BIOSAFETY AND BIOETHICS & IPR

(E CONTENT)

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Biosafety

Biosafety is the prevention of large-scale loss of biological integrity, focusing both on ecology and human health. These prevention mechanisms include the conduction of regular reviews of biosafety in laboratory settings, as well as strict guidelines to follow. Biosafety is used to protect from harmful incidents. Many laboratories handling biohazards employ an ongoing risk management assessment and enforcement process for biosafety. Failures to follow such protocols can lead to increased risk of exposure to biohazards or pathogens. Human error and poor technique contribute to unnecessary exposure and compromise the best safeguards set into place for protection.

The international Cartagena Protocol on Biosafety deals primarily with the agricultural definition but many advocacy groups seek to expand it to include post-genetic threats: new molecules, artificial life forms, and even robots which may compete directly in the natural food chain.

Biosafety in agriculture, chemistry, medicine, exobiology and beyond will likely require the application of the precautionary principle, and a new definition focused on the biological nature of the threatened organism rather than the nature of the threat.

When biological warfare or new, currently hypothetical, threats (i.e., robots, new artificial bacteria) are considered, biosafety precautions are generally not sufficient. The new field of biosecurity addresses these complex threats.

Biosafety level refers to the stringency of biocontainment precautions deemed necessary by the Centers for Disease Control and Prevention (CDC) for laboratory work with infectious materials.

Typically, institutions that experiment with or create potentially harmful biological material will have a committee or board of supervisors that is in charge of the institution's biosafety. They create and monitor the biosafety standards that must be met by labs in order to prevent the accidental release of potentially destructive biological material. (note that in the US, several groups are involved, and efforts are being made to improve processes for government run labs, but there is no unifying regulatory authority for all labs.

Biosafety is related to several fields:

- In ecology (referring to imported life forms from beyond ecoregion borders),

- In agriculture (reducing the risk of alien viral or transgenic genes, genetic engineering or prions such as BSE/"MadCow", reducing the risk of food bacterial contamination)
- In medicine (referring to organs or tissues from biological origin, or genetic therapy products, virus; levels of lab containment protocols measured as 1, 2, 3, 4 in rising order of danger),
- In chemistry (i.e., nitrates in water, PCB levels affecting fertility)
- In exobiology (i.e., NASA's policy for containing alien microbes that may exist on space samples. See planetary protection and interplanetary contamination), and
- In synthetic biology (referring to the risks associated with this type of lab practice)

Hazards

Chemical hazards typically found in laboratory settings include carcinogens, toxins, irritants, corrosives, and sensitizers. Biological hazards include viruses, bacteria, fungi, prions, and biologically derived toxins, which may be present in body fluids and tissue, cell culture specimens, and laboratory animals. Routes of exposure for chemical and biological hazards include inhalation, ingestion, skin contact, and eye contact.

Physical hazards include ergonomic hazards, ionizing and non-ionizing radiation, and noise hazards. Additional safety hazards include burns and cuts from autoclaves, injuries from centrifuges, compressed gas leaks, cold burns from cryogens, electrical hazards, fires, injuries from machinery, and falls.

In synthetic biology

A complete understanding of experimental risks associated with synthetic biology is helping to enforce the knowledge and effectiveness of biosafety. With the potential future creation of man-made unicellular organisms, some are beginning to consider the effect that these organisms will have on biomass already present. Scientists estimate that within the next few decades, organism design will be sophisticated enough to accomplish tasks such as creating biofuels and lowering the levels of harmful substances in the atmosphere. Scientist that favor the development of synthetic biology claim that the use of biosafety mechanisms such as suicide genes and nutrient dependencies will ensure the organisms cannot survive outside of the lab setting in which they were originally created. Organizations like the ETC Group argue that regulations should control the creation of organisms that could potentially harm existing life. They also argue that the development of these organisms will simply shift the consumption of petroleum to the utilization of biomass in order to create energy. These organisms can harm existing life by affecting the prey/predator food chain, reproduction between species, as well as competition against other species

(species at risk, or act as an invasive species). Synthetic vaccines are now being produced in the lab. These have caused a lot of excitement in the pharmaceutical industry as they will be cheaper to produce, allow quicker production, as well as enhance the knowledge of virology and immunology.

Biosafety issues refer to the procedures, policies, and principles to be adopted to safeguard the environment and the human population. It refers to the containment principles, strategies, and practices that are adopted to prevent exposure to pathogens and toxins. Its main objective is to keep a check on harmful biological agents, toxins, chemicals, and radiation. With the advent of genetic engineering, biosafety measures have gained importance to ensure public and environmental safety.

The people should be made aware of the rules, regulations, and monitoring bodies. Researchers should be the first ones to have the complete knowledge of the biosafety issues and measures so that safety is ensured at the root level. A multilateral agreement "The Cartagena Protocol on Biosafety" has been adopted by 167 countries, including many countries of the United Nations. The protocol was enforced on 11th September 2003. It had the following objectives:

- It aimed at ensuring the safe movement of the living modified organisms across the boundaries.
- Frame and share the principles and methodologies for risk assessment through Biosafety Clearing House.

Also read: Biotechnology and Its principles

Importance Of Biosafety issues

The areas where genetic engineering practices are being carried out require prior approval from the regulatory authorities of the country.

It is mandatory to follow the guidelines to minimize biosafety.

The awareness about biosafety has been increased among the researchers, producers of Genetically Modified Organisms, policymakers, administrators and environmentalists.

Efforts have been made by the OMICS Publishing Group USA, on publishing Biosafety journals and organizing international conferences to make everyone aware of the biosafety issues and the measures to rectify them.

Though modern research is a boon to human population yet can be dangerous if not used wisely.

Biosecurity and bioterrorism are emerging issues nowadays that need to be checked upon in the interest of human and environmental safety. Biosafety is therefore important to ensure the safe utilization of technology.

Certain biosafety levels have been proposed for the laboratories depending upon the pathogenicity of the microbes being worked upon. These protect the environment and the surroundings from the hazards of such microbes.

Biosafety Levels

Biosafety levels is a set of biocontainment precautions designed to protect laboratory personnel as well as the surrounding environment and the community. They are ranked based on the organisms that are being researched on in a laboratory.

Following are the biosafety levels described in detail:

Biosafety Level 1

This is the lowest biosafety level and is applied to the agents which pose the least threat to the laboratory workers and the environment. These are not isolated from the general building. The non-pathogenic strain of *E.coli* is worked at a Biosafety level 1.

The research is carried out on the benches without any special contaminant equipment. The biosafety level 1 facility are as follows:

- mechanical pipetting
- safe handling of sharps
- avoiding splashes or aerosols
- washing hands
- prohibition on drinking, smoking and food in the laboratories
- signs of biohazards
- protective equipment such as gloves, goggles, lab coats, gowns

All the infectious materials should be decontaminated before being disposed of.

Biosafety Level 2

This includes agents that cause human diseases. For eg., encephalitis virus, HIV, Staphylococcus aureus. Personnel working in these labs requires greater attention to prevent any injuries such as cuts, ingestions, etc.

The following practices should be carried out in a Biosafety Level 2 laboratory:

- Use of protective equipment such as goggles, glasses, face shields, etc.
- The procedures that can cause infections are carried out in biological safety cabinets.
- The waste material should be decontaminated before disposal.
- An eyewash and a sink should be readily available.
- Biohazard signs should be provided.

Biosafety Level 3

This includes working on such pathogenic microbes that can cause serious disease through inhalation. For eg., West Nile virus, yellow fever virus, bacteria causing tuberculosis, etc.

The common requirements in a BSL 3 laboratory include:

- Protective equipment including respirators is required.
- All the work should be performed under proper biosafety cabinets.
- The door should have access away from the general building.
- The researchers are under medical surveillance and are immunized against certain microbes.

Biosafety Level 4

This includes work with highly dangerous and exotic microbes. Infections through these microbes cannot be treated or immunized and are usually fatal. For eg., Ebola and Marburg virus.

The common requirements in a biosafety level 4 laboratory are as follows:

- The researchers should change their clothes and shower while exiting.
- All the materials should be decontaminated.

- All the experiments should be carried out under class III safety cabinets.

The laboratory is isolated present in a separate building and the entry to this zone is restricted.

PRINCIPLES OF BIOSAFETY

2.1 Biohazard Awareness and Risk Assessment

Biological laboratories are special work environments that can pose infectious disease or toxin exposure risks to persons working or entering these laboratories. In fact, there is a clear historical record of infections having been acquired in laboratory settings. More than 4,000 laboratory-acquired infections (LAIs) have been reported since the 1920s and many others have likely occurred. Some LAIs have been associated with morbidity & mortality, expensive remediation costs, and/or damaging publicity.

