Biosafety and Risk Assessment

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List of contacts

Week	*Subject*
1	Biological Safety Biosafety and Biosecurity Regulations
2	Laboratory-acquired infections
3	Biosafety Foundation: Risk Assessment
4	Biosafety Program Management
5	Regulation, Standards and Guidelines of Biological Safety
6	Control in Biosafety: Facility Design (Basic Laboratories – Biosafety Levels 1 and 2 and Containment Equipment)
7	Control in Biosafety: Facility Design (The containment laboratory – Biosafety Level 3 and level 4)
8	Documentation and Emergency Response
9	Animal Biosafety; Laboratory Animal Facilities
10	Human gene transfer
11	Security Sensitive Biological Agent (SSBA)

References:

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Biological Safety

Biosafety and Biosecurity Regulations

Laboratory Biosafety And Biosecurity Definitions

Multiple definitions of biosafety and biosecurity exist. Moreover, in some languages, biosafety and biosecurity are translated into the same word, and the terms are often not differentiated

Laboratory Biosafety:

The WHO definition of **laboratory biosafety** is **"the containment principles, technologies and practices that are implemented to prevent unintentional exposure to pathogens and toxins, or their accidental release."** More simply, it is a set of practices to reduce the risks of accidental infection. The principles of laboratory biosafety were first introduced by the WHO in 1983 with the first edition of their *Laboratory biosafety manual*; consequently, many laboratories are familiar with the concepts of biosafety and have integrated it to varying degrees into their daily laboratory work in efforts to reduce laboratory-acquired infections.

In conclusion, Biosafety: is the prevention of exposures, occupationally acquired infections, and release of organisms to the environment by laboratory workers in the biomedical environment.

Elements of Biosafety:

- ✓ Standard microbiological practices
- ✓ Special practices
- ✓ Safety equipment (Primary Containment Barriers)
- ✓ Laboratory facilities (Secondary Containment Barriers)

Laboratory Biosecurity:

The *Laboratory biosafety manual* has in the past focused on traditional biosafety guidance for laboratories. The manual emphasizes 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 is 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.

Laboratory biosecurity is a newer concept and is much less well known to many laboratories around the world. **Laboratory biosecurity**, as defined by the WHO, **is "the protection, control and accountability for Valuable biological materials (VBM) within laboratories, in order to prevent their unauthorized access, loss, theft, misuse, diversion or intentional release."** It is alternatively described as the set of practices to reduce the risks of intentional infection as a result of malicious intent, ***or*** it is the protection of pathogens, toxins, and sensitive information from loss, theft and subsequent misuse.

The Difference Between Biosafety And Biosecurity

It is important to understand the distinction between **"laboratory biosafety"** and **"laboratory biosecurity".**

"Laboratory biosafety" is the term used to describe the set of 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 protect and control valuable biological materials (e.g. pathogens and toxins) and other valuable items (e.g. equipment) within laboratories, in order to prevent their loss, theft, misuse, diversion of, and/or unauthorized access or intentional unauthorized release.

Biosafety and biosecurity are related, but not identical, concepts. Biosafety programs reduce or eliminate exposure of individuals and the environment to potentially hazardous biological agents. Biosafety is achieved by implementing various degrees of laboratory control and containment, through laboratory design and access restrictions, personnel expertise and training, use of containment equipment, and safe methods of managing infectious materials in a laboratory setting.

The objective of biosecurity is to prevent loss, theft or misuse of microorganisms, biological materials, and research-related information. This is accomplished by limiting access to facilities, research materials and information. While the objectives are different, biosafety and biosecurity measures are usually complementary.

Biosafety and biosecurity programs share common components. Both are based upon risk assessment and management methodology; personnel expertise and responsibility; control and accountability for research materials including microorganisms and culture stocks; access control elements; material transfer documentation; training; emergency planning; and program management.

Both programs assess personnel qualifications. The biosafety program ensures that staff are

qualified to perform their jobs safely through training and documentation of technical expertise. Staff must exhibit the appropriate level of professional responsibility for management of research materials by adherence to appropriate materials management procedures. Biosafety practices require laboratory access to be limited when work is in progress. Biosecurity practices ensure that access to the laboratory facility and biological materials are limited and controlled as necessary. An inventory or material management process for control and tracking of biological stocks or other sensitive materials is also a component of both programs. For biosafety, the shipment of infectious biological materials must adhere to safe packaging, containment and appropriate transport procedures, while biosecurity ensures that transfers are controlled, tracked and documented commensurate with the potential risks.

A specific laboratory biosecurity programme 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 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 reference materials, clinical and epidemiological specimens and related information necessary for clinical or public health investigations.

Terms related to biosafety and Biosecurity

Biohazard: The potential source of harm caused by biological agents or toxins.

Biorisk: A combination of the probability of occurrence of harm and the severity of that harm

where the source of harm is a biological agent or toxin.

Valuable Biological Material: Biological materials that require (according to their owners, users, custodians, caretakers or regulators) administrative oversight, control, accountability, and specific protective and monitoring measures in laboratories to protect their economic and historical (archival) value, and/or the population from their potential to cause harm. VBM may include pathogens and toxins, as well as non-pathogenic organisms, vaccine strains, foods, genetically modified organisms (GMOs), cell components, genetic elements, and extraterrestrial samples.

Valuable Laboratory Material: Material of value to the laboratory due to its replacement cost and its necessity for the laboratory operational purposes. An example of VLM is laboratory equipment. Such material may be of interest to individuals outside the laboratory for other purposes (e.g. monetary or resource value, illegal drug production, etc.).

Laboratory-acquired infections

Thousands of infectious biological agents and toxins are handled and processed in an assortment of laboratory types for diagnostic, clinical, research, and commercial purposes around the world (e.g. *Mycobacterium tuberculosis*, *Brucella abortus*, *Escherichia coli* O157:H7, *Salmonella spp*., *Vibrio cholera*, foot-and-mouth disease virus, dengue fever virus). The type, number, and quantity of such materials are dependent upon the scope and nature of the work conducted in the laboratory. Each agent and toxin handled is a potential hazard posing a risk to personnel in the laboratory and facility, and likely to surrounding animal and human communities beyond the laboratory.

Classification of infective microorganisms by risk group

References are made to the relative hazards of infective microorganisms by risk group (WHO Risk Groups 1, 2, 3 and 4). **This risk group classification is to be used for laboratory work only**. Table 1 describes the risk groups.

Table 1. ***Classification of infective microorganisms by risk group***

Risk Group 1 *(no or low individual and community risk)* A microorganism that is unlikely to cause human or animal disease.
Risk Group 2 *(moderate individual risk, low community risk)* A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.
Risk Group 3 *(high individual risk, low community risk)* A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
Risk Group 4 *(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.

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 groups to biosafety levels, practices and equipment***

RISK GROUP	BIOSAFETY LEVEL	LABORATORY TYPE	LABORATORY PRACTICES	SAFETY EQUIPMENT
1	Basic – Biosafety Level 1	Basic teaching, research	GMT	None; open bench work
2	Basic – Biosafety Level 2	Primary health services; diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus BSC for potential aerosols
3	Containment – Biosafety Level 3	Special diagnostic services, research	As Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4	Maximum containment – Biosafety Level 4	Dangerous pathogen units	As Level 3 plus airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double-ended autoclave (through the wall), filtered air

BSC, biological safety cabinet; GMT, good microbiological techniques (see Part IV of this manual)

Countries (regions) should draw up a national (regional) classification of microorganisms, by risk group, taking into account:

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, antivirals and chemotherapeutic agents,

and should take into consideration the possibility of the emergence of drug-resistant strains.

