or stored in the laboratory.
3. No articles should be placed in the mouth – pens, pencils, chewing gum – in the laboratory.
4. Cosmetics should not be applied in the laboratory.
5. The face, eyes and mouth should be shielded or otherwise protected during any operation that may result in the splashing of potentially infectious materials.

Avoiding injection of infectious materials

1. Accidental inoculation resulting from injury with broken or chipped glassware can be avoided through careful practices and procedures. Glassware should be replaced with plastic ware whenever possible.
2. Accidental injection may result from sharps injuries e.g. with hypodermic needles (needle-sticks), glass Pasteur pipettes, or broken glass.
3. Needle-stick injuries can be reduced by: (a) minimizing the use of syringes and needles (e.g. simple devices are available for opening septum-stoppered bottles so that pipettes can be used instead of syringes and needles; or (b) using engineered sharp safety devices when syringes and needles are necessary.
4. Needles should never be recapped. Disposable articles should be discarded into puncture-proof/puncture-resistant containers fitted with covers.
5. Plastic Pasteur pipettes should replace those made of glass.
6. Fill the syringe carefully to minimize air bubbles and frothing of the inoculum.
7. Expel excess air and liquid from the syringe vertically into a disinfectant-soaked piece of gauze or cotton.
8. When removing a syringe and needle from a rubber-stoppered bottle, wrap the needle and stopper in a disinfectant-soaked piece of gauze or cotton. If there is a danger of the disinfectant contaminating sensitive experimental materials, use a sterile dry piece of gauze then discard immediately into a disinfectant solution.
Separation of serum

1. Only properly trained staff should be employed for this work.
2. Gloves and eye and mucous membrane protection should be worn.
3. Splashes and aerosols can only be avoided or minimized by good laboratory technique. Blood and serum should be pipetted carefully, not poured. Pipetting by mouth is forbidden.
4. After use, pipettes should be completely submerged in suitable disinfectant. They should remain in the disinfectant for the appropriate time before disposal or sterilization and washing for reuse.
5. Discarded specimen tubes containing blood clots, etc. (with caps replaced) should be placed in suitable leakproof containers for autoclaving and/or incineration.
6. Suitable disinfectants should be available for clean-up of splashes and spillages.

Use of centrifuges

Centrifugation presents two serious hazards: mechanical failure and dispersal of aerosols.

1. Satisfactory mechanical performance is a prerequisite of microbiological safety in the use of laboratory centrifuges.
2. Centrifuges should be operated according to the manufacturer’s instructions.
3. Centrifuges should be placed at such a level that workers can see into the bowl to place trunnions and buckets correctly.
4. Centrifuge tubes and specimen containers for use in the centrifuge should be made of thick-walled glass or preferably of plastic and should be inspected for defects before use.
5. Tubes and specimen containers should always be securely capped (screw-capped if possible) for centrifugation.
6. The buckets must be loaded, equilibrated, sealed and opened in a biological safety cabinet.
7. Buckets and trunnions should be paired by weight and, with tubes in place, correctly balanced.
8. The amount of space that should be left between the level of the fluid and the rim of the centrifuge tube should be given in manufacturer’s instructions.
9. Distilled water or alcohol (propanol, 70%) should be used for balancing empty buckets. Saline or hypochlorite solutions should not be used as they corrode metals.
10. Sealable centrifuge buckets (safety cups) must be used for microorganisms in Risk Groups 3 and 4.
11. When using angle-head centrifuge rotors, care must be taken to ensure that the tube is not overloaded as it might leak.
12. The interior of the centrifuge bowl should be inspected daily for staining or soiling at the level of the rotor. If staining or soiling are evident then the centrifugation protocols should be re-evaluated.
13. Centrifuge rotors and buckets should be inspected daily for signs of corrosion and for hair-line cracks.
14. Buckets, rotors and centrifuge bowls should be decontaminated after each use.
15. After use, buckets should be stored in an inverted position to drain the balancing fluid.
16. Infectious airborne particles may be ejected when centrifuges are used. These particles travel at speeds too high to be retained by the cabinet airflow if the centrifuge is placed in a traditional open-fronted Class I or Class II biological safety cabinet. Enclosing centrifuges in Class III safety cabinets prevents emitted aerosols from dispersing widely. However, good centrifuge technique and securely capped tubes offer adequate protection against infectious aerosols and dispersed particles.
17. Avoid pouring the supernatant from the tube, but instead use a pipette to remove. If you must decant, wipe off the outer rim with a disinfectant afterwards otherwise hazardous material may be spun off as droplets that form an aerosol or the droplet serves as a source of contamination during subsequent handling of the tube.
Use of homogenizers, shakers, blenders and sonicators

1. Domestic (kitchen) homogenizers should not be used in laboratories as they may leak or release aerosols. Laboratory blenders and stomachers are safer.
2. Caps and cups or bottles should be in good condition and free from flaws or distortion. Caps should be well-fitting and gaskets should be in good condition.
3. Pressure builds up in the vessel during the operation of homogenizers, shakers and sonicators. Aerosols containing infectious materials may escape from between the cap and the vessel. Plastic, in particular, polytetrafluoroethylene (PTFE) vessels are recommended because glass may break, releasing infectious material and possibly wounding the operator.
4. When in use, homogenizers, shakers and sonicators should be covered by a strong transparent plastic casing. This should be disinfected after use. Where possible, these machines should be operated, under their plastic covers, in a biological safety cabinet.
5. At the end of the operation the containers should be opened in a biological safety cabinet.
6. Hearing protection should be provided for people using sonicators.

Use of tissue grinders

1. Glass grinders should be held in absorbent material in a gloved hand. Plastic (PTFE) grinders are safer.
2. Tissue grinders should be operated and opened in a biological safety cabinet.

Care and use of refrigerators and freezers

1. Refrigerators, deep-freezers and solid carbon dioxide (dry-ice) chests should be defrosted and cleaned periodically, and any ampules, tubes, etc. that have broken during storage removed. Face protection and heavy duty rubber gloves should be worn during cleaning. After cleaning, the inner surfaces of the cabinet should be disinfected.
2. All containers stored in refrigerators, etc. should be clearly labeled with the scientific name of the contents, the date stored and the name of the individual who stored them. Unlabeled and obsolete materials should be autoclaved and discarded.
3. An inventory of the freezer’s contents should be maintained.
4. Flammable solutions must not be stored in a refrigerator unless it is explosion proof. Notices to this effect should be placed on refrigerator doors.

Opening of ampules containing lyophilized infectious materials

Care should be taken when ampules of freeze-dried materials are opened, as the contents may be under reduced pressure and the sudden inrush of air may disperse some of the materials into the atmosphere. Ampules should always be opened in a biological safety cabinet. The following procedures are recommended for opening ampules.

1. First decontaminate the outer surface of the ampule.
2. Make a file mark on the tube near to the middle of the cotton or cellulose plug, if present.
3. Hold the ampule in alcohol-soaked cotton to protect hands before breaking it at a file scratch.
4. Remove the top gently and treat as contaminated material.
5. If the plug is still above the contents of the ampule, remove it with sterile forceps.
6. Add liquid for resuspension slowly to the ampule to avoid frothing.

Storage of ampules containing infectious materials

Ampules containing infectious materials should never be immersed in liquid nitrogen because cracked or imperfectly sealed ampules may break or explode on removal. If very low temperatures are required, ampules should be stored only in the gaseous phase above the liquid nitrogen. Otherwise, infectious materials should be stored in mechanical deep-freeze cabinets or on dry ice. Laboratory workers should wear eye and hand protection when removing ampules from cold storage. The outer surfaces of ampules stored in these ways should be disinfected when the ampules are removed from storage.
Standard Precautions with Blood and Other Body Fluids, Tissues and Excreta
Standard precautions (also known as “universal precautions”) are designed to reduce the risk of transmission of microorganisms from both recognized and unrecognized sources of infection.

Collection, labeling and transport of specimens

1. Standard precautions should always be followed; gloves should be worn for all procedures.
2. Blood should be collected from patients and animals by trained staff.
3. For phlebotomies, conventional needle and syringe systems should be replaced by single-use safety vacuum devices that allow the collection of blood directly into stoppered transport and/or culture tubes, automatically disabling the needle after use.
4. The tubes should be placed in adequate containers for transport to the laboratory and within the laboratory facility.

Opening specimen tubes and sampling contents

1. Specimen tubes should be opened in a biological safety cabinet.
2. Gloves must be worn. Eye and mucous membrane protection is also recommended (goggles or face shields).
3. Protective clothing should be supplemented with a plastic apron.
4. The stopper should be grasped through a piece of paper or gauze to prevent splashing.

Glass and “sharps”

1. Plastics should replace glass wherever possible. Only laboratory grade (borosilicate) glass should be used, and any article that is chipped or cracked should be discarded.
2. Hypodermic needles must not be used as pipettes.

Films and smears for microscopy
Fixing and staining of blood and tissue samples for microscopy do not necessarily kill all organisms or viruses on the smears. These items should be handled with forceps, stored appropriately, and decontaminated and/or autoclaved before disposal.

Automated equipment (sonicators, vortex mixers)

1. Equipment should be of the closed type to avoid dispersion of droplets and aerosols.
2. Effluents should be collected in closed vessels for further autoclaving and/or disposal.
3. Equipment should be disinfected at the end of each session, following manufacturers’ instructions.

Tissues

1. Formalin fixatives should be used.
2. Frozen sectioning should be avoided. When necessary, the cryostat should be shielded and the operator should wear a safety face shield.

Precautions with materials that may contain prions
Prions are associated with the transmissible spongiform encephalopathies (TSEs), notably Creutzfeldt-Jakob disease (CJD; including the new variant form), Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia and kuru in humans; scrapie in sheep and goats; bovine spongiform encephalopathy (BSE) in cattle; and other transmissible encephalopathies of deer, elk and mink. Although CJD has been transmitted to humans, there appear to be no proven cases of laboratory-associated infections with any of these agents. Nevertheless, it is prudent to observe certain precautions in the handling of material from infected or potentially infected humans and animals.
The selection of a biosafety level for work with materials associated with TSEs will depend on the nature of the agent and the samples to be studied, and should be undertaken in consultation with federal authorities. The highest concentrations of prions are found in central nervous system tissue. Animal studies suggest that it is likely that high concentrations of prions are also found in the spleen, thymus, lymph nodes and lung. Recent studies indicate that prions in lingual and skeletal muscle tissue may also present a potential infection risk.

As complete inactivation of prions is difficult to achieve, it is important to stress the use of disposable instruments whenever possible, and to use a disposable protective covering for the work surface of the biological safety cabinet. The main precaution to be taken is to avoid ingestion of contaminated materials or puncture of the laboratory worker’s skin. The following additional precautions should be taken, as the agents are not killed by the normal processes of laboratory disinfection and sterilization.