There may be instances where agent or procedural characteristics create unique hazards such as the potential for aerosolization of pathogens cultured in concentrations higher than found in nature, inadvertent contamination of surfaces, etc. Given the microscopic nature of most biological hazards, it is difficult-to-impossible to directly evaluate the risk in real time. Consequently, only ~15% of all LAIs are attributable to a known or identifiable accident or exposure. Therefore, understanding the potential risks and proactive/preventative measures to mitigate those risks is essential.

This section outlines the biosafety principles related to risk assessment, agent risk groups, and prevention paradigms (biosafety levels).

2.1.1 Biological Hazards

Biological hazards are any agents, materials, or conditions that pose a threat to human, animal, plant, or environmental health.

Biological hazards include the following:

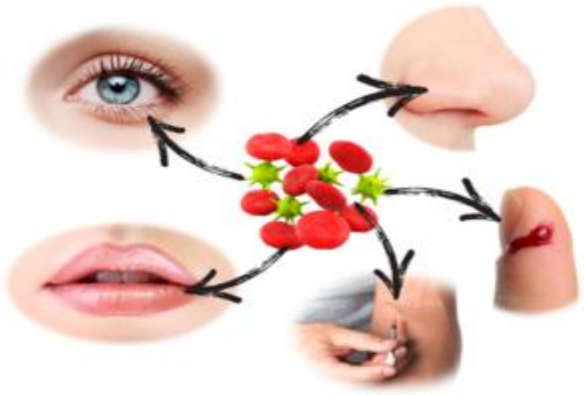
- **Biological agents**, including bacteria, viruses, fungi, protozoa, helminths, and prions. These are also referred to as infectious agents, etiological agents or pathogens. Biological agents are propagative, cause a broad range of diseases (asymptomatic-to-fatal), and may take hours-to-years to manifest as disease in the host.
- **Recombinant or synthetic nucleic acid molecules**. While nucleic acids do not pose an explicit risk, the macromolecules they encode (or interact with) and resulting phenotypes may. Recombinant or synthetic constructs that encode toxins, viruses, oncogenes, antibiotic resistance (of clinical relevance), or any other molecule that contributes to disease are of particular concern. Host cells/systems, method/control of gene expression, potential for horizontal/vertical transfer, and/or research procedures may contribute to the risk of recombinant/synthetic nucleic acid molecules.
- **Biological toxins, venoms** or other molecules derived from biological systems that may cause or contribute to disease. These are non-propagative, but often have acute and serious-to-fatal effects.
- **Blood, blood products, tissues, secretions, excretions, or cell lines derived from humans or animals.** The risk profile is *generally*: human > primate/simian > other mammals > avian > reptile/amphibian > arthropods (other invertebrates)
- **Novel nanoparticles conjugated to biologically active or cell-modifying molecules** (e.g. siRNA, antibodies, effector proteins, etc.).
- **Environmental specimens**, particularly plant, soil, or water samples that are likely reservoirs of high-risk biological agents or toxins.

2.1.2 Routes of Transmission

One of the unfortunate consequences of working with biological hazards is the potential for acquiring an infection. History has shown that such infections occur and that laboratory workers are clearly at higher risk for infection with certain agents, such as the hepatitis B virus, than the general population.

Although work-related infections can occur via routes that differ from those in naturally occurring disease, there are limited routes of exposure and modes of entry into the body. A worker exposed to an infectious aerosol could inhale respirable particles. Larger droplets of that aerosol could fall on skin, mucous membranes, or environmental surfaces. The worker could then inadvertently inhale or ingest the agent without experiencing an overt accident. On the other hand, a needle stick or an animal bite would usually be noticed. Providing awareness and barriers for these routes of infection is a preventative approach to biosafety. The following are the primary routes of transmission that can result in laboratory acquired infections.

1. Injection (percutaneous)
    1. Contaminated sharp objects (e.g. needle, scalpel)
    2. Animal bites, scratches
    3. Through broken or abraded skin (including rashes, eczema, split cuticles, etc.)
2. Absorption (mucous membrane contact)



    1. Splashes to the eyes, nose, mouth
    2. Hand to face movements (i.e., applying cosmetics, cell phone usage, etc.)
3. Ingestion
    1. Eating/drinking
    2. Applying cosmetics
    3. Contact with tear ducts
4. Inhalation (aerosols)
    1. Liquid disturbance
    2. Syringe preparation
    3. Dried animal excretions
    4. Leakage from injection site

2.1.3 Host Factors

In addition to the biohazard and route of transmission, host factors play an important role in the outcome of an exposure/infection. Factors that can increase susceptibility include:

- Underlying diseases, particularly those affecting the immune system
- Age (children and elderly are at higher risk)
- Treatment with antimicrobials, steriods, or anticancer drugs
- Vaccination status
- Type of pathogen/agent exposure
    - Opportunistic pathogens can cause disease only when introduced into an unusual location or an immunocompromised host (e.g. normal flora, most environmental yeasts/molds, etc.).
    - A primary pathogen (also known as true or frank pathogen) can cause disease in an otherwise healthy individual (e.g. Staphylococcus aureus, Streptococcus pyogenes, hepatitis B virus, influenza virus, etc.).

2.1.4 The Biological Risk Assessment

A thorough biological risk assessment determines the proper safety and containment precautions given the intrinsic risk of the biohazard(s), procedures, and health of laboratory workers.

The risk assessment is a guide for the selection of appropriate controls and microbiological practices, safety equipment, and facility safeguards. The risk assessment will be used to alert others to the hazards of working in the lab and to the need for developing proficiency in the use of safe practices and containment equipment. Successful control of hazards in the laboratory also protects persons not directly associated with the laboratory, such as other occupants in the building, infrequent visitors (e.g., facilities services), and the public.

Risk assessments generally begin with the question, "Is the biological material that I'm working with capable of causing human disease or environmental harm?" If so, the PI should work with the BSO to determine the best safety practices and level of containment to reduce the chance of accidental exposure or release of infectious agents, recombinant agents/organisms, or other relevant biohazard. If you are unsure, contact the Biosafety Office for guidance.

Basic considerations for the risk assessment are listed it the table below:

Agent	Procedure
Host range	Concentration of organisms
Pathogenicity/virulence	Scale of procedures
Availability of prophylaxis	Use of animals
Route of transmission	Use of sharps
Viability in the environment	Potential for the generation of aerosols
Origin of the source	Field procedures
For recombinant DNA: 1) Nature of insert; 2) method of delivery; 3) recombinant host; and 4) safety/environmental impact	Experience level of personnel

2.1.5 Risk Groups

Infectious agents are grouped according to their intrinsic biological properties, particularly their pathogenicity and virulence in humans. Similar principles are used to categorize the risk of recombinant materials, which could impact human (or animal or plant) health and/or the environment if released.

Risk groups consider the following:

- Pathogenicity of the organism
- Virulence of the organism
- Mode of transmission and host range
- Availability of effective preventive measures (e.g., vaccines)
- Availability of effective treatment (e.g., antibiotics, antivirals)
- For recombinant materials: type/pathogenicity of host, gene/transgene products, gene expression, targeted effects (and consideration of off-target effects), phenotypes, biological/environmental impacts

Risk groups range from 1-4, with RG1 posing minimal risk to healthy individuals and public health and RG 4 posing a very serious risks to individuals **and** public. The Biosafety Office follows the World Health Organization's (WHO) *Laboratory Biosafety Manual*, 3rd ed., *NIH Guidelines*, and BMBL categorization of risk groups as follows:

- **RG1** – Are rarely associated with disease in healthy adult humans or animals and pose no-to-little public health risk (e.g. *Saccharomyces cerevisiae, E. coli* K-12 strain derivatives often used for recombinant/molecular biology, many environmental organisms).
- **RG2** – Are associated with mild to moderate disease for which preventative measures or post exposure treatments are often available; public health impact is limited (e.g. *Streptococcus pyogenes, Salmonella* , hepatitis B virus)
- **RG3** – Are associated with serious to lethal human disease for which preventative vaccines or post exposure therapies ***may be*** Public health impact is limited-to-moderate (e.g. *Mycobacterium tuberculosis*, hantaviruses, West Nile virus)
- **RG4** – Are associated with serious to lethal human disease for which preventative vaccines or post exposure therapies are ***not*** Public health impact is high (e.g. Ebola virus, Marburg virus, smallpox virus, etc.).

RG4 agents are not permitted at UTK!