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, 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 facility requirements at the four biosafety levels.

Table 3. ***Summary of biosafety level requirements***

	BIOSAFETY LEVEL			
	1	**2**	**3**	**4**
Isolation[a] of laboratory	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation:				
— inward airflow	No	Desirable	Yes	Yes
— controlled ventilating system	No	Desirable	Yes	Yes
— HEPA-filtered air exhaust	No	No	Yes/No[b]	Yes
Double-door entry	No	No	Yes	Yes
Airlock	No	No	No	Yes
Airlock with shower	No	No	No	Yes
Anteroom	No	No	Yes	—
Anteroom with shower	No	No	Yes/No[c]	No
Effluent treatment	No	No	Yes/No[c]	Yes
Autoclave:				
— on site	No	Desirable	Yes	Yes
— in laboratory room	No	No	Desirable	Yes
— double-ended	No	No	Desirable	Yes
Biological safety cabinets	No	Desirable	Yes	Yes
Personnel safety monitoring capability[d]	No	No	Desirable	Yes

[a] Environmental and functional isolation from general traffic.
[b] Dependent on location of exhaust (see Chapter 4).
[c] Dependent on agent(s) used in the laboratory.
[d] For example, window, closed-circuit television, two-way communication.

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

Biosafety Foundation: Risk Assessment

It is the responsibility of all laboratories that work with valuable biological material (VBM) and other valuable laboratory material (VLM) to operate safely and securely. The first step in achieving this operational goal is to assess the safety and security risks present in the laboratory. This is important during both routine work and unexpected situations. A risk assessment is the fundamental process to aid in the determination, management, and mitigation of laboratory risks. Mitigation of these risks will ultimately protect the individuals in the laboratory, in the facility and/or institution, as well as those outside the biological laboratory, including both the human and animal communities.

So biosafety risk assessment can be defined as An analytical procedure designed to characterize safety risks in a laboratory. A biosafety risk assessment allows a laboratory to determine the relative level of risks and helps guide risk mitigation decisions so they are targeted to the most important risk. A biosafety risk assessment should consider every activity and procedure in a laboratory that involves infectious disease agents.

Biosecurity risk assessment An analytical procedure designed to characterize security risks in a laboratory. A biosecurity risk assessment should consider every asset as well as every vulnerability in an institution and its component laboratories and units.

The backbone of the practice of biosafety is risk assessment. 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 institution's safety committee 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. 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 regimens.
11. Local availability of effective prophylaxis or therapeutic interventions.

On the basis of the information discovered 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.

Specimens for which there is limited information

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 wise to take a cautious approach to specimen manipulation.

- ✓ Standard precautions should always be followed, and barrier protections applied (gloves, gowns, eye protection), whenever samples are obtained from patients.

- ✓ Basic containment – Biosafety Level 2 practices and procedures should be the minimum requirement for handling specimens.

- ✓ 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 on the patient
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 (formed, arranged, or done for a particular purpose only) may be generated and posted by national competent authorities and/or WHO on the World Wide Web to indicate how specimens should be delivered for shipment and the biosafety level at which they should be analysed.

The importance and benefits of risk assessment

The benefits of risk assessment in the laboratory extend beyond risk reduction and mitigation. Laboratory risk assessments can also help to provide the following:

1. Effective allocation of resources to mitigate risks
2. Identification of training needs and supervision
3. Advance planning for renovation

4. Evaluation of procedural changes
5. Compliance with governmental regulations
6. Justification for space and equipment needs
7. Evaluation of emergency plans
8. Planning for preventative maintenance
9. Evaluation of exchanges and workflow with other laboratories/units

When to Perform and Review a Laboratory Risk Assessment

A periodic assessment of laboratory risk is important. When experiments, processes, and technology change, so does the risk. A risk assessment should therefore be performed and reviewed periodically, perhaps annually. Although an organization should consider conducting a risk assessment more often as circumstances warrant, for example, following the occurrence of problems or if laboratory practices change.

Ideally, a laboratory should perform an initial risk assessment before any work is started. A risk assessment should also be done whenever a change occurs. Examples of activities or events that will change risk and warrant a reassessment include:

1. New infectious agents, toxins, reagents or other dangerous substances
2. New animal species, model, or route of administration of biological agents
3. New procedures and practices
4. New equipment
5. Personnel changes
6. Aging of equipment
7. Advances in scientific understanding and technology
8. A relocation or renovation
9. A recent or "near-miss" accident, laboratory-acquired infection (LAI), theft, or security violation
10. National or regional changes in disease status (endemicity of disease or disease eradication)
11. National, regional or local changes in threat environment or security environment
12. New local or national regulations

After reviewing the results of the risk assessment, measures should be made to modify or update, as necessary. It is important to perform a risk assessment regularly - do not wait until an adverse event happens. Further, each modification or update, as well as each step in the risk assessment process, must be fully documented. Documentation is critical for future reviews when evaluating performance.

Laboratory Risk Assessment Methodology

A laboratory risk assessment should be a structured process to identify and manage the biorisks present within a biological laboratory. A risk assessment reviews all aspects of the work environment, including location, proposed work activities, personnel, storage, sample transfer and transport, destruction, access, and security, among others.

The basic risk assessment process is as follows:

1.	Define the situation	*What work is occurring?*
2.	Define the risks	*What can go wrong?*
3.	Characterize the risks	*How likely is it to happen? What are the consequences?*
4.	Determine if the risks are acceptable	*Engage management and other key stakeholders*
5.	Implement risk mitigation measures	*Ensure all risks are acceptable post implementation of mitigation measures*

Biosafety Risk Assessment

A biosafety risk assessment should adhere to a structured and repeatable process and should follow the five-step technical approach described and illustrated below. Factors that affect the likelihood of the biosafety risk are captured in the first step of the risk assessment process, ("Define the situation").

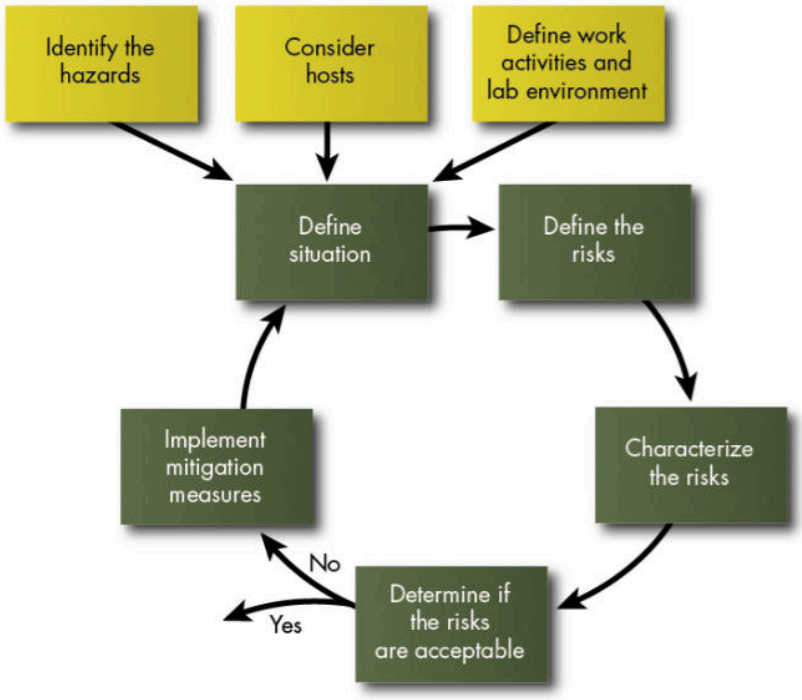


Figure 6. Biosafety Risk Assessment Process. Yellow boxes indicate biosafety specific steps of the risk assessment process; green boxes illustrate common steps shared between biosafety and biosecurity risk assessments.