1. The use of dedicated equipment, i.e. equipment not shared with other laboratories, is highly recommended.
2. Disposable laboratory protective clothing (gowns and aprons) and gloves must be worn (steel mesh gloves between rubber gloves for pathologists).
3. Use of disposable plastic ware, which can be treated and discarded as dry waste, is highly recommended.
4. Tissue processors should not be used because of the problems of disinfection. Jars and beakers (plastic) should be used instead.
5. All manipulations must be conducted in biological safety cabinets.
6. Great care should be exercised to avoid aerosol production, ingestion, and cuts and punctures of the skin.
7. Formalin-fixed tissues should be regarded as still infectious, even after prolonged exposure to formalin.
8. Histological samples containing prions are substantially inactivated after exposure to 96% formic acid for 1 hour followed by 48 hours in fresh 10% formalin.
9. Bench waste, including disposable gloves, gowns and aprons, should be autoclaved using a porous load steam sterilizer at 134–137°C for a single cycle of 18 min, or six successive cycles of 3 min each, followed by incineration.
10. Non-disposable instruments, including steel mesh gloves, must be collected for decontamination.
11. Infectious liquid waste contaminated with prions should be treated with 1 N NaOH or sodium hypochlorite (20,000 ppm) for 1 hour. 20,000 ppm sodium hypochlorite equals a 2% solution. Most commercial household bleach contains 5.25% sodium hypochlorite therefore a 1:2.5 dilution (1 part 5.25% bleach plus 1.5 parts water) will produce a 20,000 ppm solution. Prepare fresh daily.
12. Paraformaldehyde vaporization procedures do not diminish prion titers and prions are resistant to ultraviolet irradiation. However, the cabinets must continue to be decontaminated by standard methods (i.e. formaldehyde gas) to inactivate other agents that may be present.
13. Prion-contaminated biological safety cabinets and other surfaces can be decontaminated with 1 N NaOH or sodium hypochlorite (20,000 ppm) for 1 hour.
14. High-efficiency particulate air (HEPA) filters should be incinerated at a minimum temperature of 1000 °C after removal. Recommended additional steps prior to incineration include:
   a. spraying of the exposed face of the filter with lacquer hairspray prior to removal,
   b. “bagging” of filters during removal, and
   c. removal of the HEPA filter from the working chamber so that the inaccessible plenum of the cabinet is not contaminated.
15. Instruments should be soaked in 1 N NaOH or sodium hypochlorite (20,000 ppm) for 1 hour and then rinsed well in water before autoclaving.
16. Instruments that cannot be autoclaved can be cleaned by repeated wetting with 1 N NaOH or sodium hypochlorite (20,000 ppm) for 1 hour. Appropriate washing to remove residual sodium hypochlorite or NaOH is required.
Use of human and nonhuman primate cell and/or tissue culture

The potential hazards associated with the handling of human/nonhuman primate cell culture are mainly the contamination of the cells with pathogenic agents and/or the tumorigenicity of the cells.

Of the pathogenic agents, viruses are of particular concern. Other agents such as bacteria, fungi, and mycoplasmas generally cause some kind of visual effect on the cells or culture media allowing for detection of contamination prior to work with the cells. However, many viruses do not cause cytopathic effect (CPE), can be latent or are undetectable with current technology.

Contamination of the cell culture may stem from the donor (endogenous) or from contamination by the user or from the materials used in the culture process (e.g. serum, proteins, fetal extracts, hormones, etc.).

Human cell lines are most likely to be contaminated with the highly pathogenic viruses including hepatitis B virus and HIV (human immunodeficiency virus). It must be understood though, that primate and other mammalian cell lines can harbor viruses with a broad host range. Primate cells can contain dangerous pathogens, most notably herpes B virus and Marburg virus both of which have caused fatal infections in humans. Rodent cell lines can carry lymphocytic choriomeningitis virus, Reo-3 virus and hantavirus with documented cases of human disease and death.

Some of the bovine sourced culture media products may contain bovine viral diarrhea virus (BVDV), infectious bovine rhinotracheitis virus, and parainfluenza virus type 3. BVDV can bind to cells from many species and noncytopathic strains may establish persistent infections in bovine and nonbovine cell lines.

Oncogenic viruses (e.g. Hepatitis B and C viruses, HIV, Epstein Barr virus (EBV), human T-lymphotropic virus (HTLV), human herpesvirus 8, simian virus 40) are able to transform cells into malignant forms. HTLV-1 is known to be oncogenic for humans and others are known to be oncogenic for primates including feline sarcoma virus, EBV, and the human papillomavirus.

The other hazard associated with human cell lines is the tumorigenicity of continuous cell lines. There has been one reported case of a laboratory worker who developed a tumor after an accidental needlestick with a human colonic adenocarcinoma cell line.

In 1991, the Occupational Safety and Health Administration (OSHA) issued the Bloodborne Pathogens (BBP) Standard to protect employees who have occupational exposure to human blood or other potentially infectious materials. While human blood, most body fluids, unfixed human tissues and organs were clearly included within the scope and application of the standard, the inclusion of human cell lines was ambiguous.

In 1994, OSHA issued an interpretation of the applicability of the BBP Standard towards human cell lines. According to the interpretation, human cell lines are considered to be potentially infectious and within the scope of the BBP Standard unless the specific cell line has been characterized to be free of hepatitis viruses, HIV, Epstein-Barr virus, papilloma viruses and other recognized bloodborne pathogens. In alignment with this interpretation, the American Type Culture Collection (ATCC) recommends that all human cell lines be accorded the same level of biosafety consideration as a line known to carry HIV (BSL-2). Moreover, the 5th Edition of the NIH/CDC publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) recommends that human and other primate cells should be handled using Biosafety Level 2 (BSL2) practices and containment.

Please read the MSU policy on the use of human and nonhuman primate cell lines available on the biosafety web page.

If you are using or plan to use human or nonhuman primate cell lines, you may be required to do the following:
1. Submit an IBC application.
2. Take bloodborne pathogen training
3. Conduct the work with the cell line is a BSL-2 certified laboratory.

The CDC’s stance on cell cultures taken from the 5th edition of the BMBL:

“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,\textsuperscript{13,14} the inadvertent transmission of disease to organ recipients,\textsuperscript{15,16} and the persistence of human immunodeficiency virus (HIV), HBV, and hepatitis C virus (HCV) within infected individuals in the U.S. population.\textsuperscript{17} 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.\textsuperscript{18} 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.\textsuperscript{19} 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.\textsuperscript{17}


A Note About ATCC: ATCC \textbf{DOES NOT} test their cell lines for the presence of human viruses. Because of this ATCC recommends that all cell lines be treated at the same level as a line known to contain HIV which is BSL-2. Also, the biosafety designation that the ATCC gives to their lines refers to the shipping requirements not the laboratory requirements. So if a cell line is rated BSL-1, that means it can be shipped as a biological substance, category B, UN 3373 per DOT/IATA. Category B substances are those that generally are not capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals upon exposure.

It is best to consult with the BSO to determine the appropriate actions.

\textbf{Use of water baths}

Water baths used to inactivate, incubate, or test infectious substances should contain a disinfectant. For cold water baths, 70% propylene glycol is recommended. Sodium azide should not be used as a bacteriostatic agent. It creates a serious explosion hazard.

\textbf{Use of shaking incubators}

A variety of shaking machines are commercially available for aerating and mixing cultures, disrupting cells, culturing or homogenizing tissues, and mixing reactants in serological and biochemical studies. Since there is the possibility of glass breakage, closures becoming loose or dislodged, and the leakage of containers with the consequent release of aerosols or liquids to the environment, these machines should be used with caution and examined carefully for possible hazards associated with their use. Screw capped durable plastic or heavy walled glass containers should be used. If used for handling infectious or
hazardous materials, the flasks, bottles, tubes, etc., should be held securely in place without undue strain on the container in leakproof trays. A plastic bag with or without absorbent material could be used to enclose the container as an additional safety precaution unless aeration requirements restrict enclosure of this type.

To prevent escape of infectious microorganisms during shaking, stoppers and cotton plugs of containers should be held in place with tape. Screw caps can be modified to allow diffusion of gases by drilling out the top and inserting an appropriate filter pad between the cap and the gasket.
12. Contingency Plans and Emergency Procedures

Every laboratory that works with biohazardous materials should institute safety precautions appropriate to the hazard being handled.

Contingency plan
The contingency plan should provide operational procedures for:
1. Precautions against natural disasters, e.g. fire, flood, tornado and explosion
2. Biohazard risk assessment
3. Incident-exposure management and decontamination
4. Emergency evacuation of people and animals from the premises
5. Emergency medical treatment of exposed and injured persons
6. Medical surveillance of exposed persons
7. Clinical management of exposed persons
8. Epidemiological investigation

In the development of this plan the following items should be considered for inclusion:
1. Identification of high-risk organisms
2. Location of high-risk areas, e.g. laboratories, storage areas, animal facilities
3. Identification of at-risk personnel and populations
4. Identification of responsible personnel and their duties, e.g. biosafety officer, safety personnel, local health authority, clinicians, microbiologists, veterinarians, epidemiologists, and fire and police services
5. Lists of treatment and isolation facilities that can receive exposed or infected persons
6. Transport of exposed or infected persons
7. Lists of sources of immune serum, vaccines, drugs, special equipment and supplies
8. Provision of emergency equipment, e.g. protective clothing, disinfectants, chemical and biological spill kits, decontamination equipment and supplies.

Emergency Procedures for Microbiological Laboratories

Puncture wounds, cuts and abrasions
The affected individual should remove protective clothing, wash the hands and any affected area(s), apply an appropriate skin disinfectant, and seek medical attention as necessary. The cause of the wound and the organisms involved should be reported, and appropriate and complete medical records kept.

Ingestion of potentially infectious material
Protective clothing should be removed and medical attention sought. Identification of the material ingested and circumstances of the incident should be reported, and appropriate and complete medical records kept.

Potentially infectious aerosol release (outside a biological safety cabinet)
All persons should immediately vacate the affected area and any exposed persons should be referred for medical advice. The laboratory supervisor and the biosafety officer should be informed at once. No one should enter the room for an appropriate amount of time (e.g. 1 h), to allow aerosols to be carried away and heavier particles to settle. If the laboratory does not have a central air exhaust system, entrance should be delayed (e.g. for 24 h).

Signs should be posted indicating that entry is forbidden. After the appropriate time, decontamination should proceed, supervised by the biosafety officer. Appropriate protective clothing and respiratory protection should be worn.
**Broken containers and spilled infectious substances**

Broken containers contaminated with hazardous substances and spilled hazardous substances should be covered with a cloth or paper towels. Disinfectant should then be poured over these and left for the appropriate amount of time. The cloth or paper towels and the broken material can then be cleared away; glass fragments should be handled with forceps. The contaminated area should then be swabbed with disinfectant. If dustpans are used to clear away the broken material, they should be autoclaved or placed in an effective disinfectant. Cloths, paper towels and swabs used for cleaning up should be placed in a contaminated-waste container. Gloves should be worn for all these procedures.

If laboratory forms or other printed or written matter are contaminated, the information should be copied onto another form and the original discarded into the contaminated-waste container.

**Breakage of tubes containing potentially infectious material in centrifuges not having sealable buckets**

If a breakage occurs or is suspected while the machine is running, the motor should be switched off and the machine left closed (e.g. for 30 min) to allow settling. If a breakage is discovered after the machine has stopped, the lid should be replaced immediately and left closed (e.g. for 30 min). In both instances, the biosafety officer should be informed.

Strong (e.g. thick rubber) gloves, covered if necessary with suitable disposable gloves, should be worn for all subsequent operations. Forceps, or cotton held in the forceps, should be used to retrieve glass debris.

All broken tubes, glass fragments, buckets, trunnions and the rotor should be placed in a noncorrosive disinfectant known to be active against the organisms concerned. Unbroken, capped tubes may be placed in disinfectant in a separate container and recovered.

The centrifuge bowl should be swabbed with the same disinfectant, at the appropriate dilution, and then swabbed again, washed with water and dried. All materials used in the clean-up should be treated as infectious waste.

**Breakage of tubes inside sealable buckets (safety cups)**

All sealed centrifuge buckets should be loaded and unloaded in a biological safety cabinet. If breakage is suspected within the safety cup, the safety cap should be loosened and the bucket autoclaved. Alternatively, the safety cup may be chemically disinfected.