2.1.6 Risk Reduction for Biohazardous Agents

Once the RG and other procedural factors are determined, basic considerations for risk reduction include:

Risk awareness

- Risk assessments are performed and communicated
- Materials and procedures are reviewed by the IBC as required
- Containment recommendations are implemented
- Standard Operating Procedures (SOPs) are developed and communicated

Control of materials

- Containment requirements per the risk assessment are followed
- Materials are labeled and securely stored per risk assessment

- Biohazard inventory is documented, maintained and controlled
- Secondary containment is used for storage and transport of biohazards

Good Practices

- Follow prescribed personal & lab hygiene principles, also known as Standard Microbiological Practices (SMP; see Module 4 for details)
- Wear and maintain personal protective equipment (PPE) per the risk assessment and manufacturers recommendations
- Communicate hazards via door placard, biohazard labels, etc.
- Segregate and dispose biohazardous wastes per Biosafety Office guidelines
- Decontamination must be routine and effective based on the risk assessment and prudent practices
- Communicate and follow emergency response procedures (spills, personal exposures, injuries, etc.)

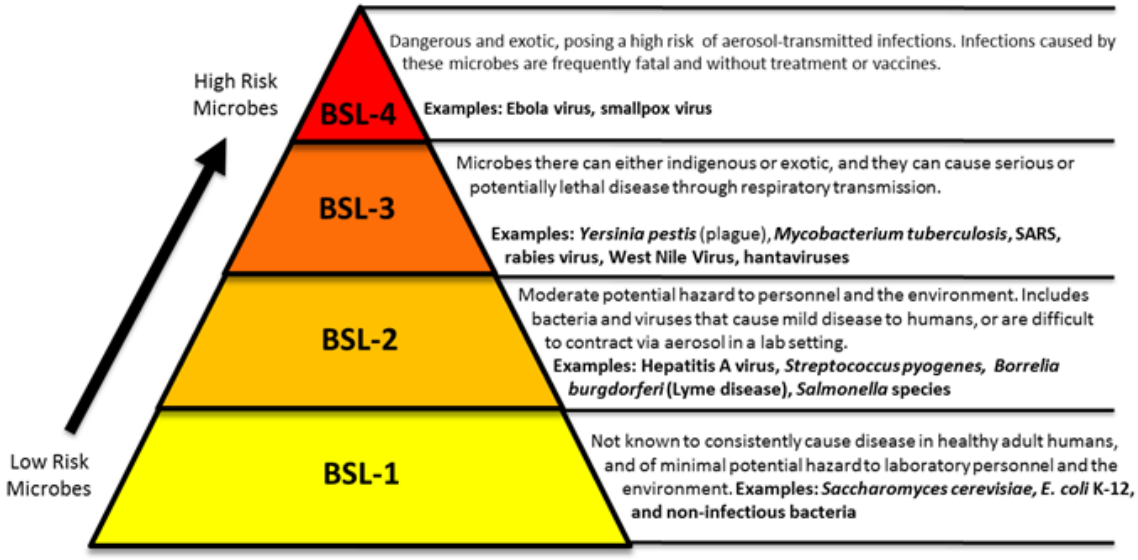
Restriction of Access

- Follow prescribed laboratory security procedures per institutional policy
- Secure storage equipment in common areas and placard with content owners name and contact information

2.1.7 Biosafety Levels

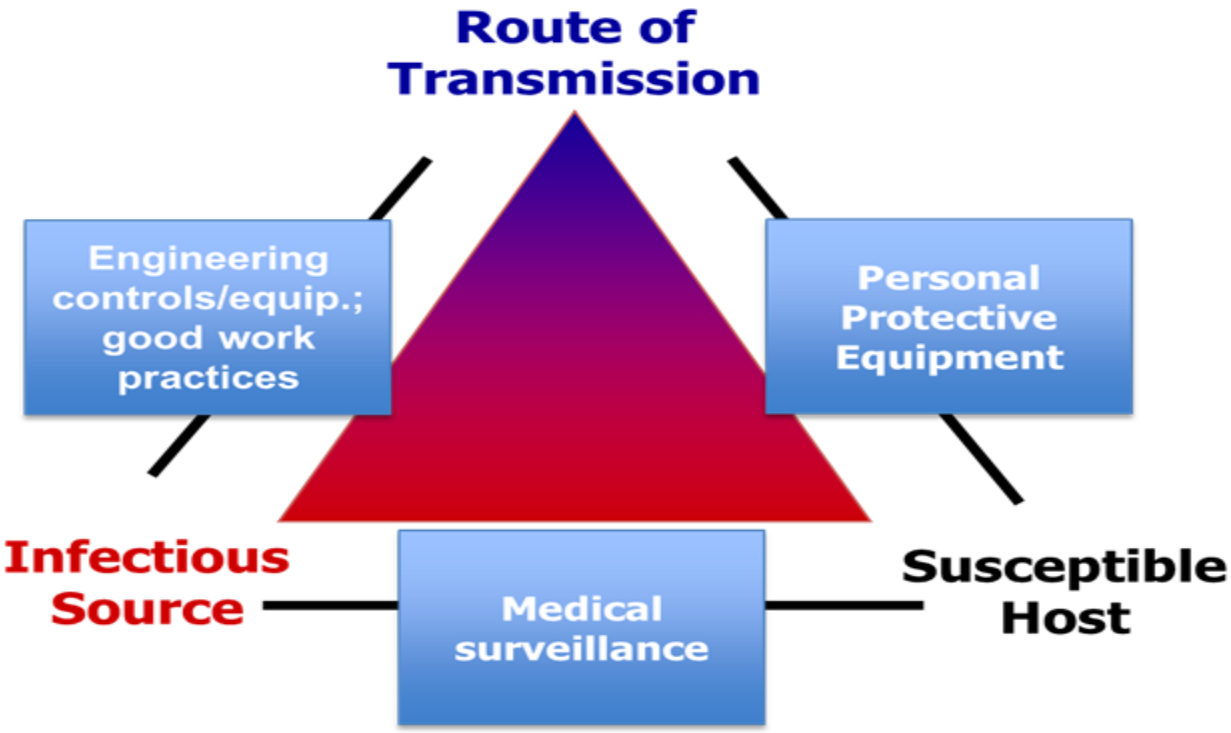
Biosafety Levels (BSL) prescribe procedures and levels of containment for the particular microorganism or material (including research involving recombinant or synthetic nucleic acid molecules) and associated procedures. In addition to the risk reduction strategies highlighted above, BSLs also consider primary barriers (e.g. biosafety cabinets), secondary barriers, facility design, air handling, laboratory security, etc. BSLs are graded from 1 – 4; as the BSL increases so does the relative risk of the agent/procedures as well the stringency of procedures and facility design. Generally, these correlate with RGs (e.g. a RG2 agent is worked with at BSL-2), but there are exceptions (e.g. production volumes, high-risk procedures, etc.).

The majority of work at UTK involves Biosafety Level 1 & 2 practices.



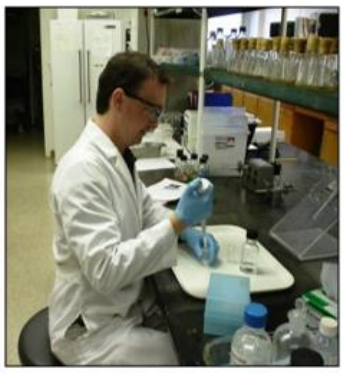
Biosafety Levels: Breaking the Chain

Another way to think about biosafety levels is that they are a systematic approach to disrupting the chain of infection (or release). By disrupting or eliminating the pathways between an infectious source and a susceptible host (or environment), safety, containment, and security can be assured. Examples are provided in the diagram below.



Biosafety Levels Permitted at UT

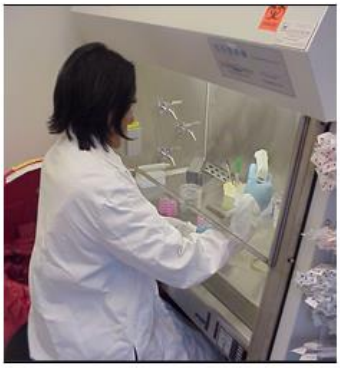
BSL-1



Used for work with biological agents and materials that pose **minimal risk** to people or the environment.

Features:
Work on open bench
Lab coat & gloves recommended
Decontamination procedures in place

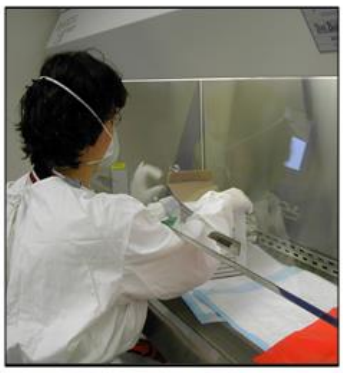
BSL-2



Used for work with biological agents or materials that pose **moderate risk** to people or the environment.