Biosafety Risk Assessment Process Summary

A summary of the biosafety risk assessment process and its steps is presented in Table 1.

	Step 1	Step 2	Step 3
1. Define the situation	**1a. Identify the hazards**	**1b. Consider the hosts**	**1c. Define work activities and lab environment**
	Create a list of biological agents currently used. What are the biological characteristics of the agents (hazards)?	• Lab staff? • Human community? • Animal community?	• Locations? • Procedures? • Equipment? • Manuals and guidelines?
2. Define the risks	**2a. Consider all risks and determine which risks should be defined.**		
	Should more than one risk assessment be conducted?		
3. Characterize the risks	**3a. Perform a hazard assessment**	**3b. Perform a host assessment**	**3c. Perform a work activities/environment assessment**
	Define likelihood of infection of the agent. Consider consequences to a host of this agent following infection?	Define likelihood of infection for staff? Consider consequences of disease from infection.	Define likelihood of infection considering the work activities and lab processes used with the hazard. Consider consequences of an exposure and infection to work practices.
4. Determine if the risks are acceptable	**4a. Must be discussed with management and other stakeholders.**		
	Must consider lab personnel, institutional personnel and the community		
5. Implement mitigation measures, if needed	**5a. Must be discussed with management and other stakeholders.**		
	Must consider lab personnel, institutional personnel and the community		

Table 1. Summary of Biosafety Risk Assessment Methodology Process

Biosafety Program Management

It is essential that each laboratory organization have a comprehensive safety policy, a safety manual, and supporting programs for their implementation. 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.

Biosafety Program:

A successful biosafety program requires:

1. **Institutional oversight & acceptance:** Support from the highest administrative body at your institution

2. **Institutional Biosafety Committee (IBC):** A group of individuals who will help forge policy and make decisions for the leaders of the institution

3. **Biosafety Office:** A person or group of people to manage the program and disseminate information regarding program requirements; and

4. **Faculty, staff, students & visitors:** Individuals that buy into and accept the program's information and requirements

Each group is as important as the other. If the end users, the trainees, those at risk, etc. don't "use" or "follow" basic biosafety procedures, the program will likely fail (exposures, incidents, spills/releases, laboratory acquired infections, non-compliance events, increased regulatory visits or inspections by outside agencies, bad press, etc.)

Related Compliance Entities

In a research environment there are ties to multiple groups, Cross-training between these groups are recommended.

- **IRB – Institutional Review Board –** oversight for research involving human subjects (they are the 1st group to see research rDNA research involving human subjects or human gene transfer research).
- **IACUC – Institutional Animal Care and Use Committee –** oversight for research involving living vertebrate animals (their registration forms should capture research with animals involving biohazards, such as the creation of Transgenic animals, other rDNA experiments, such as the use of replication defective vectors, the use of human or animal pathogens, work with human and animal oncogenic material, human materials, and a review of the animals themselves from a zoonotic disease risk potential)
- **ICC – Infection Control Committee –** involved in the protection of patients and employees in hospital and outpatient settings to prevent nosocomial infections and outbreaks of diseases in these settings and limit spread within the community. Cross-over activities include inspection of facilities, examination of compliance with work

practices such as hand washing, use of personal protective equipment, decontamination, and disinfection, evaluation of isolation facilities, development of medical waste programs, training, and control of the spread of contaminants during construction of renovation activities. The Infection Control Practitioner is also a valuable asset to the Institutional Biosafety Committee (IBC) in the review of protocols involving human pathogens and measures to minimize exposure.

- **Grants & Contracts Offices:** review proposed research protocols that are going out for funding requests and those successful applications that are coming back in with funding allocated to start the projects. If this Office serves as the "funnel" for new and ongoing research projects, their education in biohazard projects that require institutional registration is essential in ensuring that all related groups are aware of the proposed research before it is initiated. Their registration forms should also include check boxes for the major categories of research that requires this registration (rDNA, Human Pathogens, Human Gene Transfer, Select Agents, Animals, Human Subjects, etc.)

- **Police, Security:** Biosecurity has become a big issue and interactions with local groups responsible for background checks, providing access to secure areas and investigating suspicious activity is now a standard component of biosafety programs involving high risk biohazardous materials.

- **Other:** the other groups that can help to identify potential biosafety needs or catch potential exposure to biohazards:

- ✓ Food Service Managers and Sanitation Officials.
- ✓ Area Hospital and/or Health Clinic to catch foodborne outbreaks from the same location.
- ✓ Undergraduate and Graduate Student advisors who sign off on student research protocols.
- ✓ Fellowship and other Program Committees that fund student and employee travel abroad to work on public health related research projects.
- ✓ Employee and Student Health healthcare providers (including those responsible for athletic programs) to catch MRSA, VRE and other outbreaks
- ✓ Grounds Maintenance personnel who can help control conditions that allow water build up (for mosquito control), this group also needs info on how to protect themselves from exposure to ticks, and zoonotic diseases from wild animals (as well as many physical related hazards)

The biosafety officer and biosafety committee

Biosafety officer

Wherever possible a biosafety officer should be appointed to ensure that biosafety policies and programs are followed consistently throughout the laboratory. The biosafety officer executes these duties on behalf of the head of the institute or laboratory. In small units, the biosafety officer may be a microbiologist or a member of the technical staff. The person designated should possess the professional competence necessary to suggest, review and approve specific activities

that follow appropriate biocontainment and biosafety procedures. The biosafety officer should apply relevant national and international rules, regulations and guidelines, as well as assist the laboratory in developing standard operating procedures. The person appointed must have a technical background in microbiology, biochemistry and basic physical and biological sciences. Knowledge of laboratory and clinical practices and safety, including containment equipment, and engineering principles relevant to the design, operation and maintenance of facilities is highly desirable. The biosafety officer should also be able to communicate effectively with administrative, technical and support personnel.

The activities of the biosafety officer should include the following:

1. Biosafety, biosecurity and technical compliance consultations.
2. Periodic internal biosafety reviews on technical methods, procedures and protocols, biological agents, materials and equipment.
3. Discussions of violation of biosafety protocols or procedures with the appropriate persons.
4. Verification that all staff have 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 laboratory director and biosafety committee.
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. Ensuring appropriate 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 laboratory, according to national regulations.
13. Reviewing the biosafety aspects of all plans, protocols and operating procedures for research work involving infectious agents prior to the implementation of these activities.
14. Institution of a system to deal with emergencies.

Biosafety committee

A biosafety committee should be established to develop institutional biosafety policies and codes of practice. The biosafety committee should also review research protocols for work involving infectious agents, animal use, recombinant DNA and genetically modified materials. Other functions of the committee may include risk assessments, formulation of new safety policies and arbitration in disputes over safety matters.

The composition of a basic biosafety committee may include:

1. Biosafety officer(s)
2. Scientists
3. Medical personnel
4. Veterinarian(s) (if work with animals is conducted)
5. Representatives of technical staff
6. Representatives of laboratory management.

The biosafety committee should seek advice from different departmental and specialist safety officers (e.g. with expertise in radiation protection, industrial safety, fire prevention, etc.) and may at times require assistance from independent experts in various associated fields, local authorities and national regulatory bodies. Community members may also be helpful if there is a particularly contentious or sensitive protocol under discussion.

Training programs

A continuous, on-the-job safety-training program is essential to maintain safety awareness among laboratory and support staff. Laboratory supervisors, with the assistance of the biosafety officer and other resource persons, play the key role in staff training. The effectiveness of biosafety training, indeed all safety and health training, depends on management commitment, motivational factors, adequate initial job training, good communications, and ultimately the organization's goals and objectives. WHO provides various tools for microbiological safety training.