**Fire and natural disasters**

Fire and other services should be involved in the development of emergency preparedness plans. They should be told in advance which rooms contain potentially infectious materials. It is beneficial to arrange for these services to visit the laboratory to become acquainted with its layout and contents.

After a natural disaster, local or national emergency services should be warned of the potential hazards within and/or near laboratory buildings. They should enter only when accompanied by a trained laboratory worker. Biohazardous materials should be collected in leakproof boxes or strong disposable bags.

Contact the Biological Safety Officer for disposal options.
Emergency services: whom to contact
The telephone numbers and addresses of the following should be prominently displayed in the facility:
1. The institution or laboratory itself (the address and location may not be known in detail by the caller or the services called)
2. Director of the institution or laboratory
3. Laboratory supervisor
4. Responsible technician
5. Biosafety officer: 662-325-3294 or 662-325-0620
6. Fire services: 911
7. Hospitals/ambulance services/medical staff
   For emergencies: 911
   Oktibbeha County Hospital: 662-323-4320
8. Police
   For emergencies: 911
   MSU Police: 662-325-2121
   Starkville Police: 662-323-4131
   Oktibbeha County Sheriff’s Office: 662-323-2421
9. Medical Officer
   Longest Student Health Center: 662-325-2431
10. Water, gas and electricity services
    MSU Facilities Management: 662-325-2005

Emergency equipment
The following emergency equipment must be available:
1. First-aid kit
2. Appropriate fire extinguishers, fire blankets

The following are also suggested but may be varied according to local circumstances:
1. Full protective clothing
2. Full-face respirators with appropriate chemical and particulate filter canisters
3. Room disinfection apparatus, e.g. sprays and formaldehyde vaporizers
4. Stretcher
5. Tools, e.g. hammers, axes, spanners, screwdrivers, ladders, ropes
6. Hazard area demarcation equipment and notices.
13. Disinfection and Sterilization

A basic knowledge of disinfection and sterilization is crucial for biosafety in the laboratory. The following general principles apply to all known classes of microbial pathogens.

Specific decontamination requirements will depend on the type of experimental work and the nature of the material(s) being handled. The generic information given here can be used to develop both standardized and more specific procedures to deal with biohazard(s) involved in a particular laboratory.

Contact times for disinfectants are specific for each material and manufacturer. Therefore, all recommendations for use of disinfectants should follow manufacturers’ specifications.

Definitions
Many different terms are used for disinfection and sterilization. The following are among the more common in biosafety:

- **Antimicrobial** – An agent that kills microorganisms or suppresses their growth and multiplication.
- **Antiseptic** – A substance that inhibits the growth and development of microorganisms without necessarily killing them. Antiseptics are usually applied to body surfaces.
- **Biocide** – A general term for any agent that kills organisms.
- **Chemical germicide** – A chemical or a mixture of chemicals used to kill microorganisms.
- **Decontamination** – Any process for removing and/or killing microorganisms. The same term is also used for removing or neutralizing hazardous chemicals and radioactive materials.
- **Disinfectant** – A chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects.
- **Disinfection** – A physical or chemical means of killing microorganisms, but not necessarily spores.
- **Microbicide** – A chemical or mixture of chemicals that kills microorganisms. The term is often used in place of “biocide”, “chemical germicide” or “antimicrobial”.
- **Sporocide** – A chemical or mixture of chemicals used to kill microorganisms and spores.
- **Sterilization** – A process that kills and/or removes all classes of microorganisms and spores.

Chemical germicides
Many types of chemicals can be used as disinfectants and/or antiseptics. As there is an ever-increasing number and variety of commercial products, formulations must be carefully selected for specific needs.

The germicidal activity of many chemicals is faster and better at higher temperatures. At the same time, higher temperatures can accelerate their evaporation and also degrade them.

Many germicides can be harmful to humans or the environment. They should be selected, stored, handled, used and disposed of with care, following manufacturers’ instructions. For personal safety, gloves, aprons and eye protection are recommended when preparing dilutions of chemical germicides.

Chemical germicides are generally not required for regular cleaning of floors, walls, equipment and furniture. However, their use may be appropriate in cases of spills.

Proper use of chemical germicides will contribute to workplace safety while reducing the risk from infectious agents. As far as possible, the number of germicidal chemicals to be used should be limited for economic reasons, inventory control and to limit environmental pollution.

Commonly used classes of chemical germicides are described below, with generic information on their applications and safety profiles. Unless otherwise indicated, the germicide concentrations are given in weight/volume (w/v). Table 10 summarizes commonly used working dilutions of household bleach. Table 11 summarizes the recommended dilutions of other chlorine-releasing compounds.
Chlorine (sodium hypochlorite)
Chlorine, a fast-acting oxidant, is a widely available and broad-spectrum chemical germicide. It is normally sold as bleach, an aqueous solution of sodium hypochlorite (NaOCl), which can be diluted with water to provide various concentrations of available chlorine.

Chlorine, especially as bleach, is highly alkaline and can be corrosive to metal. Its activity is considerably reduced by organic matter (protein). Storage of stock or working solutions of bleach in open containers, particularly at high temperatures, releases chlorine gas thus weakening their germicidal potential. The frequency with which working solutions of bleach should be changed depends on their starting strength, the type (e.g. with or without a lid) and size of their containers, the frequency and nature of use, and ambient conditions. As a general guide, solutions receiving materials with high levels of organic matter several times a day should be changed at least daily, while those with less frequent use may last for as long as a week.

A general all-purpose laboratory disinfectant should have a concentration of 1 g/l available chlorine. A stronger solution, containing 5 g/l available chlorine, is recommended for dealing with biohazardous spillage and in the presence of large amounts of organic matter. Sodium hypochlorite solutions, as domestic bleach, contain 50 g/l available chlorine and should therefore be diluted 1:50 or 1:10 to obtain final concentrations of 1 g/l and 5 g/l, respectively. Industrial solutions of bleach have a sodium hypochlorite concentration of nearly 120 g/l and must be diluted accordingly to obtain the levels indicated above.

Granules or tablets of calcium hypochlorite (Ca(ClO)₂) generally contain about 70% available chlorine. Solutions prepared with granules or tablets, containing 1.4 g/l and 7.0 g/l, will then contain 1.0 g/l and 5 g/l available chlorine, respectively.

Bleach is not recommended as an antiseptic, but may be used as a general-purpose disinfectant and for soaking contaminated metal-free materials. In emergencies, bleach can also be used to disinfect water for drinking, with a final concentration of 1–2 mg/l available chlorine.

Chlorine gas is highly toxic. Bleach must therefore be stored and used in well ventilated areas only. Also, bleach must not be mixed with acids to prevent the rapid release of chlorine gas. Many by-products of chlorine can be harmful to humans and the environment, so that indiscriminate use of chlorine-based disinfectants, in particular bleach, should be avoided.

### Table 10. Household bleach dilutions

<table>
<thead>
<tr>
<th>Concentration of bleach</th>
<th>Concentration of sodium hypochlorite</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percent %</td>
</tr>
<tr>
<td>Undiluted</td>
<td>5.25</td>
</tr>
<tr>
<td>10 %</td>
<td></td>
</tr>
<tr>
<td>10 ml in 90 ml H₂O</td>
<td>0.525</td>
</tr>
<tr>
<td>1 %</td>
<td></td>
</tr>
<tr>
<td>1 ml in 99 ml H₂O</td>
<td>0.0525</td>
</tr>
</tbody>
</table>

### Table 11. Recommended dilutions of chlorine-releasing compounds

<table>
<thead>
<tr>
<th>Available chlorine required</th>
<th>“CLEAN” CONDITIONS[^a]</th>
<th>“DIRTY” CONDITIONS[^b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household bleach (5% available chlorine)</td>
<td>0.1% (1 g/l)</td>
<td>0.5% (5 g/l)</td>
</tr>
<tr>
<td>Households bleach l</td>
<td>20 ml/l</td>
<td>100 ml/l</td>
</tr>
</tbody>
</table>
Calcium hypochlorite (70% available chlorine) | 1.4 g/l | 7 g/l
Sodium dichloroisocyanurate powder (60% available chlorine) | 1.7 g/l | 8.5 g/l
Sodium dichloroisocyanurate tablets (1.5 g available chlorine per tablet) | 1 tablet per liter | 4 tabs per liter
Chloramine (25% available chlorine) | 20 g/l | 20 g/l

| After removal of bulk material | For flooding, e.g. on blood or before removal of bulk material | See text.

**Sodium dichloroisocyanurate**

Sodium dichloroisocyanurate (NaDCC) in powder form contains 60% available chlorine. Solutions prepared with NaDCC powder at 1.7 g/l and 8.5 g/l will contain 1 g/l or 5 g/l available chlorine, respectively. Tablets of NaDCC generally contain the equivalent of 1.5 g available chlorine per tablet. One or four tablets dissolved in 1 l of water will give approximately the required concentrations of 1 g/l or 5 g/l, respectively.

NaDCC as powder or tablets is easy and safe to store. Solid NaDCC can be applied on spills of blood or other biohazardous liquids and left for at least 10 min before removal. Further cleaning of the affected area can then take place.

**Chloramines**

Chloramines are available as powders containing about 25% available chlorine. Chloramines release chlorine at a slower rate than hypochlorites. Higher initial concentrations are therefore required for efficiencies equivalent to those of hypochlorites. On the other hand, chloramine solutions are not inactivated by organic matter to the same extent as hypochlorite solutions, and concentrations of 20 g/l are recommended for both “clean” and “dirty” situations.

Chloramine solutions are virtually odor-free. However, items soaked in them must be thoroughly rinsed to remove any residue of the bulking agents added to chloramine powders.

**Chlorine dioxide**

Chlorine dioxide (ClO₂) is a strong and fast-acting germicide, disinfectant agent and oxidizer, often reported to be active at concentrations levels lower than those needed by chlorine as bleach. Chlorine dioxide is unstable as a gas and will undergo decomposition into chlorine gas (Cl₂), oxygen gas (O₂), giving off heat. However, chlorine dioxide is soluble in water and stable in an aqueous solution. Chlorine dioxide can be obtained in two ways: (1) on-site generation by mixing of two separate components, hydrochloric acid (HCl) and sodium chlorite (NaClO₂); and (2) ordering its stabilized form, which is then activated on-site when required.

Of the oxidizing biocides, chlorine dioxide is the most selective oxidant. Ozone and chlorine are much more reactive than chlorine dioxide, and they will be consumed by most organic compounds. Chlorine dioxide, however, reacts only with reduced sulfur compounds, secondary and tertiary amines, and some other highly reduced and reactive organic compounds. A more stable residue can therefore be achieved with chlorine dioxide at much lower doses than when using either chlorine or ozone. Generated properly, chlorine dioxide can be used more effectively than ozone or chlorine in cases of higher organic loading because of its selectivity.

**Formaldehyde**

Formaldehyde (HCHO) is a gas that kills all microorganisms and spores at temperatures above 20°C. However, it is not active against prions. Formaldehyde is relatively slow-acting and needs a relative humidity level of about 70%. It is marketed as the solid polymer, paraformaldehyde, in flakes or tablets, or as formalin, a solution of the gas in water of about 370 g/l (37%), containing methanol (100 ml/l) as a
stabilizer. Both formulations are heated to liberate the gas, which is used for decontamination and disinfection of enclosed volumes such as safety cabinets and rooms.