Features:
Aerosol-generating procedures performed in a biosafety cabinet (BSC)
Lab coat & gloves required
Biosafety manual with lab-specific procedures/training and restricted access

BSL-3



Used with indigenous or exotic biological agents with potential for airborne transmission or for procedures involving aerosolization, concentration or large quantities of moderate risk materials.

Features:

- Lab designed to contain airborne hazard (i.e., double door entry, negative airflow relative to surrounding areas, no recirculation of air
- All open manipulations of materials in BSC
- Respiratory protection usually required
- Facility design and operational procedures documented; annual functional verification

2.1.8 Risk Assessment Resources

Resources that are helpful in determining risk group and biosafety levels include:

- Agent summary statements in the **CDC** (Centre for Disease Control and Prevention) / **NIH** (National Institute of Health) document Biosafety in Microbiological and Biomedical Research Laboratories (BMBL).
- Appendix B Classification of Human Etiologic Agents on the Basis of Hazard from the NIH Guidelines.
- The OSHA Bloodborne Pathogen Standard interpretation letter regarding risk for all human cell lines **(must be used at BSL-2).**

- The American Biological Safety Association's (ABSA) database of Risk Group Classification of Infectious Agents.
- The Public Health Agency of Canada's PSDS for Infectious Substances.
- The World Health Organization (WHO).
- Research publications (various)
- This Biosafety Manual

An overview of Biosafety and its regulation

B. iosafety (biosafety regulation) means the need to protect human and animal health and environment from the possible adverse effects of the products of modern biotechnology. Biosafety defines the containment conditions under which infectious agents can be safely manipulated. Biosafety is the prevention of large-scale loss of biological integrity, focusing both on ecology and human health. These prevention mechanisms include conduction of regular reviews of the biosafety in laboratory settings, as well as strict guidelines to follow. Biosafety also means safety from exposure to infectious agents.

Need of biosafety:

In the past century, medical research has led to improved health and increased life expectancy largely because of success in preventing and treating infectious diseases. This success has come about through the use of antibiotics and vaccines, improved hygiene, and increased public awareness. New threats to health continually emerge naturally, however, as bacteria and viruses evolve, are transported to new environments, or develop resistance to drugs and vaccines. Some familiar examples of these so-called emerging or re-emerging infections include HIV/AIDS, West Nile virus, severe acute respiratory syndrome (SARS), and annual outbreaks of influenza and recently most dangerous Corona virus which claimed thousands of lives worldwide. To control epidemics and protect the public health, medical researchers must quickly identify naturally occurring microbes and then develop diagnostic tests, treatments, and vaccines for them. Preparing for bioterrorism—the deliberate release of a microbe into a community in which it is not a current health concern—calls for the identical scientific skills and strategies. Hence biosafety is used to protect from harmful incidents. Many laboratories handling biohazards employ an ongoing risk management assessment and enforcement process for biosafety. Failures to follow such protocols can lead to increased risk of exposure to biohazards or pathogens.

The Wuhan Virology Institute is the highest biosafety lab in China and the country's first Biosafety Level 4 laboratory. Scientists at the institute conduct research on some of the most dangerous pathogens or biological agents in the world. The scientists believe that while the pathogens for Coronavirus may have leaked from the Wuhan Virology Institute, the nearby seafood market may have aided its spread within and beyond the city. The

scientists said they have genomic evidence that the outbreak of the COVID-19 virus from the laboratory may have occurred around November 2019.

Biosafety guidelines aiming at-

• Regulating rDNA research with organisms that have least or no adverse effect.

• Minimizing the possibilities of occasional release of GEOs from the lab.

• Banning the release of GEOs if they are supposed to be causing potential risks in the environment.

Biosafety Guidelines for Laboratories

• Food storage, eating, drinking and smoking are prohibited in lab.

• Mouth pipetting is prohibited

• Laboratory coats are obligatory and should be removed when exiting the lab.

• Working surfaces must be decontaminated using soap and alcohol after each working day.

• Waste products must be decontaminated by incineration or by autoclaving.

• Frequent hand wash is obligatory.

• Avoid contact with GMO's and other exotic biological agents, disposable gloves should be worn when handling such items. • Laboratory door should be closed at all times.

• Working with fume-producing chemicals must be under the laboratory hood.

• Biohazard warning signs should be always posted in labs.

Based upon ICGEB's long-standing activities in biosafety, identified the main issues derived from the deliberate introduction of GM crops (and their derived products) into the environment or onto the market of concern today. These have been classified as:

• Risks for animal and human health

• Toxicity & food quality/safety

• Allergies;

• Pathogen drug resistance (antibiotic resistance)

• Risks for the environment:

• Susceptibility of non-target organisms;

• Change in use of chemicals in agriculture

• Unpredictable gene expression or transgene instability (gene silencing).

Risks for agriculture:

• Weeds or superweeds

• Alteration of nutritional value (attractiveness of the organism to pests)

• Change in cost of agriculture

• Unpredictable variation in active product availability

• Loss of changes in agricultural practise

General concerns:

• Detection and analytical methods

• Ethical issues (eg. labelling)

• Public attitudes, perception; legislation monitoring

• Socio-economics (eg. situation of poor farmers in developing countries)

Bio-Safety Levels

Biosafety levels are defined in terms of using specific laboratory practices and techniques, safety equipment and laboratory facilities required for different category of infectious agents based on their hazardous nature. The guidelines for Microbiological and Biomedical Laboratories suggest four Biosafety levels in incremental order depending on the nature of work. The proposed safety levels for projects involving recombinant DNA techniques take into consideration the source of the donor DNA and its disease-producing potential. Based on this, four levels are defined which correspond to (P1<P2<P3<P4) or BSL1 to BSL4 facilities.

1. **Biosafety level-1**

BSL-1 level is suitable for working with well characterized agents which are not known to cause

any disease in healthy human beings and are of minimal hazard to workers in the laboratory as well as to the environment e.g. non-pathogenic *E. coli.* No special equipment is required. The safety precautions and requirement of BSL-1

Features:

1. Following of good microbiological practices i.e using laminar flows, washing hands with anti-bacterial soap, cleaning working benches of the lab with disinfectants,
2. Decontamination of bacterial cultures by autoclaving etc.
3. The laboratory personnel should be imparted specific training and also be supervised by a scientist with general training in microbiology.

2. Biosafety level 2 (BSL-2)

BSL-2 level is suitable for working with agents of moderate potential hazard to laboratory personnel and the environment e.g. *Salmonella* spp., *E. coli* 0157:H7, *Listeria monocytogenes*, mumps, measles, influenza etc. including genetically modified organisms.

Features of BSL-2

1. BSL-2 facility limits the release of modified organisms in the environment.
2. Class II safety cabinets are required to be used in handling the high risk organisms under this category. Thus, the Class II biosafety cabinet provides personnel, environment and product protection.
3. Laboratory personnel are to be provided with specific training in handling pathogenic agents and to be supervised by competent scientists.
4. The access to the laboratory is limited where work is being conducted.
5. Each and everything used should be decontaminated either by autoclaving or putting them in disinfectants.

4. Biosafety level 3 (BSL-3)

BSL-3 level facility is required for working with agents such as bacteria and viruses which can cause severe to fatal disease in humans on inhalation e.g. *Mycobacterium tuberculosis, Bacillus anthracis,* Q fever, and SARS coronavirus. However, such diseases can be treated with vaccines or other treatments.

Important feature of BSL-3

1. BSL-3 laboratory has special engineering and design features e.g. double door access zone and sealed penetration.

2. Laboratory personnel need to be specifically trained in handling pathogenic and potentially lethal agents and should be supervised by competent scientists having adequate expertise in working with these agents.
3. It is mandatory to conduct all procedures involving the manipulation of infectious materials within biological safety cabinets or other physical containment facilities or by personnel wearing appropriate personal protective clothing and equipment.
4. Specially designed laboratories (BSL-3 laboratory with double door access zone and sealed penetration) and precautions including the use of safety cabinets are prescribed and the access is strictly controlled.
5. Class III cabinets are generally used for working with the pathogens falling in this category. It is a totally enclosed ventilated cabinet of gas-tight construction. The work within this cabinet is conducted through attached rubber gloves. When in use, the Class III cabinet is maintained through negative air pressure of at least 0.5 inches water gauge. The supply air is drawn into the cabinet through HEPA filters. The cabinet exhaust air is filtered by two HEPA filters, installed in series, before its discharge outside the facility. The exhaust fan for the Class III cabinet is generally separate from the exhaust fans of the facility's ventilation system.