Regulation, Standards and Guidelines of Biological Safety

Regulations and Guidelines:

Protection of personnel from exposure to biohazards is the first target in Biosafety. However, compliance with regulations standards and guidelines is equally for your institution.

The following is a summary of federal, state, and local agency (in USA) **regulations and guidelines** that either regulate or provide guidelines covering the use of biological agents:

1. **Centers for Disease Controls and Prevention and the National Institutes of Health (CDC/NIH):**

   - ✓ ***Biosafety in Microbiological and Biomedical Laboratories*** **(BMBL) (CDC/NIH BMBL):** This document contains guidelines for microbiological practices, safety equipment, and facilities that constitute the four established biosafety levels. The BMBL is generally considered the standard for biosafety and is the basis for this manual. This biosafety guidebook updated every 5 years or so that provides a framework for the control of biohazards in research settings.
   - ✓ **CDC/TB: (Tuberculosis)** the CDC along with OSHA (Occupational Safety and Health Administration) and each State provides guidance on the control of Tuberculosis in healthcare and laboratory settings.

2. **National Institutes of Health:** *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* **(NIH Guidelines).** This document provides guidelines for constructing and handling recombinant or synthetic nucleic acid molecules and organisms containing those molecules. Although these guidelines are not subject to regulatory enforcement, institutions that receive any NIH funding for research involving recombinant or synthetic nucleic acid molecules are required to comply with these guidelines as a condition of funding. This document requires that each institution establish an **IBC** (Institutional Biosafety Committee) with the authority to approve proposed research involving recombinant or synthetic nucleic acid molecules using the NIH guidelines as the minimum standard. This document was first drafted in 1976 by scientists concerned about developing standards for the safe practice of research involving rDNA as well as with organisms containing rDNA.

3. **Occupational Safety and Health Administration (OSHA):** This regulation covers occupational exposure to human blood and other potentially infectious materials, including human tissue and cells. OSHA specifies a combination of engineering controls, work

practices, and training to reduce the risk of infection. Personnel potentially exposed to human blood and other potentially infectious material must be offered immunization against hepatitis B and receive annual training. Personnel who work with HIV or hepatitis B in a research laboratory must receive additional training and demonstrate proficiency in working with human pathogens.

A few specific Occupational Safety and Health Administration (OSHA) Standards have been developed for biohazards or related topics and each have prescriptive criteria that must be followed to protect workers, for examples:

- ✓ PPE (personal protective equipment) laws are detailed in OSHA.
- ✓ Blood borne Pathogens Standard are the major compliance rules to address for Biosafety Officers.
- ✓ Biohazard labeling requirements.
- ✓ Control of exposure to Ethylene Oxide (usually used in hospitals for a cold sterilization process for items that cannot be autoclaved)
- ✓ Standards that address emergency response to spills and releases.

Other Guidelines Included:

- **Association of Practitioners in Infection Control (APIC):** overview of infection control precautions in healthcare settings.
- **World Health Organization (WHO)**: recommendations, guidance documents related to Biosafety and the control of infectious diseases in developing nations (they also publish a Biosafety Laboratory Guidelines text similar to the CDC/NIH BMBL).
- **National Standard Foundation Standard #49 (NSF 49):** for Class II Biological Safety Cabinets (the primary engineering control device used to confine biohazards in research and clinical laboratories).

Transport Regulation Requirements:

Transport regulation requirements must be widely disseminated across an institution to prevent the improper packaging and shipment of biohazards. All "shippers" must be trained and specialized packaging is required. Shipping Declaration Forms and Permits may also be required.

1. **U.S. Department of Transportation (DOT):** legal body for all forms of hazardous material transport in the U.S.

2. **International Air Transport Association (IATA):** Determine requirements for air transport of dangerous goods.

These two organizations have strict requirements governing the shipment and transportation of hazardous materials, including biological agents.

3. **U.S. Centers for Disease Control and Prevention/Public Health Service (CDC/PHS):** importation, possession and transfer of certain human pathogens. The CDC has established specific regulatory requirements for importation or transportation of etiologic agents, which include a permit application that must be submitted and approved *prior* to any such importations.

4. **U.S. Department of Agriculture, Animal and Plant Health Inspection Service, and Veterinary Services (USDA, APHIS, and VS):** importation, possession and transfer of certain animal pathogens. The USDA, APHIS, and VS regulate the importation of animals and animal derived materials to ensure that strange animal and poultry diseases are not introduced into the United States. Generally, a USDA veterinary permit is needed for materials derived from animals or exposed to animal source materials. Materials that require a permit include animal tissues, blood, cells or cell lines of livestock or poultry origin, RNA/DNA extracts, hormones, enzymes, monoclonal antibodies for *in vivo* use in non-human species, certain polyclonal antibodies, antisera, bulk shipments of test kit reagents, and microorganisms, including bacteria, viruses, protozoa, and fungi.

5. **Department of Commerce (DoC):** govern the export of materials outside the U.S. The DOC has specific regulatory requirements for exportation of biological materials. These regulations are both agent and country specific and must be followed strictly. Basic overview of U.S. Export Controls for Biological Materials.

6. **Plant Protection and Quarantine (PPQ).**

7. **Department of Defense (DoD) (U.S. Army).**

Other Agencies and Regulatory Requirements:

- **U.S. Food and Drug Administration (FDA):** Human Subjects, Approve Human Gene Transfer research protocols
- **U.S. Environmental Protection Agency (EPA):** provide guidance to states on medical waste rules and requirements
- **Office of Human (subjects) Research Protection (NIH OHRP)**
- **State – Department of Public Health:** variety of regulations that impact human health

sanitation, patient care, clinical lab standards, use of human pathogens in clinical and research labs, etc., Department of Environmental Protection (medical waste, releases/spills that may reach the environment).

- **City – work with local Dept. of Public Health to see what rules, regulations or ordinances may apply to your institution.**
- **Massachusetts Department of Public Health (MADPH):** The MADPH regulates the management of biological wastes in the state and also inspects BSL-3 laboratory spaces on a regular basis.
- **United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern (DURC):** The policy addresses institutional oversight of DURC, which includes policies, practices, and procedures to ensure DURC is identified and risk mitigation measures are implemented, where applicable. The fundamental aim of this oversight is to preserve the benefits of life sciences research while minimizing the risk of misuse of the knowledge, information, products, or technologies provided by such research.
- **Institutional Biosafety Committee (IBC):** is an institutional committee created under the NIH Guidelines to review research involving recombinant DNA and synthetic nucleic acid research. The IBC also has promulgated a number of specific policies and procedures that are incorporated into this document as requirements or have been included as appendices.

Training:

The standards and guidelines noted above also require many of training programs to protect personnel

Control in Biosafety: Facility Design (Basic Laboratories –Biosafety Levels 1 and 2 and Containment Equipment)

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.

Basic Laboratories –Biosafety Levels 1 and 2

Diagnostic and health-care laboratories (public health, clinical or hospital-based) must all be designed for Biosafety Level 2 or above. As no laboratory has complete control over the specimens it receives, laboratory workers may be exposed to organisms in higher risk groups than anticipated.