Formaldehyde (5% formalin in water) may be used as a liquid disinfectant. Formaldehyde is a suspected carcinogen. It is a dangerous, irritant gas that has a pungent smell and its fumes can irritate eyes and mucous membranes. It must therefore be stored and used in a fume-hood or well-ventilated area.

**Glutaraldehyde**
Like formaldehyde, glutaraldehyde (OHC(CH₂)₃CHO) is also active against vegetative bacteria, spores, fungi and lipid- and nonlipid-containing viruses. It is non-corrosive and faster acting than formaldehyde. However, it takes several hours to kill bacterial spores.

Glutaraldehyde is generally supplied as a solution with a concentration of about 20 g/l (2%) and some products may need to be “activated” (made alkaline) before use by the addition of a bicarbonate compound supplied with the product. The activated solution can be reused for 1–4 weeks depending on the formulation and type and frequency of its use. Dipsticks supplied with some products give only a rough indication of the levels of active glutaraldehyde available in solutions under use. Glutaraldehyde solutions should be discarded if they become turbid.

Glutaraldehyde is toxic and an irritant to skin and mucous membranes, and contact with it must be avoided. It must be used in a fume-hood or in well-ventilated areas. It is not recommended as a spray or solution for the decontamination of environmental surfaces.

**Phenolic compounds**
Phenolic compounds, a broad group of agents, were among the earliest germicides. However, more recent safety concerns restrict their use. They are active against vegetative bacteria and lipid-containing viruses and, when properly formulated, also show activity against mycobacteria. They are not active against spores and their activity against nonlipid viruses is variable. Many phenolic products are used for the decontamination of environmental surfaces, and some (e.g. triclosan and chloroxylenol) are among the more commonly used antiseptics.

Triclosan is common in products for hand-washing. It is active mainly against vegetative bacteria and safe for skin and mucous membranes. However, in laboratory based studies, bacteria made resistant to low concentrations of triclosan also show resistance to certain types of antibiotics. The significance of this finding in the field remains unknown.

Some phenolic compounds are sensitive to and may be inactivated by water hardness and therefore must be diluted with distilled or deionized water. Phenolic compounds are not recommended for use on food contact surfaces and in areas with young children. They may be absorbed by rubber and can also penetrate the skin.

**Quaternary ammonium compounds**
Many types of quaternary ammonium compounds are used as mixtures and often in combination with other germicides, such as alcohols. They have good activity against some vegetative bacteria and lipid-containing viruses. Certain types (e.g. benzalkonium chloride) are used as antiseptics.

The germicidal activity of certain types of quaternary ammonium compounds is considerably reduced by organic matter, water hardness and anionic detergents. Care is therefore needed in selecting agents for pre-cleaning when quaternary ammonium compounds are to be used for disinfection. Potentially harmful bacteria can grow in quaternary ammonium compound solutions. Owing to low biodegradability, these compounds may also accumulate in the environment.

**Alcohols**
Ethanol (ethyl alcohol, C₂H₅OH) and 2-propanol (isopropyl alcohol, (CH₃)₂CHOH) have similar disinfectant properties. They are active against vegetative bacteria, fungi and lipid-containing viruses but not against spores. Their action on nonlipid viruses is variable. For highest effectiveness they should be used at
concentrations of approximately 70% (v/v) in water: higher or lower concentrations may not be as germicidal. A major advantage of aqueous solutions of alcohols is that they do not leave any residue on treated items.

Mixtures with other agents are more effective than alcohol alone, e.g. 70% (v/v) alcohol with 100 g/l formaldehyde, and alcohol containing 2 g/l available chlorine. A 70% (v/v) aqueous solution of ethanol can be used on skin, work surfaces of laboratory benches and biosafety cabinets, and to soak small pieces of surgical instruments. Since ethanol can dry the skin, it is often mixed with emollients. Alcohol-based hand-rubs are recommended for the decontamination of lightly soiled hands in situations where proper hand-washing is inconvenient or not possible. However, it must be remembered that ethanol is ineffective against spores and may not kill all types of nonlipid viruses.

Alcohols are volatile and flammable and must not be used near open flames. Working solutions should be stored in proper containers to avoid the evaporation of alcohols. Alcohols may harden rubber and dissolve certain types of glue. Proper inventory and storage of ethanol in the laboratory is very important to avoid its use for purposes other than disinfection. Bottles with alcohol-containing solutions must be clearly labeled to avoid autoclaving.

Iodine and iodophors
The action of these disinfectants is similar to that of chlorine, although they may be slightly less inhibited by organic matter. Iodine can stain fabrics and environmental surfaces and is generally unsuitable for use as a disinfectant. On the other hand, iodophors and tinctures of iodine are good antiseptics. Polyvidone-iodine is a reliable and safe surgical scrub and preoperative skin antiseptic. Antiseptics based on iodine are generally unsuitable for use on medical/dental devices. Iodine should not be used on aluminum or copper. Iodine can be toxic. Organic iodine-based products must be stored at 4–10°C to avoid the growth of potentially harmful bacteria in them.

Hydrogen peroxide and peracids
Like chlorine, hydrogen peroxide (H₂O₂) and peracids are strong oxidants and can be potent broad-spectrum germicides. They are also safer than chlorine to humans and the environment.

Hydrogen peroxide is supplied either as a ready-to-use 3% solution or as a 30% aqueous solution to be diluted to 5–10 times its volume with sterilized water. However, such 3–6% solutions of hydrogen peroxide alone are relatively slow and limited as germicides. Products now available have other ingredients to stabilize the hydrogen peroxide content, to accelerate its germicidal action and to make it less corrosive.

Hydrogen peroxide can be used for the decontamination of work surfaces of laboratory benches and biosafety cabinets, and stronger solutions may be suitable for disinfecting heat-sensitive medical/dental devices. The use of vaporized hydrogen peroxide or peracetic acid (CH₃COOOH) for the decontamination of heat-sensitive medical/surgical devices requires specialized equipment.

Hydrogen peroxide and peracids can be corrosive to metals such as aluminum, copper, brass and zinc, and can also decolorize fabrics, hair, skin and mucous membranes. Articles treated with them must be thoroughly rinsed before contact with eyes and mucous membranes. They should always be stored away from heat and protected from light.

Local environmental decontamination
Decontamination of the laboratory space, its furniture and its equipment requires a combination of liquid and gaseous disinfectants. Surfaces can be decontaminated using a solution of sodium hypochlorite (NaOCl); a solution containing 1 g/l available chlorine may be suitable for general environmental sanitation, but stronger solutions (5 g/l) are recommended when dealing with high-risk situations. For environmental decontamination, formulated solutions containing 3% hydrogen peroxide (H₂O₂) make suitable substitutes for bleach solutions.
Rooms and equipment can be decontaminated by fumigation with formaldehyde gas generated by heating paraformaldehyde or boiling formalin. This is a highly dangerous process that requires specially trained personnel. All openings in the room (i.e. windows, doors, etc.) should be sealed with masking tape or similar before the gas is generated. Fumigation should be conducted at an ambient temperature of at least 21 °C and a relative humidity of 70%.

After fumigation the area must be ventilated thoroughly before personnel are allowed to enter. Appropriate respirators must be worn by anyone entering the room before it has been ventilated. Gaseous ammonium bicarbonate can be used to neutralize the formaldehyde.

Fumigation of smaller spaces with hydrogen peroxide vapor is also effective but requires specialized equipment to generate the vapor.

**Decontamination of biological safety cabinets**
Use a qualified technician to decontaminate Class I and Class II cabinets with either formaldehyde gas or vaporized hydrogen peroxide. Contact the Biological Safety Officer for more information.

**Hand-washing/hand decontamination**
Whenever possible, suitable gloves should be worn when handling biohazardous materials. However, this does not replace the need for regular and proper hand-washing by laboratory personnel. Hands must be washed after handling biohazardous materials and animals, and before leaving the laboratory.

In most situations, thorough washing of hands with ordinary soap and water is sufficient to decontaminate them, but the use of germicidal soaps is recommended in high-risk situations. Hands should be thoroughly lathered with soap, using friction, for at least 10 s, rinsed in clean water and dried using a clean paper or cloth towel (if available, warm-air hand-dryers may be used). Foot- or elbow-operated faucets are recommended. Where not fitted, a paper/cloth towel should be used to turn off the faucet handles to avoid re-contaminating washed hands. As mentioned above, alcohol-based hand-rubs may be used to decontaminate lightly soiled hands when proper hand-washing is not available.

**Heat disinfection and sterilization**
Heat is the most common among the physical agents used for the decontamination of pathogens. “Dry” heat, which is totally non-corrosive, is used to process many items of laboratory ware which can withstand temperatures of 160 °C or higher for 2–4 h. Burning or incineration (see below) is also a form of dry heat. “Moist” heat is most effective when used in the form of autoclaving.

Boiling does not necessarily kill all microorganisms and/or pathogens, but it may be used as the minimum processing for disinfection where other methods (chemical disinfection or decontamination, autoclaving) are not applicable or available. Sterilized items must be handled and stored such that they remain uncontaminated until used.

**Autoclaving**
Saturated steam under pressure (autoclaving) is the most effective and reliable means of sterilizing laboratory materials. For most purposes, the following cycles will ensure sterilization of correctly loaded autoclaves:

1. 3 min holding time at 134 °C
2. 10 min holding time at 126 °C
3. **15 min holding time at 121 °C - standard**
4. 25 min holding time at 115 °C.

Examples of different autoclaves include the following.

**Gravity displacement autoclaves.** Steam enters the chamber under pressure and displaces the heavier air downwards and through the valve in the chamber drain, fitted with a HEPA filter.

**Pre-vacuum autoclaves.** These machines allow the removal of air from the chamber before steam is admitted. The exhaust air is evacuated through a valve fitted with a HEPA filter. At the end of the cycle,
the steam is automatically exhausted. These autoclaves can operate at 134 °C and the sterilization cycle can therefore be reduced to 3 min. They are ideal for porous loads, but cannot be used to process liquids because of the vacuum.

Loading autoclaves
Materials should be loosely packed in the chamber for easy steam penetration and air removal. Bags should allow the steam to reach their contents.

Precautions in the use of autoclaves
The following rules can minimize the hazards inherent in operating pressurized vessels.
1. Responsibility for operation and routine care should be assigned to trained individuals.
2. A preventive maintenance program should include regular inspection of the chamber, door seals and all gauges and controls by qualified personnel.
3. The steam should be saturated and free from chemicals (e.g. corrosion inhibitors) that could contaminate the items being sterilized.
4. All materials to be autoclaved should be in containers that allow ready removal of air and permit good heat penetration; the chamber should be loosely packed so that steam will reach the load evenly.
5. For autoclaves without an interlocking safety device that prevents the door being opened when the chamber is pressurized, the main steam valve should be closed and the temperature allowed to fall below 80 °C before the door is opened.
6. Slow exhaust settings should be used when autoclaving liquids, as they may boil over when removed due to superheating.
7. Operators should wear suitable gloves and visors for protection when opening the autoclave, even when the temperature has fallen below 80 °C.
8. In any routine monitoring of autoclave performance, biological indicators or thermocouples should be placed at the centre of each load. Regular monitoring with thermocouples and recording devices in a “worst case” load is highly desirable to determine proper operating cycles.
9. The drain screen filter of the chamber (if available) should be removed and cleaned daily.

Incineration
Incineration is useful for disposing of animal carcasses as well as anatomical and other laboratory waste, with or without prior decontamination. Incineration of infectious materials is an alternative to autoclaving only if the incinerator is under laboratory control.