5. Biosafety level 4 (BSL-4)

BSL-4 level is required for working with highly dangerous agents that pose a high risk to the workers through transmission by aerosols and lead to fatal diseases for which no treatment or vaccines are available e.g. Bird flu, swine flu, hemorrhagic fever, Ebola virus, Foot and Mouth Disease virus etc.

Important feature:

1. It requires the use of 'Hazmat suit' and a self-contained oxygen supply.
2. The entrance and exit contain multiple showers, a vacuum room, ultraviolet room as well as all the precautions designed to destroy the biohazardous waste. Multiple locks are employed which are electronically secured to prevent both doors opening at the same time. The air and water services to BSL-4 has to undergo decontamination procedures to eliminate the possibility of an accidental release.
3. BSL-4 facility has to be created in a controlled area within the premises of the institute / industry or as a separate facility outside the building which is located away the other areas.
4. The building protocols should use negative pressurized facilities. Airlocks should be provided during entry and exit of the personnel working in lab.
5. Specific facility operation manual has to be prepared.
6. The researchers / workers / personnel working in the BSL-4 facility should be given specialized training in handling hazardous infectious agents and should be well versant with the containment equipment and laboratory
7. Design so that they follow all practices religiously.

Classification of laboratories on biosafety levels

The concept of developing such laboratories resides within the principles of biosafety and biosecurity. Biosafety is achieved by implementing various degrees of laboratory control and containment, through laboratory design and access restrictions, professional expertise and training, use of containment equipment, and safe methods of managing infectious materials in a laboratory setting.

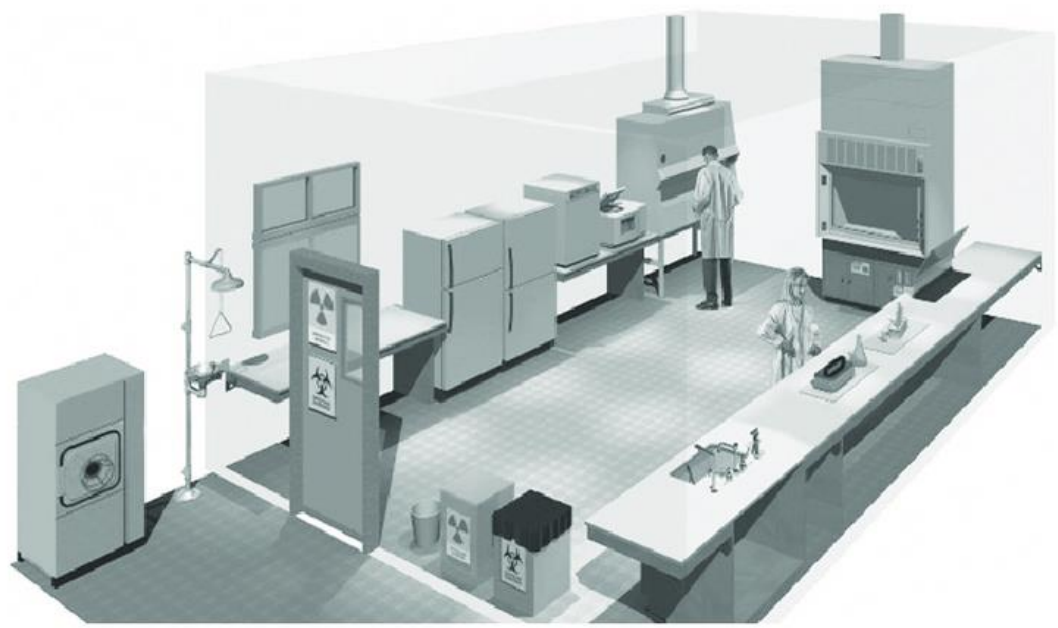
1. The lowest level, biosafety level 1 (BSL-1) laboratory is essentially a teaching laboratory that may include research involving well-characterized agents not known to consistently cause any disease in immunocompetent adult humans, and pose minimal potential hazard to laboratory personnel and the environment. Work can be performed on open-bench with good laboratory practices, aseptic techniques, and proper waste disposal; no containment facility is required.

2. Biosafety level 2 (BSL-2) laboratory involves working with agents that pose moderate hazards to personnel and the environment. Basic laboratory with restricted access and containment during certain processes (*i.e.* aerosols, large volumes, *etc*.) is required. Use of autoclaves and biological safety cabinets is desired. Use of good laboratory practices, safe waste disposal measures, and aseptic techniques are mandatory. Usually non-respiratory, non-lethal agents are handled in BSL-2 laboratory.

3. Biosafety level 3 (BSL-3) is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with agents that may cause serious or potentially lethal disease through inhalation, to the personnel, and may contaminate the environment. It requires that laboratory personnel receives specific training in handling pathogenic and potentially lethal agents, and be supervised by scientists competent in handling infectious agents and associated procedures. All work is performed in bio-contained environments using appropriate engineering controls.

4. Biosafety level 4 (BSL-4) laboratory, the highest level, is required for working with dangerous and exotic infectious agents that pose a high individual as well as environment risk of life-threatening disease, aerosol transmission, or a related agent with unknown risk of transmission. Laboratory personnel receive specific training in handling pathogenic and potentially lethal agents, and have to mandatorily work wearing positive pressure BSL-4 suits.

As per the guidelines of the Ministry of Environment & Forests, India, various animal pathogens and plant pests are classified and defined in G.S.R. 1037(E) conferred by sections 6, 8 and 25 of the Environment (Protection) Act, 1986 (29 of 1986)



A typical Biosafety Level 2 laboratory (Graphics kindly provided by 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 being taken into use. Recertification should take place at regular intervals, according to the manufacturer's instructions

Code of practice

The code of practice for basic laboratories

1. The international biohazard warning symbol and sign (see Figure 1) 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, as are sleeves that do not fully cover the forearms. Laboratory protective clothing must not be worn outside the laboratory, and it must be decontaminated before it is laundered. The removal of street clothing and change into dedicated laboratory clothing may be warranted when working with certain agents (e.g. agricultural or zoonotic agents).

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

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 prohibited 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 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. 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.

8. Written documents that are expected to be removed from the laboratory need to be protected from contamination while in the laboratory.

Biological Safety Cabinets

Biological Safety Cabinets (BSCs) form an integral part of any microbiological laboratory. These are enclosed, ventilated workspaces that are designed in a manner so as to protect the environment as well as the operator/ laboratory personnel from harmful infectious agents that are handled inside the cabinet. BSCs have been found to be really effective in reducing the incidences of laboratory acquired infections, provided the BSCs are properly operated and used. There is no substitute for good lab practices that must be performed in any laboratory, irrespective of the biosafety or containment level.

The biosafety cabinets are categorically divided into three classes based on the degree of protection provided to the worker, product and environment. In other words, the level of biocontainment required decides which BSC should be used. The various characteristic features that distinguish these cabinets from each other include:

- The velocity of air entering the cabinet,

- The amount of air recirculated or exhausted,

- exhaust system, i.e., whether the air is exhausted to the room or outside

- pressure arrangements (whether the biologically contaminated ducts and plenums are under negative pressure, or they are surrounded by negative pressure ducts and plenums).

The primary purpose of any BSC is to provide protection to the operator as well as the environment from infectious agents like bacteria and viruses; but it is not the sole function. Maintaining sterility of the product inside the cabinet is also an important function served by a BSC.