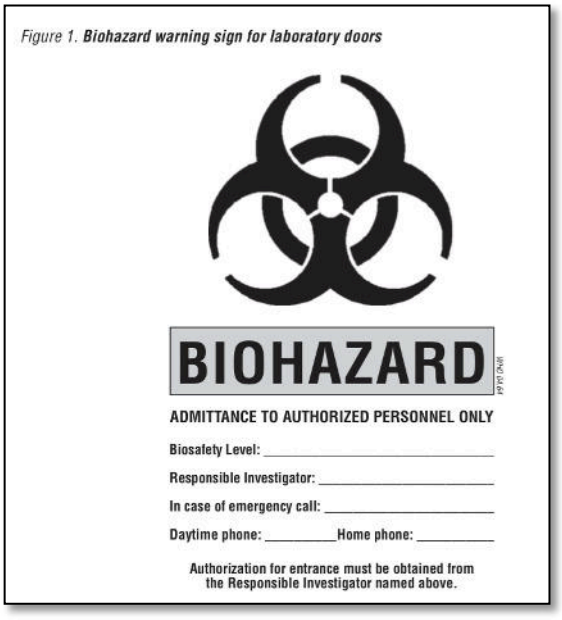
Code of practice

This code is a listing of the most essential laboratory practices and procedures that are basic to Good Microbiological Techniques (GMT). In many laboratories and national laboratory programs, this code may be used to develop written practices and procedures for safe laboratory operations.

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. Good Microbiological Techniques (GMT) are fundamental to laboratory safety. 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.
  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.

Figure 1. ***Biohazard warning sign for laboratory doors***



5. Access to animal houses should be specially authorized.
6. No animals should be admitted other than those involved in the work of the laboratory.

- ***Personal protection***

1. Laboratory coveralls, gowns or uniforms 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 forbidden to wear protective laboratory clothing outside the laboratory, e.g. in canteens, 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 forbidden in the laboratory working areas.
8. Storing human foods or drinks anywhere in the laboratory working areas is forbidden.
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. Materials 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 or for any purpose other than parenteral injection or aspiration of fluids from laboratory animals.
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 discharge to the sanitary sewer. An effluent treatment system may be required, depending on the risk assessment for the agent(s) being handled.

- ***Laboratory working areas***

1. The laboratory should be kept well-ordered, 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 arthropod-proof screens.

- ***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 arthropod and rodent control programme.
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. Invasion 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. Sufficient 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, resistant to liquids and resistant to the chemicals and disinfectants normally used in the laboratory. Floors should be slip-resistant.
3. Bench tops should be waterproof to water and resistant to disinfectants, acids, alkalis, organic solvents and moderate heat.
4. Illumination (lighting) should be suitable for all activities. Undesirable reflections and glare should be avoided.
5. Laboratory furniture should be tough. Open spaces between and under benches, cabinets and equipment should be accessible for cleaning.
6. Storage space must be suitable to hold supplies for immediate use and thus prevent mess on bench tops and in passageways. 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 clothes 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, with running water if possible, should be provided in each laboratory room, preferably near the exit door.
11. Doors should have vision boards, 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 eyewash facilities.
14. First-aid areas or rooms suitably prepared and readily accessible should be available.
15. In the planning of new facilities, consideration should be given to the providing 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 arthropod-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 compulsory. 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.

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.

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.
   - ✓ 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.

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
2. Facilitation of the early detection of laboratory-acquired infections
3. Exclusion of highly susceptible individuals (e.g. pregnant women or immunecompromised individuals) from highly hazardous laboratory work
4. Provision of effective personal protective equipment and procedures.
5. 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.

Training

continuous in-service training in safety measures is essential because it is key to the prevention of laboratory- acquired infections, incidents and accidents. 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.

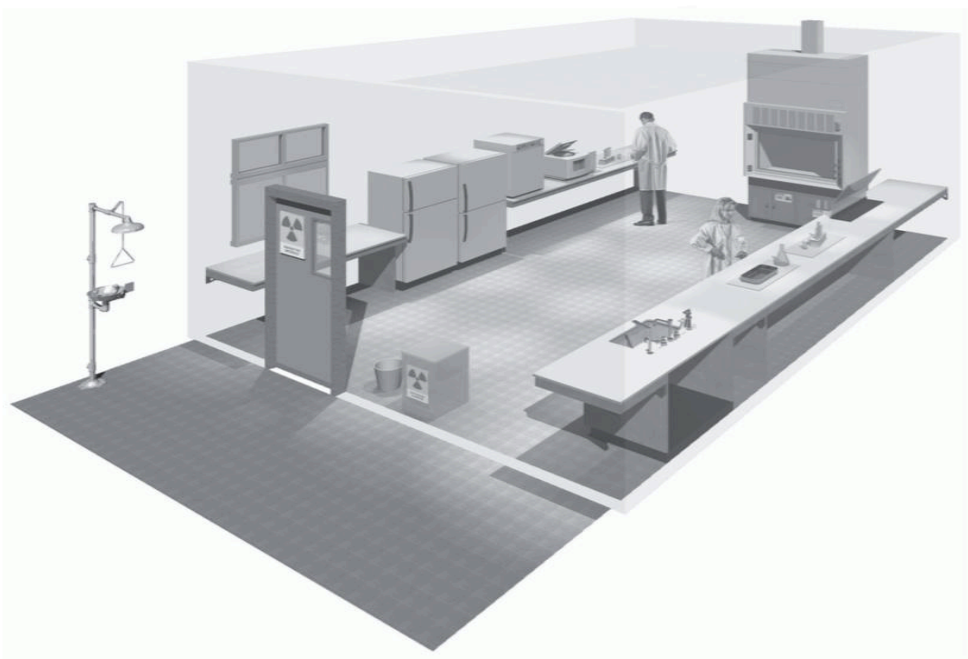


Figure 2. ***A typical Biosafety Level 1 laboratory***
(graphics kindly provided by CUH2A, Princeton, NJ, USA)

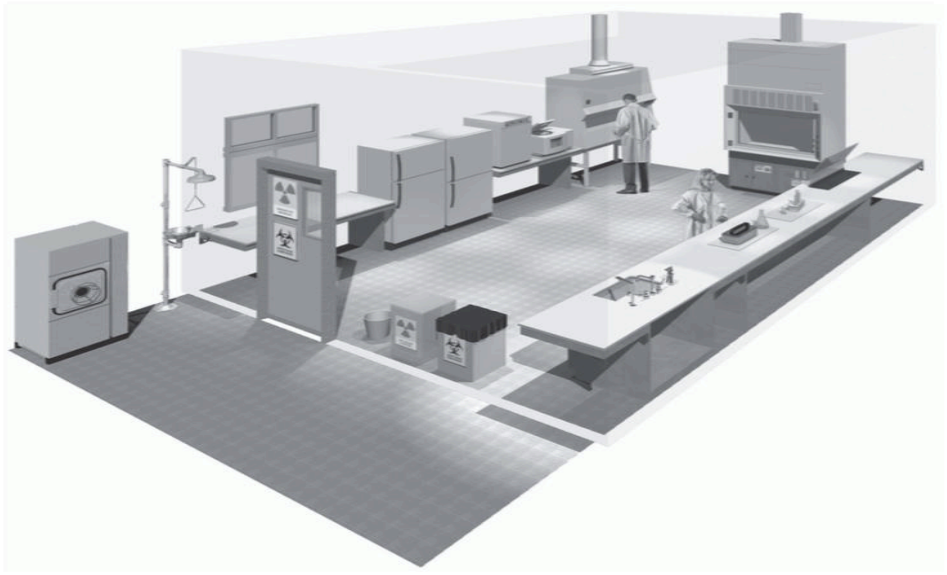


Figure 3. ***A typical Biosafety Level 2 laboratory***
(graphics kindly provided by CUH2A, Princeton, NJ, USA). Procedures likely to generate aerosols are performed within a biological safety cabinet. Doors are kept closed and are posted with appropriate hazard signs. Potentially contaminated wastes are separated from the general waste stream.