Proper incineration requires an efficient means of temperature control and a secondary burning chamber. Many incinerators, especially those with a single combustion chamber, are unsatisfactory for dealing with infectious materials, animal carcasses and plastics. Such materials may not be completely destroyed and the effluent from the chimney may pollute the atmosphere with microorganisms, toxic chemicals and smoke. However, there are many satisfactory configurations for combustion chambers. Ideally the temperature in the primary chamber should be at least 800 °C and that in the secondary chamber at least 1000 °C.

Materials for incineration, even with prior decontamination, should be transported to the incinerator in bags, preferably plastic. Incinerator attendants should receive proper instructions about loading and temperature control. It should also be noted that the efficient operation of an incinerator depends heavily on the right mix of materials in the waste being treated.

There are ongoing concerns regarding the possible negative environmental effects of existing or proposed incinerators, and efforts continue to make incinerators more environmentally friendly and energy-efficient.

Disposal
The disposal of laboratory and medical waste is subject to federal and Mississippi state regulations, and the latest versions of such relevant documents must be consulted before designing and implementing a program for handling, transportation and disposal of biohazardous waste. In general, ash from
Incinerators may be handled as normal domestic waste and removed by local authorities. Autoclaved waste may be disposed of by off-site incineration or in licensed landfill sites. Contact the Biological Safety Officer for MSU policies on disposal of biohazardous waste.
14. Introduction to the Transport of Infectious Substances

Transport of infectious and potentially infectious materials is subject to strict national and international regulations. These regulations describe the proper use of packaging materials, as well as other shipping requirements.

The shipment of any hazardous material off campus including infectious agents or clinical specimens must comply with MSU Policy 79.09. All MSU personnel should review the policy before shipping any materials.

Laboratory personnel must ship infectious substances according to applicable transport regulations. Compliance with the rules will:
1. Reduce the likelihood that packages will be damaged and leak, and thereby
2. Reduce the exposures resulting in possible infections
3. Improve the efficiency of package delivery.

Go to Appendix C of the BMBL (5th edition) for detailed information on federal and international regulations as well as permitting:

General Department of Transportation (DOT) Packaging Requirements for Transport of Infectious Substances by Aircraft

The DOT packaging for transporting infectious substances by aircraft are required by domestic and international aircraft carriers and are the basis for infectious substance packaging for motor vehicle, railcar, and vessel transport. The following is a summary of each packaging type and related transportation requirements.

Category A Infectious Substance (UN 2814 and UN 2900): A Category A material is an infectious substance that is transported in a form that is capable of causing permanent disability or life-threatening or fatal disease to otherwise healthy humans or animals when exposure to it occurs. An exposure occurs when an infectious substance is released outside of its protective packaging, resulting in physical contact with humans or animals. Category A infectious substances are assigned to identification number “UN 2814” for substances that cause disease in humans or in both humans and animals, or “UN 2900” for substances that cause disease in animals only.

Biological specimen, Category B (UN 3373): (previously known as Clinical specimen and Diagnostic Specimen). A Category B infectious substance is one that does not meet the criteria for inclusion in Category A. A Category B infectious substance does not cause permanent disability or life-threatening or fatal disease to humans or animals when exposure to it occurs. The proper shipping name for a Category B infectious substance, “Biological specimen, Category B,” is assigned to identification number “UN 3373.” The proper shipping names “Diagnostic specimen” and “Clinical specimen” may no longer be used after January 1, 2007.

Some biological specimens are exempt from federal transportation regulations. Contact the Biological Safety Officer for additional information.

The basic triple packaging system
The triple packaging system, the choice for the transport of infectious and potentially infectious substances, is shown in Figures 9 and 10 for Category A and B substances respectively. This packaging
system consists of three layers: the primary receptacle, the secondary packaging and the outer packaging.

The primary receptacle containing the specimen must be watertight, leakproof and appropriately labeled as to content. The primary receptacle is wrapped in enough absorbent material to absorb all fluid in case of breakage or leakage.

A second watertight, leakproof packaging is used to enclose and protect the primary receptacle(s). Several wrapped primary receptacles may be placed in a single secondary packaging. Volume and/or weight limits for packaged infectious substances are included in certain regulatory texts.

The third layer protects the secondary packaging from physical damage while in transit. Specimen data forms, letters and other types of information that identify or describe the specimen and identify the shipper and receiver, and any other documentation required, must also be provided according to latest regulations.

**Transport within a building or across campus**
The standard practice for campus transport is a two container system. The primary container should be sealed, leak-proof and shatter-resistant. The primary container is placed into a secondary container which is also leak- and shatter-proof and able to be sealed with a lid. The primary receptacle should be wrapped in enough absorbent material to absorb all fluid in case of breakage or leakage. The exterior of both containers are disinfected prior to transport with either 70% ethanol or a 1% fresh household bleach solution. At the end of the day's use, the secondary container should be disinfected completely (inside and out). Examples of a secondary container include a cooler with a sealable lid or a plastic Tupperware container with a lid. Cardboard boxes are not appropriate for primary or secondary containers if liquid is being transported. Fresh or dried blood, fluid or other material should not be visible on the exterior of the secondary container. The secondary container should be labeled with contact information including the name and phone number of the responsible party.

The purpose of this two container system is to provide containment of the specimen in the event of an accident. This will prevent contamination of personnel and the environment.
Figure 9. Packing and Labeling of Category A Infectious Substances

Packing and Labeling of Category A Infectious Substances

(See Packing Instruction 602)

- Watertight Primary Receptacle
- Cap
- Watertight Secondary Packaging
- UN Package Certification Mark
- Wassermann Tape Closure must be on top of absorbent material
- Infectious Substance
- Specimen ID Label
- Absorbent Packing Material
- Proper Shipping Name and UN Number
- Shipment and Consignee Identification

*If multiple primary receptacles are placed in a single secondary packaging, they must be individually wrapped or separated so as to prevent contact between them.*
Figure 10. Packing and Labeling of Category B Infectious Agents

Packing and Labeling of Category B Infectious Substances
(See Packing Instruction 650)

1. Wear gloves and protective clothing, including face and eye protection.
2. Cover the spill with cloth or paper towels to contain it.
3. Apply disinfectant concentrically beginning at the outer margin of the spill area, working toward the center. Generally, 5% bleach solutions are appropriate.
4. After the appropriate amount of time (e.g. 30 min if using bleach), clear away the materials. If there is broken glass or other sharps involved, use a dustpan or a piece of stiff cardboard to collect the material and deposit it into a puncture-resistant container for disposal.

Spill clean-up procedure
In the event of a spill of infectious or potentially infectious material, the following spill clean-up procedure should be used.

- Wear gloves and protective clothing, including face and eye protection.
- Cover the spill with cloth or paper towels to contain it.
- Apply disinfectant concentrically beginning at the outer margin of the spill area, working toward the center. Generally, 5% bleach solutions are appropriate.
- After the appropriate amount of time (e.g. 30 min if using bleach), clear away the materials. If there is broken glass or other sharps involved, use a dustpan or a piece of stiff cardboard to collect the material and deposit it into a puncture-resistant container for disposal.
6. Clean and disinfect the area of the spillage (if necessary, repeat steps 2–5).
7. Dispose of contaminated materials (except for sharps) into a red biohazard bag for subsequent sterilization.
8. After successful disinfection, inform the principal investigator that the site has now been decontaminated.
9. If a large spill occurs contact the Biological Safety Officer for guidance.
15. Biosafety and Recombinant DNA Technology

Recombinant DNA technology involves combining genetic material from different sources thereby creating genetically modified organisms (GMOs) that may have never existed in nature before. Initially there was concern among molecular biologists that such organisms might have unpredictable and undesirable properties that could represent a biohazard if they escaped from the laboratory. This concern became the focus of a scientific conference held in Asilomar, CA, USA, in 1975. At that meeting, safety issues were discussed and the first guidelines for recombinant DNA technology were proposed. The subsequent 25+ years of research experience have demonstrated that genetic engineering may be conducted in a safe manner when an appropriate risk assessment is performed and adequate safety measures are used.

Recombinant DNA technology or genetic engineering was first used to clone DNA segments in bacterial hosts in order to over-express specific gene products for further studies. Recombinant DNA molecules have also been used to create GMOs such as transgenic and “knock-out” animals and transgenic plants.

Recombinant DNA technology has already had an enormous impact on biology and medicine, and will probably have an even greater influence now that the nucleotide sequence of the entire human genome is available. Tens of thousands of genes of yet unknown functions will be studied using recombinant DNA technology. Gene therapy may become a routine treatment for certain diseases, and new vectors for gene transfer are likely to be devised using genetic engineering techniques. Also, transgenic plants produced by recombinant DNA technology may play an increasingly important role in modern agriculture.

Experiments involving the construction or use of GMOs should be conducted after performing a biosafety risk assessment. The pathogenic properties and any potential hazards associated with such organisms may be novel and not well-characterized. The properties of the donor organism, the nature of the DNA sequences that will be transferred, the properties of the recipient organism, and the properties of the environment should be evaluated. These factors should help determine the biosafety level that is required for the safe handling of the resulting GMO, and identify the biological and physical containment systems that should be used.

Biosafety considerations for biological expression systems
Biological expression systems consist of vectors and host cells. A number of criteria must be satisfied to make them effective and safe to use. An example of such a biological expression system is plasmid pUC18. Frequently used as a cloning vector in combination with *Escherichia coli* K12 cells, the pUC18 plasmid has been entirely sequenced. All genes required for expression in other bacteria have been deleted from its precursor plasmid pBR322. *E. coli* K12 is a non-pathogenic strain that cannot permanently colonize the gut of healthy humans or animals. Routine genetic engineering experiments can safely be performed in *E. coli* K12/pUC18 at Biosafety Level 1, provided the inserted foreign DNA expression products do not require higher biosafety levels.

Biosafety considerations for expression vectors
Higher biosafety levels may be required when:
1. The expression of DNA sequences derived from pathogenic organisms may increase the virulence of the GMO
2. Inserted DNA sequences are not well characterized, e.g. during preparation of genomic DNA libraries from pathogenic microorganisms
3. Gene products have potential pharmacological activity
Viral vectors for gene transfer
Viral vectors, e.g. adenovirus vectors, are used for the transfer of genes to other cells. Such vectors lack certain virus replication genes and are propagated in cell lines that complement the defect.

Stocks of such vectors may be contaminated with replication-competent viruses, generated by rare spontaneous recombination events in the propagating cell lines, or may derive from insufficient purification. These vectors should be handled at the same biosafety level as the parent adenovirus from which they are derived.

Transgenic and “knock-out” animals
Animals carrying foreign genetic material (transgenic animals) should be handled in containment levels appropriate to the characteristics of the products of the foreign genes. Animals with targeted deletions of specific genes (“knock-out” animals) do not generally present particular biological hazards.

Examples of transgenic animals include animals expressing receptors for viruses normally unable to infect that species. If such animals escaped from the laboratory and transmitted the transgene to the wild animal population, an animal reservoir for that particular virus could theoretically be generated.

This possibility has been discussed for poliovirus and is particularly relevant in the context of poliomyelitis eradication. Transgenic mice expressing the human poliovirus receptor generated in different laboratories were susceptible to poliovirus infection by various inoculation routes and the resulting disease was clinically and histopathologically similar to human poliomyelitis. However, the mouse model differs from humans in that alimentary tract replication of orally administered poliovirus is either inefficient or does not occur. It is therefore very unlikely that escape of such transgenic mice to the wild would result in the establishment of a new animal reservoir for poliovirus. Nevertheless, this example indicates that, for each new line of transgenic animal, detailed studies should be conducted to determine the routes by which the animals can be infected, the inoculum size required for infection, and the extent of virus shedding by the infected animals. In addition, all measures should be taken to assure strict containment of receptor transgenic mice.