The following factors reduce the efficiency of the BSC

- Poor location
- Room air currents
- Decreased airflow
- Leakage in HEPA filters
- Working with raised sashes
- Overcrowding the work surface
- Improper user methodology

The different classes of BSC are explained as follows:

Class I cabinets (Figure 2)

- Provides personnel protection

- Provides environmental protection

- No product protection is provided (as room air which is not sterile passes over the work surface through the front opening)

- Inward flow of air maintained at a minimum velocity of 0.38 m/s

- The air from the cabinet is HEPA filtered before being exhausted

- The cabinet may be ducted or non-ducted

- Generally used for procedures that generate aerosols

- Can also be used for work with radionuclides and volatile toxic chemicals

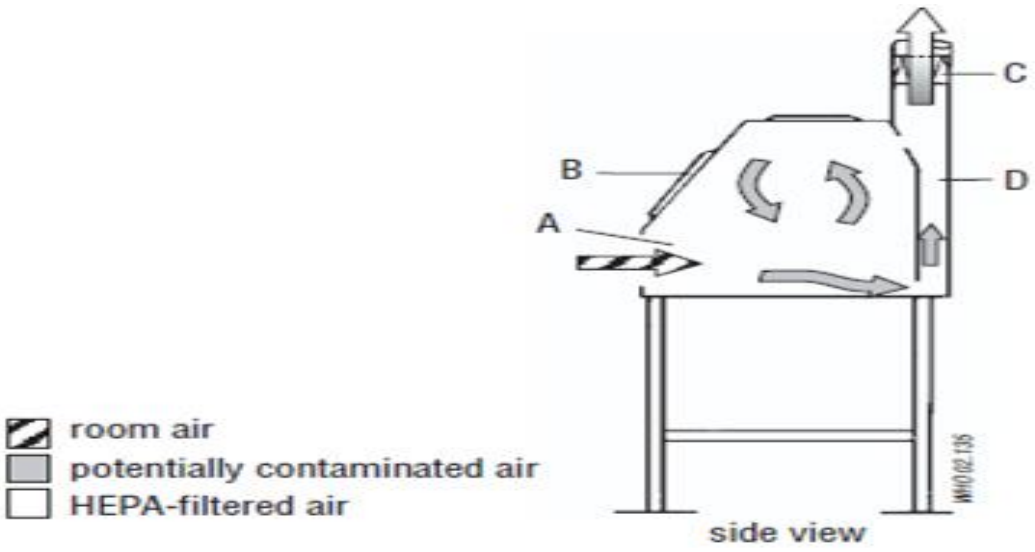


Figure 2: Schematic diagram of a Class I biological safely cabinet. (A) Front opening; (B) Sash; (C) Exhaust HEPA filter; (D) Exhaust plenum

Class II cabinets

- Provides personnel, product and environmental protection

- Class II cabinets are further divided into types A1, A2, B1 and B2.

BSC class II cabinets can be used for infectious agents belonging to the risk level 2 and 3. They may also be used for level 4 organisms, provided a positive-pressure suit is used by the laboratory worker.

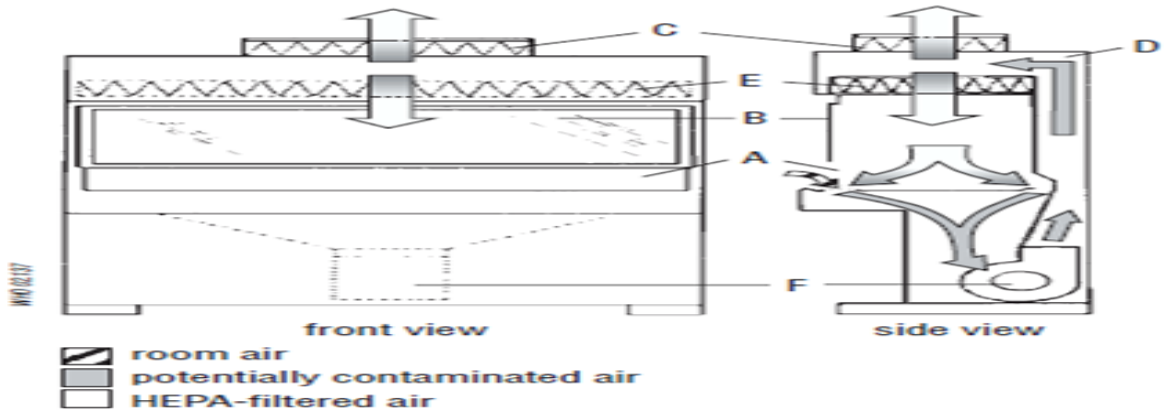


Figure 3**: Schematic representation of class-II biological safety cabinet**. a) Front opening; b) Sash; c) Exhaust HEPA filter; d) Rear plenum; f) Supply HEPA filter d) Blower

Class III BSC

The class III BSC provides for maximum containment and is used for handling of BSL-4 pathogenic agents having high risk of infection. The cabinet is a gas-tight enclosure with negative pressure having HEPA filters through which the air passes. There's a single supply filter and double exhaust filters. The access to the cabinet is through heavy duty arm length rubber gloves that provide added protection by avoiding contact with the pathogen. The BSC III cabinet is thus, also referred to as glove box.

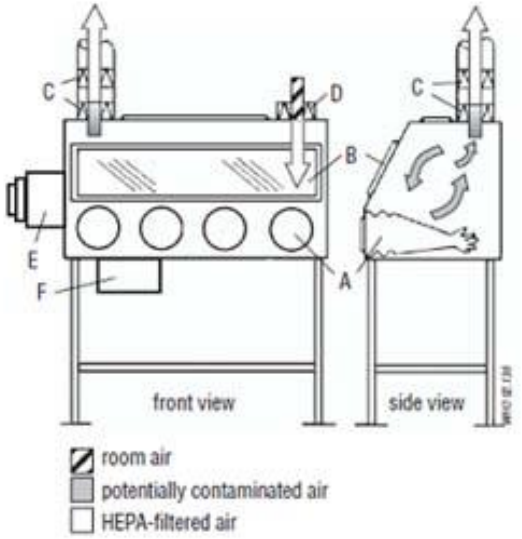


Figure 4: **Schematic representation of class-III biological safety cabinet (glove box**). a) Glove ports of arm-length; b) Sash; c) double exhaust HEPA filter; d) double ended autoclave or pass through box; f) chemical dunk tank

Classification of pathogenic microorganisms on the basis of their risk of causing threat or disease.

1. *No risk group/ minimum causing risk group pathogen*

Risk group I

A pathogen that is unlikely to cause any disease in humans or animals.

All bacterial, fungal and parasitic agents not included in higher groups.

2. *High risk group pathogen*

Risk group II

A pathogen that can cause disease in humans or animals but is unlikely to be a serious hazard. Effective treatment and preventive measures are available and the risk of spread of infection is limited.

• Bacterial- *Vibrio cholerae* • Fungal- *Aspergillus fumigatus, Actinomycetes* • Parasitic- *P.falciparum, Plasmodium thcilera* • Viral and Rickettssial -Vole rickettsia, Mumps virus

Risk group III

A pathogen that can cause serious human or animal disease, but does not ordinarily spread from one infected person to another. Effective treatment and preventive measures are available.

• Bacterial - *Clostridium botulium, Francisella tularensis* • Fungal - *Coccidioides immitis,Histoplasma capsulatum* • Parasitic- *Schisistosoma mansomi* • Viral and Rickettssial - Foot-and- Mouth disease virus

Risk group IV

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.

• Korean hemorrhagic fever • Omsk hemorrhagic fever and • Central European Encephalitis viruses