Control in Biosafety: Facility Design (The containment laboratory – Biosafety Level 3 and level 4)

The containment laboratory – Biosafety Level 3

The containment laboratory – Biosafety Level 3 is designed and provided for work with Risk Group 3 microorganisms and with large volumes or high concentrations of Risk Group 2 microorganisms that pose an increased risk of aerosol spread. Biosafety Level 3 containment requires the strengthening of the operational and safety programs over and above those for basic laboratories – Biosafety Levels 1 and 2.

The guidelines given here are presented in the form of additions to those for basic laboratories – Biosafety Levels 1 and 2, which must therefore be applied before those specific for the containment laboratory – Biosafety Level 3. The major additions and changes are in:

1. Code of practice
2. Laboratory design and facilities
3. Health and medical surveillance.

Code of practice

The code of practice for basic laboratories – Biosafety Levels 1 and 2 applies except where modified as follows:

1. The international biohazard warning symbol and sign displayed on laboratory access doors must identify the biosafety level and the name of the laboratory supervisor who controls access, and indicate any special conditions for entry into the area, e.g. immunization.
2. Laboratory protective clothing must be of the type with solid-front or wrap-around gowns, scrub suits, coveralls, head covering and, where appropriate, shoe covers or dedicated shoes. Front-buttoned standard laboratory coats are unsuitable. Laboratory protective clothing must not be worn outside the laboratory, and it must be decontaminated before it is laundered.
3. Open manipulations of all potentially infectious material must be conducted within a biological safety cabinet or other primary containment device.
4. Respiratory protective equipment may be necessary for some laboratory procedures or working with animals infected with certain pathogens.

Laboratory design and facilities

The laboratory design and facilities for basic laboratories – Biosafety Levels 1 and 2 apply except where modified as follows:

1. The laboratory must be separated from the areas that are open to unrestricted traffic flow within the building. Additional separation may be achieved by placing the laboratory at the blind end of a corridor, or constructing a barrier and door or access through an anteroom (e.g. a double-door entry or basic laboratory – Biosafety Level 2). The anteroom should have facilities for separating clean and dirty clothing and a shower may also be necessary.
2. Anteroom doors may be self-closing and interlocking so that only one door is open at a time. A break-through panel may be provided for emergency exit use.
3. Surfaces of walls, floors and ceilings should be water-resistant and easy to clean. Openings through these surfaces (e.g. for service pipes) should be sealed to facilitate decontamination of the room(s).
4. The laboratory room must be sealable for decontamination. Air-ducting systems must be constructed to permit gaseous decontamination.
5. Windows must be closed, sealed and break-resistant.
6. A hand-washing station with hands-free controls should be provided near each exit door.
7. There must be a controlled ventilation system that maintains a directional airflow into the laboratory room. A visual monitoring device with or without alarm(s) should be installed so that staff can at all times ensure that proper directional airflow into the laboratory room is maintained.
8. The building ventilation system must be so constructed that air from the containment laboratory – Biosafety Level 3 is not recirculated to other areas within the building. Air may be high-efficiency particulate air (HEPA) filtered, reconditioned and recirculated within that laboratory. When exhaust air from the laboratory (other than from biological safety cabinets) is discharged to the outside of the building, it must be dispersed away from occupied buildings and air intakes. Depending on the agents in use, this air may be discharged through HEPA filters. A heating, ventilation and air-conditioning (HVAC) control system may be installed to prevent continued positive pressurization of the laboratory. Consideration should be given to the installation of audible or clearly visible alarms to notify personnel of HVAC system failure.
9. All HEPA filters must be installed in a manner that permits gaseous decontamination and testing.
10. Biological safety cabinets should be sited away from walking areas and out of cross-currents from doors and ventilation systems.
11. The exhaust air from Class I or Class II biological safety cabinets, which will have

been passed through HEPA filters, must be discharged in such a way as to avoid interference with the air balance of the cabinet or the building exhaust system.

12. An autoclave for the decontamination of contaminated waste material should be available in the containment laboratory. If infectious waste has to be removed from the containment laboratory for decontamination and disposal, it must be transported in sealed, unbreakable and leakproof containers according to national or international regulations, as appropriate.
13. The containment laboratory – Biosafety Level 3 facility design and operational procedures should be documented.

An example of laboratory design for Biosafety Level 3 is shown in Figure below.

Laboratory equipment

The principles for the selection of laboratory equipment, including biological safety cabinets are the same as for the basic laboratory – Biosafety Level 2. However, at Biosafety Level 3, manipulation of all potentially infectious material must be conducted within a biological safety cabinet or other primary containment device. Consideration should be given to equipment such as centrifuges, which will need additional containment accessories, for example, safety buckets or containment rotors. Some centrifuges and other equipment, such as cell-sorting instruments for use with infected cells, may need additional local exhaust ventilation with HEPA filtration for efficient containment.

Health and medical surveillance

The objectives of health and medical surveillance programs for basic laboratories – Biosafety Levels 1 and 2 also apply to containment laboratories – Biosafety Level 3, except where modified as follows: Medical examination of all laboratory personnel who work in containment laboratories – Biosafety Level 3 is mandatory. This should include recording of a detailed medical history and an occupationally targeted physical examination.

The maximum containment laboratory – Biosafety Level 4

The maximum containment laboratory – Biosafety Level 4 is designed for work with Risk Group 4 microorganisms. Before such a laboratory is constructed and put into operation, intensive consultations should be held with institutions that have had experience of operating a similar facility. Operational maximum containment laboratories – Biosafety Level 4 should be under the control of national or other appropriate health authorities.

Code of practice

The code of practice for Biosafety Level 3 applies except where modified as follows:

1. The two-person rule should apply, whereby no individual ever works alone. This is particularly important if working in a Biosafety Level 4 suit facility.
2. A complete change of clothing and shoes is required prior to entering and upon exiting the laboratory.
3. Personnel must be trained in emergency extraction procedures in the event of personnel injury or illness.
4. A method of communication for routine and emergency contacts must be established between personnel working within the maximum containment laboratory – Biosafety Level 4 and support personnel outside the laboratory.