Transgenic plants
Transgenic plants expressing genes that confer tolerance to herbicides or resistance to insects are currently a matter of considerable controversy in many parts of the world. The discussions focus on the food-safety of such plants, and on the long-term ecological consequences of their cultivation.

Transgenic plants expressing genes of animal or human origin are used to develop medicinal and nutritional products. A risk assessment should determine the appropriate biosafety level for the production of these plants.

Risk assessments for genetically modified organisms
Risk assessments for work with GMOs should consider the characteristics of donor and recipient/host organisms.

Examples of characteristics for consideration include the following.

Hazards arising directly from the inserted gene (donor organism)
Assessment is necessary in situations where the product of the inserted gene has known biologically or pharmacologically active properties that may give rise to harm, for example:

1. Toxins
2. Cytokines
3. Hormones
4. Gene expression regulators
5. Virulence factors or enhancers
6. Oncogenic gene sequences
7. Antibiotic resistance
8. Allergens
The consideration of such cases should include an estimation of the level of expression required to achieve biological or pharmacological activity.

**Hazards associated with the recipient/host**
1. Susceptibility of the host
2. Pathogenicity of the host strain, including virulence, infectivity and toxin production
3. Modification of the host range
4. Recipient immune status
5. Consequences of exposure

**Hazards arising from the alteration of existing pathogenic traits**
Many modifications do not involve genes whose products are inherently harmful, but adverse effects may arise as the result of alteration of existing non-pathogenic or pathogenic traits. Modification of normal genes may alter pathogenicity. In an attempt to identify these potential hazards, the following points may be considered (the list is not exhaustive).
1. Is there an increase in infectivity or pathogenicity?
2. Could any disabling mutation within the recipient be overcome as a result of the insertion of the foreign gene?
3. Does the foreign gene encode a pathogenicity determinant from another organism?
4. If the foreign DNA does include a pathogenicity determinant, is it foreseeable that this gene could contribute to the pathogenicity of the GMO?
5. Is treatment available?
6. Will the susceptibility of the GMO to antibiotics or other forms of therapy be affected as a consequence of the genetic modification?
7. Is eradication of the GMO achievable?

**Recombinant DNA Experiments at Mississippi State University**
Any rDNA research requires, at a minimum, notification of the IBC Chair or Biological Safety Officer. The Chair or BSO can assist in determining the oversight requirements for the specific project.
16. Hazardous Chemicals

Workers in biological research laboratories are not only exposed to biohazardous materials but also to chemical hazards. It is important that they have proper knowledge of the toxic effects of these chemicals, the routes of exposure and the hazards that may be associated with handling and storage. Material safety data sheets or other chemical hazard information are available from chemical manufacturers and/or suppliers. These should be accessible in laboratories where these chemicals are used, e.g. as part of a safety or operations manual.

Routes of exposure
Exposure to hazardous chemicals may occur by:
1. Inhalation
2. Contact
3. Ingestion
4. Needle-sticks
5. Through broken skin

Storage of chemicals
Only amounts of chemicals necessary for daily use should be stored in the laboratory. Bulk stocks should be kept in specially designated rooms or buildings.

Disposal of chemicals
Many common chemicals such as ethanol, methanol, acetone, acids, bases, phenol, and sodium azide must be disposed of in accordance with Applicable EPA regulations. Contact the Office of Regulatory Compliance for additional information or to arrange a pickup for waste chemicals. Evaporation, disposal as regular trash, or discharge into the sewer system is not permitted.

Chemicals should not be stored in alphabetical order.

General rules regarding chemical incompatibilities
To avoid fire and/or explosions, substances in the left-hand column of Table 12 should be stored and handled so that they cannot come into contact with the corresponding substances in the right-hand column of the table.

<table>
<thead>
<tr>
<th>SUBSTANCE CATEGORY</th>
<th>INCOMPATIBLE SUBSTANCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkali metals, e.g. sodium, potassium, cesium and lithium</td>
<td>Carbon dioxide, chlorinated hydrocarbons, water</td>
</tr>
<tr>
<td>Halogens</td>
<td>Ammonia, acetylene, hydrocarbons</td>
</tr>
<tr>
<td>Acetic acid, hydrogen sulfide, aniline, hydrocarbons, sulfuric acid</td>
<td>Oxidizing agents, e.g. chromic acid, nitric acid, peroxides, permanganates</td>
</tr>
</tbody>
</table>

Toxic effects of chemicals
Some chemicals adversely affect the health of those who handle them or inhale their vapors. Apart from overt poisons, a number of chemicals are known to have various toxic effects. The respiratory system, blood, lungs, liver, kidneys and the gastrointestinal system, as well as other organs and tissues may be adversely affected or seriously damaged. Some chemicals are known to be carcinogenic or teratogenic.

Some solvent vapors are toxic when inhaled. Apart from the more serious effects noted above, exposure may result in impairments that show no immediate discernible effects on health, but can include lack of coordination, drowsiness and similar symptoms, leading to an increased proneness to accidents.
Prolonged or repeated exposure to the liquid phase of many organic solvents can result in skin damage. This may be due to a de-fatting effect, but allergic and corrosive symptoms may also arise.

**Explosive chemicals**
Azides, often used in antibacterial solutions, should not be allowed to come into contact with copper or lead (e.g. in waste pipes and plumbing), as they may explode violently when subjected even to a mild impact.

Ethers that have aged are extremely unstable, and potentially explosive.

Perchloric acid, if allowed to dry on woodwork, brickwork or fabric, will explode and cause a fire on impact.

Picric acid and picrates are detonated by heat and impact.

**Chemical spills**
Most manufacturers of laboratory chemicals issue charts describing methods for dealing with spills. Spillage charts and spillage kits are also available commercially. Appropriate charts should be displayed in a prominent position in the laboratory. The following equipment should also be provided:

1. Chemical spill kits
2. Protective clothing, e.g. heavy-duty rubber gloves, overshoes or rubber boots, respirators
3. Scoops and dustpans
4. Forceps for picking up broken glass
5. Mops, cloths and paper towels
6. Buckets
7. Soda ash (sodium carbonate, Na₂CO₃) or sodium bicarbonate (NaHCO₃) for neutralizing acids and corrosive chemicals
8. Sand (to cover alkali spills)
9. Non-flammable detergent

Spill cleanup debris may be subject to RCRA disposal requirements. Contact the ORC before disposing of waste generated from a spill cleanup.

The following actions should be taken in the event of a chemical spill:

1. Notify the Chemical Hygiene Officer: 325-3294. After hours contact the MSU Police department.
2. Evacuate non-essential personnel from the area.
3. Attend to persons who may have been contaminated.
4. If the spilled material is flammable, extinguish all open flames, turn off gas in the room and adjacent areas, open windows (if possible), and switch off electrical equipment that may spark.
5. Avoid breathing vapor from spilled material.
6. Establish exhaust ventilation if it is safe to do so.
7. Secure the necessary items (see above) to clean up the spill.
Compressed and liquefied gases
Information regarding storage of compressed and liquefied gases is given in Table 13.

Table 13. Storage of compressed and liquefied gases

<table>
<thead>
<tr>
<th>CONTAINER</th>
<th>STORAGE INFORMATION</th>
</tr>
</thead>
</table>
| Compressed gas cylinders and liquefied gas containers A,B | • Should be securely fixed (e.g. chained) to the wall or a solid bench so that they are not inadvertently dislodged.  
• Must be transported with their caps in place and supported on trolleys.  
• Should be stored in bulk in an appropriate facility at some distance from the laboratory. This area should be locked and appropriately identified.  
• Should not be placed near radiators, open flames, other heat sources, sparking electrical equipment or in direct sunlight. |
| Small, single-use gas cylinders A,B | • Must not be incinerated. |

A The main high-pressure valve should be turned off when the equipment is not in use and when the room is unoccupied.  
B Rooms where flammable gas cylinders are used and/or stored should be identified by warning notices on the doors.

Storage and use of flammable, toxic, corrosive, or oxidizing gases must comply with MSU fire codes. Contact the ORC or University Safety Officer for guidance.
17. Additional Laboratory Hazards

Laboratory personnel may confront hazards posed by forms of energy including fire, electricity, radiation and noise. Basic information about each of these is presented in this chapter.

Fire hazards
Close cooperation between safety officers and local fire prevention officers is essential. Apart from chemical hazards, the effects of fire on the possible dissemination of biohazardous material must be considered. This may determine whether it is best to extinguish or contain the fire.

Flammable materials must be stored in UL approved solvent storage cabinets. Flammable materials must be kept segregated from oxidizers and other reactive materials.

Flammable solvents and gases should not be used in a biological safety cabinet unless the cabinet is approved for such use.

The assistance of local fire prevention officers in the training of laboratory staff in fire prevention, immediate action in case of fire and the use of fire-fighting equipment is desirable.

Fire warnings, instructions and escape routes should be displayed prominently in each room and in corridors and hallways.

Common causes of fires in laboratories are:
1. Electrical circuit overloading
2. Poor electrical maintenance, e.g. poor and deteriorated insulation on cables
3. Excessively long gas tubing or long electrical leads
4. Equipment unnecessarily left switched on
5. Equipment that was not designed for a laboratory environment
6. Open flames
7. Deteriorated gas tubing
8. Improper handling and storage of flammable or explosive materials
9. Improper segregation of incompatible chemicals
10. Sparking equipment near flammable substances and vapors
11. Improper or inadequate ventilation

Fire-fighting equipment should be placed near room doors and at strategic points in corridors and hallways. This equipment may include hoses, buckets (of water or sand) and a fire extinguisher. Fire extinguishers should be regularly inspected and maintained, and their shelf-life kept up to date. Specific types and uses of fire extinguishers are shown in Table 14.

Table 13. Types and uses of fire extinguishers

<table>
<thead>
<tr>
<th>TYPE</th>
<th>USED FOR</th>
<th>DO NOT USE FOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Paper, wood, fabric</td>
<td>Electrical fires, flammable liquids, burning metals</td>
</tr>
<tr>
<td>Carbon dioxide (CO₂)</td>
<td>Flammable liquids and gases,</td>
<td>Alkali metals, paper</td>
</tr>
<tr>
<td>extinguisher gases</td>
<td>electrical fires</td>
<td></td>
</tr>
<tr>
<td>Dry powder</td>
<td>Flammable liquids and gases,</td>
<td>Reusable equipment and instruments, as residues are very difficult to remove</td>
</tr>
<tr>
<td></td>
<td>alkali metals, electrical fires</td>
<td></td>
</tr>
<tr>
<td>Foam</td>
<td>Flammable liquids</td>
<td>Electrical fires</td>
</tr>
</tbody>
</table>
**Electrical hazards**
It is essential that all electrical installations and equipment are inspected and tested regularly, including earth/grounding systems.

Circuit-breakers and earth-fault-interrupters should be installed in appropriate laboratory electrical circuits. Circuit-breakers do not protect people; they are intended to protect wiring from being overloaded with electrical current and hence to prevent fires. Earth-fault-interrupters are intended to protect people from electric shock.

All laboratory electrical equipment should be earthed/grounded, preferably through three-prong plugs. All laboratory electrical equipment and wiring should conform to national electrical safety standards and codes.