Animal and human pathogens

Pathogens	Risk group II	Risk group III	Risk group IV
Bacterial agents	*Bacillus anthracis, Borrelia recurrentis, B. vincenti, Mycobacterum-* all species, *M. bovis M. tuberculosis, M. leprae, Mycoplasma*-all species except *M. mycoides* and *M. angalactiae,Neisseriagonorrhoea, N. meningitis,*	*Actinobacillus mallei, Bartonella* species, *Brucella* species, *Clostridium botulium, Cl. tetani, Francisella tularensis, Mycobacterium avium, M. bovis, M. tuberculosis, M. leprae, Pasteurellamultocida* type B, *Pseudomonas pseudomallai, Yersinia pestis*	
Viral, Rickettsial and Chlamydial agents	Adenoviruses-Human, Avian leukosis, Cache Valley virus, CELO (avian adenovirus) Coxsackievirus A and B viruses Corona viruses, Cytomegalo viruses, Dengue virus, Echoviruses,Encephalomyocarditis virus, Flanders virus, Hart Past virus, Hepatitis A and B viruses, non A and non B hepatitis D virus, Herpes viruses - except herpes virus simiae (monkey B virus) which is in Risk Group IV, Infections Bovine Rhinotracheitis virus, Infections Bursal diseases of poultry and Infectious bronchitis, Infectious Laryngotracheitis (ILT), Influenza virus-all types, except A/PR8/34 which in Risk Group I, Langat virus Leucosis Complex, Lymphogranuloma venereum agent, Mark's Disease virus, Measles virus, Mumps virus, Newcastle disease virus (other than vaccine strain); Parainfluenza viruses-all types except parainfluenza virus 3, SF4 strain, which is in Risk Group I, Polio viruses, Poxvirus - all types except Mastrim, monkey pox, sheep pox and white pox, which depending on experiments are in Risk Groups III or IV, Rabies virus - all strains except rabies street virus, which should be classified in Risk Group III when inoculated into carnivores, Reoviruses, Respiratory syncytial virus, Rhinoviruses, Rinderpest (other than vaccine strain in use), Rubella virus, Stimian viruses-all types except herpeavirus simae (Monkey virus) which is in Risk Group IV, Simian virus 40, Ad 7 SV 40 (defective), Sindbis virus, Tensaw virus, Turlock virus, Vaccinia virus, Varicella virus,Yellow fever virus (17 D vaccine strain), Vole *Rickettsia, Chlamydia psittaci, C. trachomatis.*	African House Sickness (attenuated strain except animal passage), Alastrim, monkey pox and white pox, when used *in vitro*, Arboviruses - all strains except those in Risk Groups II and IV, Blue tongue virus (only serotypes reported in India), Ebola fever Virus Epstein-Barr virus, Feline leukaemia, Feline sarcoma, Foot and Mouth Disease (FMD) virus, Gibbon Ape lymphosarcoma, Herpesvirus ateles, Herpevirus saimiri, herpes Simplex 2, HIV-1 & HIV-2 and strains of SIV, equine infectious anaemia, Lymphocytic choriomeningitis virus (LCMV), Monkey pox, when used *in vitro*, Nondefective Adenovirus-2 Simian Virus -40 hybrids, Psettacosis-ornithosis-trachoma group of agents, Pseudorabies virus, Rabies street virus, when used inoculations of carnivores; Rickettsia - all species except Vole Rickettsia and *Coxiella burnetti* when used for vector transmission or animal inoculating experiments; Sheep pox (field strain), African Swine Fever virus, Vesicular stomatitis virus, Woolly monkeyFibrosarcoma,Yaba pox virus	Alastrim, monkey pox, whitepox, when used for transmission or animal inoculation experiments; Hemorrhagic fever agents, including Crimean hemorrhagic fever (Congo), Korean hemorrhagic fever and others as yet undefined,Herpesvirus simiae (monkey B virus),Tick-borne encephalitis virus complex, including-Russian Spring Summer Encephalitis, Kyasanur Forest Disease, Omsk hemorrhagic fever and Central European encephalitis viruses SPECIAL CATEGORY (EXOTIC Pathogens) African Horse Sickness virus (serotypes not reported in Indian and challenge strains), African Swine Fever, Bat rabies virus, Blue tongue virus (serotypes not reported in India) Exotic FMD virus types and sub-types, Junin and Machupo viruses, Lassa virus, Marburg virus, Murrey valley encephalitis virus, Rift Valley Fever virus, Smallpox virus-Archival storage and propagation Swine Vesicular Disease,Venezuelan equine encephalitis virus epidemic strains, Western Equine encephalitis virus, Yellow fever virus-Wild strain, Other Arboviruses causing enzootics and so far not recorded in India

Only certain examples listed, for complete list see Rules for the manufacture, use, import, export and storage of hazardous micro organisms genetically engineered organisms or cells. Ministry of Environment and Forests, Department of Environment, Forests and Wildlife, Government of India, New Delhi. *http://www.moef.nic.in/legis/hsm/hsm3.html*[5].

Biological hazards,

Also known as **biohazards**, refer to biological substances that pose a threat to the health of living organisms, primarily that of humans. This can include samples of a microorganism, virus or toxin (from a biological source) that can affect human health. It can also include substances harmful to other animals.

The term and its associated symbol are generally used as a warning, so that those potentially exposed to the substances will know to take precautions. The biohazard symbol was developed in 1966 by Charles Baldwin, an environmental-health engineer working for the Dow Chemical Company on the containment products.[1]

It is used in the labeling of biological materials that carry a significant health risk, including viral samples and used hypodermic needles.

There are four circles within the symbol, signifying the chain of infection.

1. Agent: The type of microorganism, that causes infection or hazardous condition.
2. Host: The organism in which the microorganism Infect. The new host must be susceptible.
3. Source: The host from which the microorganism originate. The carrier host might not show symptoms.
4. Transmission: The means of transmission, mostly direct or indirect. Some routes of transmission include air, insect, direct contact and contaminated surfaces.

Type of hazard	Image
Generic caution	
Poison	
Ionizing radiation	
Radiation – high-level source	
Non-ionizing radiation	
Biological hazard	
Carcinogen	
High voltage	
Laser hazard	
Chemical weapon	

Figure 1: Hazards and their symbol

Bio hazardous agents are classified for transportation by UN number

- Category A, UN 2814 – Infectious substance, affecting humans: An infectious substance in a form capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to it occurs.
- Category A, UN 2900 – Infectious substance, affecting animals (only): An infectious substance that is not in a form generally capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans and animals when exposure to themselves occurs.
- Category B, UN 3373 – Biological substance transported for diagnostic or investigative purposes.
- Regulated Medical Waste, UN 3291 – Waste or reusable material derived from medical treatment of an animal or human, or from biomedical research, which includes the production and testing.

UN numbers (United Nations numbers)

UN numbers (United Nations numbers) are four-digit numbers that identify hazardous materials, and articles (such as explosives, Flammable Liquids to oxidizing solid or toxic liquids, etc.) in the framework of international transport.

UN numbers range from UN 0004 to about UN 3534 (UN 0001 – UN 0003 no longer exist) and are assigned by the United Nations Committee of Experts on the Transport of Dangerous Goods. They are published as part of their *Recommendations on the Transport of Dangerous Goods*, also known as the *Orange Book*. These recommendations are adopted by the regulatory organization responsible for the different modes of transport.

There are 4 levels of biohazards, according to the Center for Disease Control:

- **Biohazard Level 1:** Bacteria and viruses including *Bacillus subtilis*, canine hepatitis, *Escherichia coli*, varicella (chicken pox), as well as some cell cultures and non-infectious bacteria. At this level precautions against the biohazardous materials in question are minimal, most likely involving gloves and some sort of facial protection.
- **Biohazard Level 2:** Bacteria and viruses that cause only mild disease to humans, or are difficult to contract via aerosol in a lab setting, such as hepatitis A, B, and C, some influenza A strains, Lyme disease, salmonella, mumps, measles, scrapie, dengue fever, HIV. Routine diagnostic work with clinical specimens can be done safely at Biosafety Level 2, using Biosafety Level 2 practices and procedures..
- **Biohazard Level 3:** Bacteria and viruses that can cause severe to fatal disease in humans, but for which vaccines or other treatments exist, such as anthrax, West Nile virus, Venezuelan equine encephalitis, SARS virus, MERS coronavirus, hantaviruses, tuberculosis, typhus, Rift Valley fever, Rocky Mountain spotted fever, yellow fever, and malaria.
- **Biohazard Level 4:** Viruses that cause severe to fatal disease in humans, and for which vaccines or other treatments are *not* available, such as Bolivian hemorrhagic fever, Marburg virus, Ebola virus, Lassa fever virus, Crimean–Congo hemorrhagic fever. When dealing with biological

hazards at this level the use of a positive pressure personnel suit, with a segregated air supply, is mandatory. The entrance and exit of a Level Four bio-lab will contain multiple showers, a vacuum room, an ultraviolet light room, autonomous detection system, and other safety precautions designed to destroy all traces of the biohazard. Multiple airlocks are employed and are electronically secured to prevent both doors opening at the same time. Biosafety Level 4. Currently there are no bacteria classified at this level.

Containment

The safety measures which prevent the escaping of GEOs from the laboratory are called containment. They help to destroy harmful GEOs within the laboratory itself. Hence there is no chance for the microbes to come out of the laboratory.

Types of containment:

Biological containment: The biological principles used in laboratories to prevent the escape of GEOs or microbes are called biological containment. Biological containment makes the organisms unable to survive in the outside environment. It prevents the spreading of vector DNAs to the organisms outside the laboratory by usual conjugation, transformation or transduction.

Bacteria which cannot grow outside unless suitable nutrients have to be supplied are used for gene manipulations. Such bacteria are made by inducing gene mutation. This is a mutant bacterium that survive only in the culture.

Physical containment: The physical methods being adopted inside the laboratories to prevent escaping of GEOs to the environment are called physical containment and achieved by a) Laboratory practices b) Containment equipment c) Special laboratory design.

It include:

1. Air filtration

2. Sterilization lights

3. Waste disposal

4. Protective handling

1. **Air filtration**: The exhaust air from the laboratory is filtered through exhaust filters. It prevents the escaping of GEOs from the lab.

2. **Sterilization lights**: Florescent tube lights which emit UV light, are fitted in the laboratory to sterilize the work areas and exposed surfaces of the lab. This technique destroys microbial containment inside the lab.

3. **Waste disposal**: All waste coming from the laboratory are sterilized by autoclaving or by incinerating them in an incinerator. This will prevent the escaping of contaminated wastes from the lab.