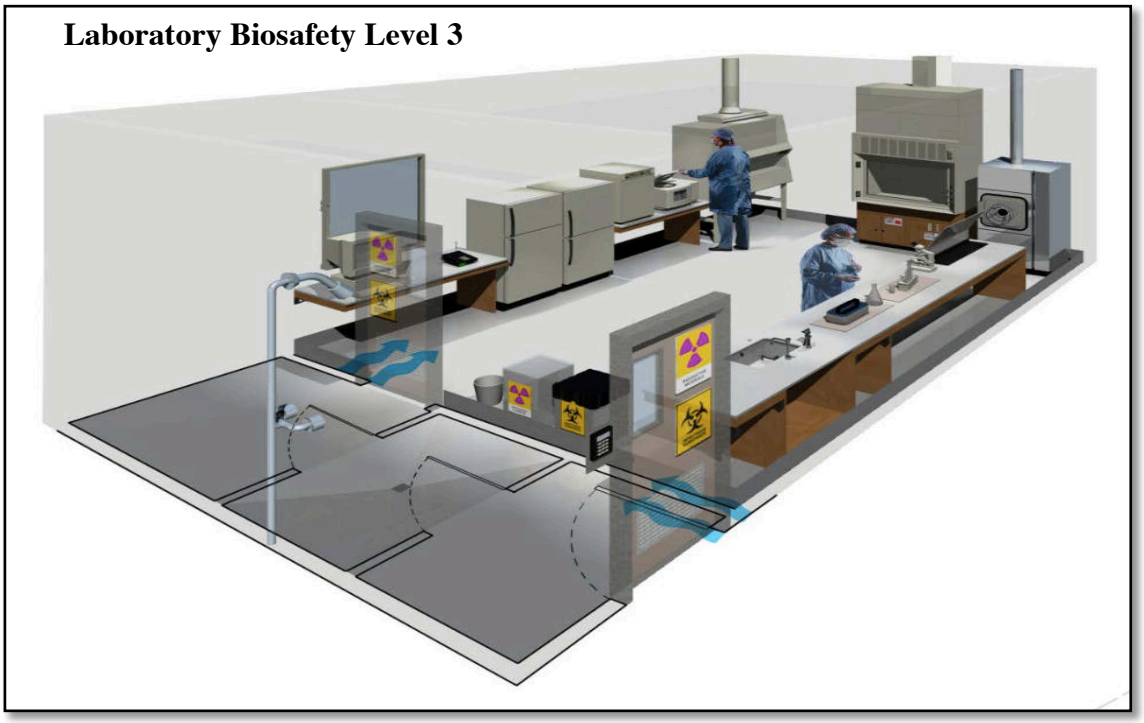
Laboratory design and facilities

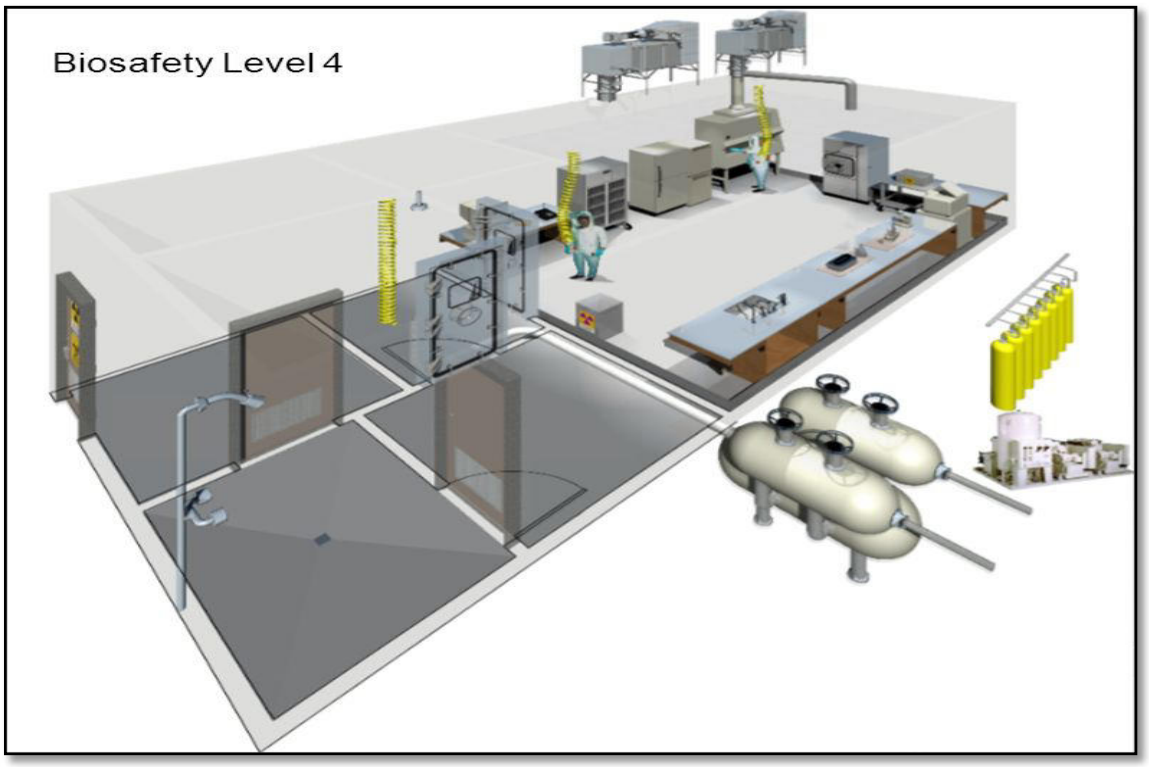
The features of a containment laboratory – Biosafety Level 3 also apply to a maximum containment laboratory – Biosafety Level 4 with the addition of the following:

1. **Primary containment**: An efficient primary containment system must be in place, consisting of one or a combination of the following:
   - ✓ ***Class III cabinet laboratory***: Passage through a minimum of two doors prior to entering the rooms containing the Class III biological safety cabinet(s) (cabinet room) is required. In this laboratory configuration the Class III biological safety cabinet provides the primary containment. A personnel shower with inner and outer changing rooms is necessary. Supplies and materials that are not brought into the cabinet room through the changing area are introduced through a double-door autoclave or fumigation (disinfection) chamber. Once the outer door is securely closed, staff inside the laboratory can open the inner door to save the materials. The doors of the autoclave or fumigation chamber are interlocked in such a way that the outer door cannot open unless the autoclave has been operated through a sterilization cycle or the fumigation chamber has been decontaminated.
   - ✓ ***Suit laboratory***: A protective suit laboratory with self-contained breathing apparatus differs significantly in design and facility requirements from a Biosafety Level 4 laboratory with Class III biological safety cabinets. The rooms in the protective suit laboratory are arranged so as to direct personnel through the changing and decontamination areas prior to entering areas where infectious materials are manipulated. A suit decontamination shower must be provided and used by personnel leaving the containment laboratory area. A separate personnel shower with inner and outer changing rooms is also provided. Air to the suit must be provided by a system

that has a 100% excessive capability with an independent source of air, for use in the event of an emergency. Entry into the suit laboratory is through an airlock fitted with airtight doors. An appropriate warning system for personnel working in the suit laboratory must be provided for use in the event of mechanical system or air failure.

2. **Controlled access**: The maximum containment laboratory – Biosafety Level 4 must be located in a separate building or in a clearly defined zone within a secure building. Entry and exit of personnel and supplies must be through an airlock or pass-through system. On entering, personnel must put on a complete change of clothing; before leaving, they should shower before putting on their street clothing.
3. **Controlled air system**: Negative pressure must be maintained in the facility. Both supply and exhaust air must be HEPA-filtered. There are significant differences in the ventilating systems of the Class III cabinet laboratory and suit laboratory:
   - ✓ ***Class III cabinet laboratory***: The supply air to the Class III biological safety cabinet(s) may be drawn from within the room through a HEPA filter fixed on the cabinet or supplied directly through the supply air system. Exhaust air from the Class III biological safety cabinet must pass through two HEPA filters prior to release outdoors. The cabinet must be operated at negative pressure to the surrounding laboratory at all times. A dedicated non-recirculating ventilating system for the cabinet laboratory is required.
   - ✓ ***Suit laboratory:*** Dedicated room air supply and exhaust systems are required. The supply and exhaust components of the ventilating system are balanced to provide directional airflow within the suit area from the area of least hazard to the area(s) of greatest potential hazard. Excessive exhaust fans are required to ensure that the facility remains under negative pressure at all times. The differential pressures within the suit laboratory and between the suit laboratory and adjacent areas must be monitored. HEPA-filtered supply air must be provided to the suit area, decontamination shower and decontamination airlocks or chambers. Exhaust air from the suit laboratory must be passed through a series of two HEPA filters prior to release outdoors.
4. **Decontamination of effluents (waste)**: All effluents from the suit area, decontamination chamber, decontamination shower, or Class III biological safety cabinet must be decontaminated before final discharge. Heat treatment is the preferred method. Effluents may also require correction to a neutral pH prior to discharge. Water from the personnel shower and toilet may be discharged directly to the sanitary sewer without treatment.
5. **Sterilization of waste and materials**: A double-door, pass-through autoclave must be available in the laboratory area. Other methods of decontamination must be available for equipment and items that cannot withstand steam sterilization.