**Noise**
The effect of excessive noise is insidious over time. Some types of laboratory equipment, such as certain laser systems, as well as facilities where animals are housed, can produce significant noise exposure to workers. Noise measurement surveys can be conducted to determine the noise hazard. Where warranted by data, engineering controls such as enclosures or barriers around noisy equipment or between noisy areas and other work areas, can be considered. Where noise levels cannot be abated and where laboratory personnel routinely experience excessive exposures, a hearing conservation program that includes the use of hearing protection while working in hazardous noise and a medical monitoring program to determine the effect of noise on the workers should be instituted.

**Ionizing radiation**
The use of radioactive materials on campus must comply with all applicable requirements found in license MS-EBL-02.

Radiological protection is concerned with protecting humans against the harmful effects of ionizing radiation, which include:
1. Somatic effects, e.g. clinical symptoms observable in exposed individuals. Somatic effects include radiation-induced cancers, e.g. leukemia and bone, lung and skin cancers, the onset of which may occur many years after irradiation. Less severe somatic effects include minor skin damage, hair loss, blood deficiencies, gastrointestinal damage and cataract formation.
2. Hereditary effects, e.g. symptoms observed in the descendants of exposed individuals. The hereditary effects of radiation exposure to the gonads include chromosome damage or gene mutation. Irradiation of the germ cells in the gonads in high doses can also cause cell death, resulting in impaired fertility in both sexes or menstrual changes in women. Exposure of the developing fetus, particularly in weeks 8–15 of pregnancy, may increase the risk of congenital malformations, mental impairment or radiation-induced cancers in later life.

**Principles of ionizing radiation protection**
To limit the harmful effects of ionizing radiation, the use of radioisotopes should be controlled and should comply with federal and state regulations. Protection from radiation is managed on the basis of four principles:
1. Minimizing the time of radiation exposure
2. Maximizing the distance from the radiation source
3. Shielding the radiation source
4. Substituting the use of radionuclides with non-radiometric techniques

Protection activities include the following.
1. **Time.** The time of exposure experienced during manipulations of radioactive material can be reduced by:
   — Practicing new and unfamiliar techniques without using the radionuclide until the techniques are mastered
   — Working with radionuclides in a deliberate and timely manner without rushing
   — Ensuring that all radioactive sources are returned to storage immediately after use
— Removing radioactive waste from the laboratory at frequent intervals
— Spending as little time as possible in the radiation area or laboratory
— Exercising effective time management and planning of laboratory manipulations involving radioactive material.

The less time spent in a radiation field, the smaller the received personal dose, as described in the equation: \[ \text{Dose} = \text{Dose rate} \times \text{time} \]

2. **Distance.** The dose rate for most γ- and X-radiation varies as the inverse square of the distance from a point source: \[ \text{Dose rate} = \text{Constant} \times \frac{1}{\text{Distance}^2} \]

Doubling the distance from a radiation source will result in reducing the exposure by one-fourth over the same period of time. Various devices and mechanical aids are used to increase the distance between the operator and the radiation source, e.g. long-handled tongs, forceps, clamps and remote pipetting aids. Note that a small increase in distance can result in significant decrease in the dose rate.

3. **Shielding.** Radiation energy-absorbing or attenuating shields placed between the source and the operator or other occupants of the laboratory will help limit their exposure. The choice and thickness of any shielding material depends on the penetrating ability (type and energy) of the radiation. A barrier of acrylic, wood or lightweight metal, thickness 1.3–1.5 cm, provides shielding against high-energy β particles, whereas high-density lead is needed to shield against high energy γ- and X-radiation.

4. **Substitution.** Radionuclide-based materials should not be used when other techniques are available. If substitution is not possible, then the radionuclide with the least penetrating power or energy should be used.

### Safe practices when working with radioactive materials

Rules for working with radioactive substances should include considerations in four areas:

1. **Radiation area**
   — Use radioactive substances only in dedicated areas.
   — Allow the presence of essential staff only.
   — Use personal protective equipment, including laboratory coats, safety spectacles and disposable gloves.
   — Monitor personal radiation exposures

Laboratories where radionuclides are used should be designed to simplify containment, cleaning and decontamination. The radionuclide work area should be located in a small room adjoining the main laboratory, or in a dedicated area within the laboratory away from other activities. Signs displaying the international radiation hazard symbol should be posted at the entrance to the radiation area (Figure 11).

2. **Work-bench area**
   — Use spill trays lined with disposable absorbent materials.
   — Limit radionuclide quantities.
   — Shield radiation sources in the radiation, work bench and radioactive waste areas.
   — Mark radiation containers with the radiation symbol, including radionuclide identity, activity and assay date.
   — Use radiation meters to monitor working areas, protective clothing and hands after completion of work.
   — Use appropriately shielded transport containers

3. **Radioactive waste area**
   — Remove radioactive waste frequently from the working area.
— Maintain accurate records of use and disposal of radioactive materials.
— Screen dosimetry records for exposures exceeding the dose limits.
— Establish and regularly exercise emergency response plans.
— In emergencies, assist injured persons first.
— Clean contaminated areas thoroughly.
— Report all spills to the Radiological Safety Officer (325-3294).
— Write and keep incident reports
— Do not mix radioactive waste with infectious waste

Figure 11. International radiation hazard symbol
18. Safety for Support Staff

The safe and optimum operation of a laboratory is dependent to a great extent on the support staff, and it is essential that such personnel are given appropriate safety training including janitorial and building maintenance personnel.
19. Safety Checklist

This checklist is intended to assist in assessments of biological research laboratory safety and security status of laboratories working with biohazardous materials.

Laboratory premises
1. Have guidelines for commissioning and certification been considered for facility construction or post-construction evaluations?
2. Do the premises meet national and local building requirements, including those relating to natural disaster precautions if necessary?
3. Are the premises generally uncluttered and free from obstructions?
4. Are the premises clean?
5. Are there any structural defects in floors?
6. Are floors and stairs uniform and slip-resistant?
7. Is the working space adequate for safe operation?
8. Are the circulation spaces and corridors adequate for the movement of people and large equipment?
9. Are the benches, furniture and fittings in good condition?
10. Are bench surfaces resistant to solvents and corrosive chemicals?
11. Is there a hand-washing sink in each laboratory room?
12. Are the premises constructed and maintained to prevent entry and harborage of rodents and arthropods?
13. Are all exposed steam and hot water pipes insulated or guarded to protect personnel?
14. Is an independent power support unit provided in case of power breakdown?
15. Can access to laboratory areas be restricted to authorized personnel?
16. Has a risk assessment been performed to ensure that appropriate equipment and facilities are available to support the work being considered?

Storage facilities
1. Are storage facilities, shelves, etc. arranged so that stores are secure against sliding, collapse or falls?
2. Are storage facilities kept free from accumulations of rubbish, unwanted materials and objects that present hazards from tripping, fire, explosion and harborage of pests?
3. Are freezers and storage areas lockable?

Sanitation and staff facilities
1. Are the premises maintained in a clean, orderly and sanitary condition?
2. Is drinking-water available?
3. Are clean and adequate toilet and washing facilities provided separately for male and female staff?
4. Are hot and cold water, soap and towels provided?
5. Are separate changing rooms provided for male and female staff?
6. Is there accommodation (e.g. lockers) for street clothing for individual members of the staff?
7. Is there a staff room for lunch, etc.?
8. Are noise levels acceptable?
9. Is there an adequate organization for the collection and disposal of general household rubbish?

Heating and ventilation
1. Is there a comfortable working temperature?
2. Are blinds fitted to windows that are exposed to full sunlight?
3. Is the ventilation adequate, e.g. at least six changes of air per hour, especially in rooms that have mechanical ventilation?
4. Are there HEPA filters in the ventilation system?
5. Does mechanical ventilation compromise airflows in and around biological safety cabinets and fume cupboards?
**Lighting**
1. Is the general illumination adequate (e.g. 300–400 Lux)?
2. Is task (local) lighting provided at work benches?
3. Are all areas well-lit, with no dark or ill-lit corners in rooms and corridors?
4. Are fluorescent lights parallel to the benches?
5. Are fluorescent lights color-balanced?

**Services**
1. Is each laboratory room provided with enough sinks, water, electricity and gas outlets for safe working?
2. Is there an adequate inspection and maintenance program for fuses, lights, cables, pipes, etc.?
3. Are faults corrected within a reasonable time?
4. Are internal engineering and maintenance services available, with skilled engineers and craftsmen who also have some knowledge of the nature of the work of the laboratory?
5. Is the access of engineering and maintenance personnel to various laboratory areas controlled and documented?
6. If no internal engineering and maintenance services are available, have local engineers and builders been contacted and familiarized with the equipment and work of the laboratory?
7. Are cleaning services available?
8. Is the access of cleaning personnel to various laboratory areas controlled and documented?
9. Are information technology services available and secured?

**Laboratory biosecurity**
1. Has a qualitative risk assessment been performed to define risks that a security system should protect against?
2. Have acceptable risks and incidence response planning parameters been defined?
3. Is the whole building securely locked when unoccupied?
4. Are doors and windows break-proof?
5. Are rooms containing hazardous materials and expensive equipment locked when unoccupied?
6. Is access to such rooms, equipment and materials appropriately controlled and documented?

**Fire prevention and fire protection**
1. Is there a fire alarm system?
2. Are the fire doors in good order?
3. Is the fire detection system in good working order and regularly tested?
4. Are fire alarm stations accessible?
5. Are all exits marked by proper, illuminated signs?
6. Is access to exits marked where the routes to them are not immediately visible?
7. Are all exits unobstructed by decorations, furniture and equipment, and unlocked when the building is occupied?
8. Is access to exits arranged so that it is not necessary to pass through a high-hazard area to escape?
9. Do all exits lead to an open space?
10. Are corridors, aisles and circulation areas clear and unobstructed for movement of staff and fire-fighting equipment?
11. Is all fire-fighting equipment and apparatus easily identified by an appropriate color code?
12. Are portable fire extinguishers maintained fully charged and in working order, and kept in designated places at all times?
13. Are laboratory rooms with potential fire hazards equipped with appropriate extinguishers and/or fire blankets for emergency use?
14. If flammable liquids and gases are used in any room, is the mechanical ventilation sufficient to remove vapors before they reach a hazardous concentration?
15. Are personnel trained to respond to fire emergencies?
Flammable liquid storage
1. Is the storage facility for bulk flammable liquids separated from the main building?
2. Is it clearly labeled as a fire-risk area?
3. Does it have a gravity or mechanical exhaust ventilation system that is separate from the main building system?
4. Are the switches for lighting sealed or placed outside the building?
5. Are the light fittings inside sealed to protect against ignition of vapors by sparking?
6. Are flammable liquids stored in proper, ventilated containers that are made of non-combustible materials?
7. Are the contents of all containers correctly described on the labels?
8. Are appropriate fire extinguishers and/or fire blankets placed outside but near to the flammable liquid store?
9. Are “No Smoking” signs clearly displayed inside and outside the flammable liquid store?
10. Are only minimum amounts of flammable substances stored in laboratory rooms?
11. Are they stored in properly constructed flammable storage cabinets?
12. Are these cabinets adequately labeled with “Flammable Liquid – Fire Hazard” signs?
13. Are personnel trained to properly use and transport flammable liquids?

Compressed and liquefied gases
1. Is each portable gas container legibly marked with its contents and correctly color-coded?
2. Are compressed-gas cylinders and their high-pressure and reduction valves regularly inspected?
3. Are reduction valves regularly maintained?
4. Is a pressure-relief device connected when a cylinder is in use?
5. Are protection caps in place when cylinders are not in use or are being transported?
6. Are all compressed gas cylinders secured so that they cannot fall, especially in the event of natural disaster?
7. Are cylinders and liquid petroleum gas tanks kept away from sources of heat?
8. Are personnel trained to properly use and transport compressed and liquefied gases?

Electrical hazards
1. Are all new electrical installations and all replacements, modifications or repairs made and maintained in accordance with a national electrical safety code?
2. Does the interior wiring have an earthed/grounded conductor (i.e. a three-wire system)?
3. Are circuit-breakers and earth-fault interrupters fitted to all laboratory circuits?
4. Do all electrical appliances have testing laboratory approval?
5. Are the flexible connecting cables of all equipment as short as practicable, in good condition, and not frayed, damaged or spliced?
6. Is each electric socket outlet used for only one appliance (no adapters to be used)?

Personal protection
1. Is protective clothing of approved design and fabric provided for all staff for normal work, e.g. gowns, coveralls, aprons, gloves?
2. Is additional protective clothing provided for work with hazardous chemicals and radioactive and carcinogenic substances, e.g. rubber aprons and gloves for chemicals and for dealing with spillages; heat-resistant gloves for unloading autoclaves and ovens?
3. Are safety glasses, goggles and shields (visors) provided?
4. Are there eye-wash stations?
5. Are there emergency showers (drench facilities)?
6. Is radiation protection in accordance with national standards, including provision of dosimeters?
7. Are respirators available, regularly cleaned, disinfected, inspected and stored in a clean and sanitary condition?
8. Are appropriate filters provided for the correct types of respirators, e.g. HEPA filters for microorganisms, appropriate filters for gases or particulates?
9. Are respirators fit-tested?
**Health and safety of staff**

1. Is there an occupational health service (e.g. Longest Student Health Center)?
2. Are first-aid boxes provided at strategic locations?
3. Are qualified first-aiders available?
4. Are such first-aiders trained to deal with emergencies unique to the laboratory, e.g. contact with corrosive chemicals, accidental ingestion of poisons and infectious materials?
5. Are non-laboratory workers, e.g. domestic and clerical staff, instructed on the potential hazards of the laboratory and the material it handles?
6. Are notices prominently posted giving clear information about the location of first-aiders, telephone numbers of emergency services, etc.?
7. Are women of childbearing age warned of the consequences of work with certain microorganisms, carcinogens, mutagens and teratogens?
8. Are women of childbearing age told that if they are, or suspect that they are, pregnant they should inform the appropriate member of the medical/scientific staff so that alternative working arrangements may be made for them if necessary?
9. Is there an immunization program relevant to the work of the laboratory?
10. Are skin tests and/or radiological facilities available for staff who work with tuberculous materials or other materials requiring such measures?
11. Are proper records maintained of illnesses and accidents?
12. Are warning and accident prevention signs used to minimize work hazards?
13. Are personnel trained to follow appropriate biosafety practices?
14. Are laboratory staff encouraged to report potential exposures?

**Laboratory equipment**

1. Is all equipment certified safe for use?
2. Are procedures available for decontaminating equipment prior to maintenance?
3. Are biological safety cabinets and fume cupboards regularly tested and serviced?
4. Are autoclaves and other pressure vessels regularly inspected?
5. Are centrifuge buckets and rotors regularly inspected?
6. Are HEPA filters regularly changed?
7. Are pipettes used instead of hypodermic needles?
8. Is cracked and chipped glassware always discarded and not reused?
9. Are there safe receptacles for broken glass?
10. Are plastics used instead of glass where feasible?
11. Are sharps disposal containers available and being used?

**Biohazardous materials**

1. Are specimens received in a safe condition?
2. Are records kept of incoming materials?
3. Are specimens unpacked in biological safety cabinets with care and attention to possible breakage and leakage?
4. Are gloves and other protective clothing worn for unpacking specimens?
5. Are personnel trained to ship biohazardous substances according to current national and/or international regulations?
6. Are work benches kept clean and tidy?
7. Are discarded materials removed daily or more often and disposed of safely?
8. Are all members of the staff aware of procedures for dealing with breakage and spillage of cultures, infectious materials and other biohazardous substances?
9. Is the performance of sterilizers checked by the appropriate chemical, physical and biological indicators?
10. Is there a procedure for decontaminating centrifuges regularly?
11. Are sealed buckets provided for centrifuges?
12. Are appropriate disinfectants being used? Are they used correctly?
Chemicals and radioactive substances
1. Are incompatible chemicals effectively separated when stored or handled?
2. Are all chemicals correctly labeled with names and warnings?
3. Are chemical hazard warning charts prominently displayed?
4. Are spill kits provided?
5. Are staff trained to deal with spills?
6. Are flammable substances correctly and safely stored in minimal amounts in approved cabinets?
7. Are bottle carriers provided?
8. Is a radiation protection officer or appropriate reference manual available for consultation?
9. Are staff appropriately trained to safely work with radioactive materials?
10. Are proper records of stocks and use of radioactive substances maintained?
11. Are radioactivity screens provided?
12. Are personal radiation exposures monitored?
Appendix I

First aid

First aid is the skilled application of accepted principles of medical treatment at the time and place of an accident. It is the approved method of treating a casualty until he or she is placed in the care of a doctor for definitive treatment of the injury. The minimum first-aid equipment consists of a first-aid box, protective clothing and safety equipment for the person rendering the first aid, and eye irrigation equipment.

The first-aid box
The first-aid box should be constructed from materials that will keep the contents dust- and damp-free. It should be kept in a prominent position and be easily recognized. By international convention, the first-aid box is identified by a white cross on a green background.

The first-aid box should contain:
1. Instruction sheet giving general guidance
2. Individually-wrapped sterile adhesive dressings in a variety of sizes
3. Sterile eye-pads with attachment bandages
4. Triangular bandages
5. Sterile wound coverings
6. Safety pins
7. A selection of sterile but nonmedicated wound dressings
8. An authoritative first-aid manual, e.g. one issued by the International Red Cross.

Protective equipment for the person rendering first aid includes:
1. Mouthpiece for mouth-to-mouth resuscitation
2. Gloves and other barrier protections against blood exposures
3. Clean-up kit for blood spills.

Eye irrigation equipment should also be readily available and staff trained in its correct use.
Appendix II
Immunization of Staff

The risks of working with particular agents should be fully discussed with individual researchers. The local availability, licensing state and utility of possible vaccines and/or therapeutic drugs (e.g. antibiotic treatments) in case of exposure should be evaluated before work with such agents is started. Some workers may have acquired immunity from prior vaccination or infection.

If a particular vaccine or toxoid is locally licensed and available, it should be offered after a risk assessment of possible exposure and a clinical health assessment of the individual have been carried out.

Facilities for specific clinical case management following accidental infections should also be available.
Appendix III
Equipment Safety

Certain items of equipment may create microbiological hazards when they are used. Other items are specifically designed to prevent or reduce biological hazards (see Chapters 9 and 10).

Equipment that may create a hazard
Table III-1 lists equipment and operations that may create hazards and suggests how such hazards may be eliminated or reduced.

Table III-1. Equipment and operations that may create hazards

<table>
<thead>
<tr>
<th>EQUIPMENT</th>
<th>HAZARD</th>
<th>HOW TO ELIMINATE/REDUCE HAZARD</th>
</tr>
</thead>
</table>
| Hypodermic needles | Accidental inoculation, aerosol or spillage | • Do not recap or clip needles.  
• Use a needle-locking type of syringe to prevent separation of needle and syringe or use a disposable type where needle is an integral part of the syringe unit.  
• Use good lab techniques, e.g.  
  o Fill syringe carefully to minimize air bubble and frothing  
  o Avoid using syringes to mix infectious liquids  
  o Wrap the needle and stopper in a gauze square moistened with an appropriate disinfectant before withdrawing from a rubber-stoppered bottle  
  o Expel excess liquid and air bubbles from the syringe vertically into a gauze square moistened with an appropriate disinfectant  
• Use a biological safety cabinet for all operations with infectious material.  
• Restrain animals while they are being inoculated. Use blunt needles or cannulas for intranasal or oral inoculation. Use a BSC  
• Autoclave after use and ensure proper disposal into a sharps container. Do not disassemble before discarding into sharps container. |
| Centrifuges     | Aerosols, splashing and tube breakage       | • Use sealable buckets (safety cups) or sealed rotors. Open buckets or rotors after aerosols have settled (30 min) or in a BSC.  
• Maintain a log book of operating hours for each rotor and a preventative maintenance program to reduce risk of mechanical failure. |
<p>| Ultra-centrifuges| Aerosols, splashing and tube breakage       | • Install a HEPA filter between centrifuge and vacuum pump. |</p>
<table>
<thead>
<tr>
<th>Equipment Type</th>
<th>Safety Hazards/Precautions</th>
</tr>
</thead>
</table>
| Anaerobic jars        | • Maintain a log book of operating hours for each rotor and a preventative maintenance program to reduce risk of mechanical failure.  
• Load and unload buckets/rotors in a BSC.  
• Ensure integrity of wire capsule around catalyst. |
| Desiccators           | • Double contain unit.                                                                     |
| Homogenizer, tissue grinder | • Use in a BSC. If you can’t use a BSC then wait 30 minutes before opening to allow aerosols to settle.  
• Use models with O-ring gaskets or use a stomacher.  
• If manual grinders are used, hold tube in a wad of absorbent material. |
| Sonicator, ultrasonic cleaner | • Use in a BSC or use a sealed unit.  
• Buy models with sufficient insulation to protect against sub-harmonics.  
• Wear gloves to protect skin from harsh detergents. |
| Culture stirrers, shakers, agitators | • Operate in a BSC or specially designed primary containment.  
• Use heavy-duty screw-capped culture flasks that are firmly secured to platform. |
| Lyophilizers          | • Use O-ring connectors to seal unit.  
• Use air filters to protect vacuum lines.  
• Use an all-metal moisture trap and a vapor condenser.  
• Inspect all glass vacuum vessels for imperfections. Use glassware designed specifically for vacuum work. |
| Water baths           | • Ensure regular cleaning and disinfection.  
• Do not use sodium azide for preventing growth of organisms.  
• Growth of microorganisms. Sodium azide forms explosive compounds with some metals. |
The following table lists examples of some of the causes of equipment accidents.

Table III-2. **Common causes of equipment-related accidents**

<table>
<thead>
<tr>
<th>ACCIDENT</th>
<th>CAUSE</th>
<th>REMEDIES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Faulty design or construction</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrical fires in incubators</td>
<td>No over-temperature cut-out</td>
<td>Compliance with local and federal standards.</td>
</tr>
<tr>
<td>Electrical shock</td>
<td>Failure to provide adequate earth/grounding</td>
<td></td>
</tr>
<tr>
<td><strong>Improper use</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centrifuge accident</td>
<td>Failure to balance buckets on swinging rotors</td>
<td>Train and supervise staff.</td>
</tr>
<tr>
<td>Anaerobic jar explosion</td>
<td>Use of incorrect gas</td>
<td>Train and supervise staff.</td>
</tr>
<tr>
<td><strong>Improper adaptation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Explosion in vacuum flask</td>
<td>Improper transport of liquid nitrogen</td>
<td>Use specially designed equipment.</td>
</tr>
<tr>
<td>Explosion in refrigerator</td>
<td>Dangerous chemical not stored in spark/explosion proof container, e.g. diethyl ether with leaking screw cap</td>
<td>Store low flashpoint solvents and extracts in spark/explosion proof refrigerators or cabinets.</td>
</tr>
<tr>
<td><strong>Lack of proper maintenance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fire in flame photometer</td>
<td>Incorrect reassembly of parts during maintenance</td>
<td>Train and supervise staff.</td>
</tr>
</tbody>
</table>