Laboratory Biosafety Level 3


Biosafety Level 4

BSL-4 Laboratory

Cabinet Laboratory

BSL-4 Laboratory

Suit Laboratory

Documentation and Emergency Response

Every laboratory that works with infective microorganisms should institute safety precautions appropriate to the hazard of the organisms and the animals being handled. A written contingency plan for dealing with laboratory and animal facility accidents is a necessity in any facility that works with or stores Risk Group 3 or 4 microorganisms (containment laboratory – Biosafety Level 3 and maximum containment laboratory – Biosafety Level 4). National and/or local health authorities should be involved in the development of the emergency preparedness plan.

Contingency Plan (Emergency Plan)

The contingency (emergency) plan should provide operational procedures for:

1. Precautions against natural disasters, e.g. fire, flood, earthquake and explosion.
2. Biohazard risk assessment.
3. Incident-exposure management and decontamination.
4. Emergency evacuation of people and animals from the buildings.
5. Emergency medical treatment of exposed and injured persons.
6. Medical surveillance of exposed persons.
7. Clinical management of exposed persons.
8. Epidemiological investigation.
9. Post-incident continuation of operations.

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 scratches:**
  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 required. 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 evacuate the affected area.
  - ✓ 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 infectious substances and spilled infectious 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. 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 recover glass trash.
  - ✓ All broken tubes, glass fragments, buckets, 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 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. Infectious materials should be collected in leakproof boxes or strong disposable bags.

Final disposal should be determined by biosafety staff on the basis of local regulations.

Emergency Services: Whom To Contact

The telephone numbers and addresses of the following should be 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. Biosafety officer
5. Fire services
6. Hospitals/ambulance services/medical staff
7. Police
8. Medical officer
9. Responsible technician
10. Water, gas and electricity services.

Emergency Equipment

The following emergency equipment must be available:

- ✓ First-aid kit, including universal and special antidotes
- ✓ Appropriate fire extinguishers, fire blankets

The following are also suggested but may be varied according to local circumstances:

- ✓ Full protective clothing (one-piece coveralls, gloves and head covering – for incidents involving microorganisms in Risk Groups 3 and 4)
- ✓ Full-face respirators with appropriate chemical and particulate filter canisters
- ✓ Room disinfection apparatus, e.g. sprays and formaldehyde vaporizers
- ✓ Stretcher
- ✓ Tools, e.g. hammers, axes, spanners, screwdrivers, ladders, ropes

Animal Biosafety; 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 healthy food and water. At the end of the experiment they must be dealt with in a humane manner.

For security reasons, the animal house should be an independent, detached unit. If it attaches a laboratory, the design should provide for its isolation from the public parts of the laboratory should such need arise, and for its decontamination and disinfestation.

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 severity according to the animal biosafety level. These are described below and summarized in Table 4.

Animal facility – Biosafety Level 1

This is suitable for the maintenance of most stock (ordinary) animals after quarantine (except nonhuman primates, regarding which national authorities should be consulted), and for **animals that are inoculated with agents in Risk Group 1**. GMT 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 programme 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 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 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 programme.
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. Animal carcasses (animal bodies) should be incinerated.
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.

Animal facility – Biosafety Level 3

This is suitable for work with animals that are inoculated with agents in Risk Group 3, or when otherwise indicated by a risk assessment. All systems, practices and procedures need to be reviewed and recertified annually. The following safety precautions apply:

1. All the requirements for animal facilities – Biosafety Levels 1 and 2 must be met.
2. Access must be strictly controlled.

3. The facility must be separated from other laboratory and animal house areas by a room with a double-door entrance forming an anteroom.
4. Hand-washing facilities must be provided in the anteroom.
5. Showers should be provided in the anteroom.
6. There must be mechanical ventilation to ensure a continuous airflow through all the rooms. Exhaust air must pass through HEPA filters before being discharged to the atmosphere without recirculation.
7. An autoclave must be available at a location convenient for the animal house where the biohazard is contained. Infectious waste should be autoclaved before it is moved to other areas of the facility.
8. An incinerator should be readily available on site or alternative arrangements should be made with the authorities concerned.
9. Animals infected with Risk Group 3 microorganisms must be housed in cages in isolators or rooms with ventilation exhausts placed behind the cages.
10. Bedding should be as dust-free as possible.
11. All protective clothing must be decontaminated before it is laundered.
12. Windows must be closed and sealed, and resistant to breakage.
13. Immunization of staff, as appropriate, should be offered.

Animal facility – Biosafety Level 4

Work in this facility will normally be linked with that in the maximum containment laboratory – Biosafety Level 4, and national and local rules and regulations must be harmonized to apply to both. If work is to be done in a suit laboratory, additional practices and procedures must be used over and above those described here.

1. All the requirements for animal facilities – Biosafety Levels 1, 2 and 3 must be met.
2. Access must be strictly controlled; only staff designated by the director of the establishment should have authority to enter.
3. Individuals must not work alone: the two-person rule must apply.
4. Personnel must have received the highest possible level of training as microbiologists and be familiar with the hazards involved in their work and with the necessary precautions.
5. Housing areas for animals infected with Risk Group 4 agents must maintain the criteria for containment described and applied for maximum containment laboratories – Biosafety Level 4.
6. The facility must be entered by an airlock anteroom, the clean side of which must be separated from the restricted side by changing and showering facilities.
7. Staff must remove street clothing when entering and put on special, protective clothing. After work they must remove the protective clothing for autoclaving, and shower before leaving.
8. The facility must be ventilated by a HEPA-filtered exhaust system designed to ensure a negative pressure (inward directional airflow).
9. The ventilation system must be designed to prevent reverse flow and positive-pressurization.
10. A double-ended autoclave with the clean end in a room outside the containment rooms

must be provided for exchange of materials.

11. A pass-through airlock with the clean end in a room outside the containment rooms must be provided for exchange of non-autoclavable materials.
12. All manipulations with animals infected with Risk Group 4 agents must take place under maximum containment – Biosafety Level 4 conditions.
13. All animals must be housed in isolators.
14. All animal bedding and waste must be autoclaved before removal from the facility.
15. There must be medical supervision of staff.

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 disinfection.
3. Insecticide sprays should be readily available.
4. Access should be through an anteroom containing insect traps and with arthropod-proof screens on the doors.
5. All exhaust ventilation ducts and openable windows should be fitted with arthropod-proof screens.
6. All waste should be decontaminated by autoclaving, as some invertebrates are not killed by all disinfectants.
7. A check should be kept on the numbers of larval and adult forms of flying, crawling and jumping arthropods.
8. Containers for ticks and mites should stand in trays of oil.
9. Infected or potentially infected flying insects must be contained in double-netted cages.
10. Infected or potentially infected arthropods must be handled in biological safety cabinets or isolators.
11. Infected or potentially infected arthropods may be manipulated on cooling trays.

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

RISK GROUP	CONTAINMENT LEVEL	LABORATORY PRACTICES AND SAFETY EQUIPMENT
1	ABSL-1	Limited access, protective clothing and gloves.
2	ABSL-2	ABSL-1 practices plus: hazard warning signs. Class I or II BSCs for activities that produce aerosols. Decontamination of waste and cages before washing.
3	ABSL-3	ABSL-2 practices plus: controlled access. BSCs and special protective clothing for all activities.
4	ABSL-4	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.

ABSL, animal facility Biosafety Level; BSCs, biological safety cabinets