4. **Protective handling**: Persons working in the laboratory must follow certain techniques to avoid contamination and to prevent escaping of microbes. The person must wear protective clothing before entering the work area, it should not be carried outside. Mouth pipetting should be avoided.

Types of Physical containment:

1. Primary containment
2. Secondary containment

Primary containment offers protection to personnel and immediate laboratory environment. Primary containment requires using proper storage containers, good microbiological technique, and the use of appropriate safety equipment such as biological safety cabinets.

Secondary containment is the protection of the environment external to the laboratory from exposure to infectious materials and is provided by a combination of facility design and operational practices.

Biosafety Boards Operating In India

With the advent of recombinant DNA technology and its safe applications in different fields like agriculture and animal husbandry across the world, an International meeting was held at Asilomer, California wherein scientists working in the field of genetic engineering made certain recommendations to manage the safety of recombinant DNA technology experiments. These formed the basis of subsequent biosafety guidelines and regulations in USA followed by other countries. India too developed her own regulatory guidelines for genetically modified organisms (GMOs) and recombinant products. There are at present two apex regulatory bodies *viz.* Department of Biotechnology (DBT) and Ministry of Environment and Forest (MEF) which are functioning in the country to regulate rDNA products.

MEF has developed guidelines for manufacture, import, use, research and release of GMOs as well as recombinant products produced from genetically modified organisms in order to ensure that GMOs or their products are safe to human beings. Safety guidelines were developed by DBT in 1990 for carrying out research in the field of Biotechnology, field trials and commercial applications.

DBT has developed separate guidelines for research in transgenic plants in 1998 and for clinical products in 1999. Activities involving GMOs are also covered under other policies such as the Drugs and Cosmetics Act (8th Amendment), 1988, the Drug Policy, 2002, and the National Seed Policy, 2002.

Presently, there are six competent authorities under the auspices of Department of Biotechnology (DBT) and State Governments for implementation of regulations and guidelines in the country as listed below:

1. **Recombinant DNA Advisory Committee (RDAC) – DBT**
2. **Institutional Biosafety Committees (IBSC) attached to every organization engaged in rDNA research – DBT**
3. **Review Committee on Genetic Manipulation (RCGM) – DBT**
4. **Genetic Engineering Approval Committee (GEAC) – DBT**
5. **State Biosafety Coordination Committees (SBCC) – State Government**
6. **District Level Committees (DLC) - State Government**

Institutional Biosafety Committee (IBSC)

DBT has issued guidelines to all the Institutes engaged in rDNA/ genetic engineering research both in Government and Private sectors to constitute their Institutional Biosafety Committee (IBSC) comprising of following members.

i) Head of the Institution or his nominee as Chairman
ii) Three or more scientists engaged in rDNA work / molecular biology / genetic engineering
iii) An outside expert in the relevant discipline
iv) A member with medical qualifications - Biosafety Officer (in case of work with pathogenic agents/large scale use)
v) One member nominated by DBT

IBSC is the nodal body at Institute level responsible for implementation of biosafety guidelines. The projects involving rDNA work are required to be submitted to IBSC for getting clearance. IBSC is responsible for implementation of proper safety guidelines for running the projects at Institute level.

The functions of IBSC are as follows:

a) IBSC gives clearance to rDNA projects submitted by investigators at Institute level based on different Bisoafety levels
b) IBSC meets twice in a year to review the progress and follow up of the recommendations

c) IBSC provides half yearly report on the ongoing projects to RCGM regarding the observance of the safety guidelines on accidents, risks and on deviations, if any.
d) IBSC is responsible for training of personnel on biosafety.
e) IBSC is also responsible for health monitoring programme for laboratory personnel complete medical check-up of personnel working in projects involving work with potentially dangerous microorganisms are required to be carried out on regular basis prior to start of such projects. The medical checkups including pathological tests need to be followed periodically, at least annually for scientific workers involved in such projects.
f) Adopting emergency plans IBSC is also involved in creating awareness amongst the workers / students, faculty and technicians involved in RDNA research projects related to rDNA through popular lectures, seminars and workshops from time to time.

Recombinant DNA Advisory Committee (RDAC)

RADC meets once in six months and monitors the developments at National and International levels for safety regulation in India on recombinant research and applications.

The functions of Recombinant Advisory Committee include:

i) To develop long term policies for research and development in Recombinant DNA research
ii) To formulate the safety guidelines for Recombinant DNA Research to be followed in India
iii) To recommend the type of training programme for technicians and research fellows for making them adequately aware of hazards and risks involved in recombinant DNA research and also to tackle them.

Review committee on genetic manipulation (RCGM)

RCGM is comprised of members from following National bodies.
a) Department of Biotechnology
b) Indian Council of Medical Research
c) Indian Council of Agricultural Research
d) Council of Scientific & Industrial Research
e) Three Experts in Individual capacity
f) Department of Science and Technology

RCGM performs the following functions:

a) To establish procedural guidance manual / procedure for regulatory process with respect to activity involving genetically engineered organisms in research, production and applications related to environmental safety.

b) To review the reports of all the approved ongoing research projects involving high risk category and controlled field experiments to ensure that safeguards are maintained at every step as per guidelines.
c) To recommend the type of containment facility required and the special containment conditions to be followed for experimental trials and for certain experiments on case to case basis
d) To advise custom authorities on import of biologically active material, genetically engineered substances or products and on excisable items to Central Revenue and Excise
e) To assist Department of Industrial Development, Banks towards clearance of applications in setting up industries based on genetically engineered organisms
f) To assist the Bureau of Indian Standards to evolve standards for biologicals produced by rDNA technology
g) To advise on intellectual property rights with respect to rDNA technology on patents.

RCGM has a Research Monitoring function by a group consisting of 3 - 4 individuals and the committee is empowered to visit experimental facilities in any laboratory in India where experiments with biohazard potential are being pursued in order to determine the Good Laboratory practice and conditions of safety and can also recommend any alterations required in the course of experiments based on hazard considerations.

Genetic engineering approval committee (GEAC)

Genetic Engineering Approval Committee (GEAC) functions under the preview of Department of Environment (DOEn) as an apex body for review and approval of activities involving large scale application of genetically engineered organisms and their products in research and development, industrial production, environmental release and field applications. It acts as a legal and statutory body with judicial powers to inspect, investigate and take punitive action in case of violations of statutory provisions under Environment Protection Act.

The constitution of GEAC is as follows.
1. Chairman - Additional Secretary, Department of Environment
2. Co-Chairman - Expert Nominee of Secretary, DBT
3. Representatives of concerned Agencies and Departments
a) Ministry of Industrial Development
b) Department of Science & Technology
c) Department of Ocean Development
d) Department of Biotechnology

4. Expert Members:
a) Director-General, Indian Council of Agricultural Research

b) Director General, Indian Council of Medical Research
c) Director-General, Council of Scientific & Industrial Research
d) Director-General, Health Services (Ministry of Health & Family Welfare)
e) Plant Protection Adviser (Ministry of Agriculture)
f) Chairman, Central Pollution Control Board
g) Three outside experts in individual capacity

5. Member Secretary - Official of, DOEn

State biosafety coordination committees (SBCC)

The SBCC is responsible for performing following functions at state level.
a) To inspect, investigate and take action in case of violations of statutory provisions through the State Pollution Control Board or the Directorate of Health etc.
b) To periodically review the safety and control measures in various institutions handling GMOs.
c) To act as nodal agency at State level to assess the damage, if any, due to release of GMOs and to take site control measures.

District level committees (DLC)

The main functions of DLC are
a) To monitor the safety regulations in installations
b) To inspect, investigate and report to the SBCC or the GEAC about compliance or non-compliance of r-DNA guidelines or violations under EPA.
c) To act as nodal agency at District level to assess the damage, if any, due to release of GMOs and to take on site control measures

Biosafety Regulations and Guidelines

There are several local, state, and federal agencies that either regulate or provide guidelines covering the use of biological agents. A summary of these regulations and guidelines is provided below. Copies of these documents can be obtained from EH&S.

1. Centers for Disease Controls and Prevention (CDC) and the National Institutes of Health (NIH): Biosafety in Microbiological and Biomedical Laboratories (BMBL). This document contains guidelines for microbiological safe work 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. Compliance with the BMBL is a regulatory requirement for work involving select agents and toxins.
2. National Institutes of Health (NIH): Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines). This document provides guidelines for constructing and handling recombinant and synthetic nucleic acid molecules, and organisms containing such nucleic acid. Although these guidelines are not subject to regulatory enforcement (with the exception of work involving select agents and toxins), 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 Institutional Biosafety Committee with the authority to approve proposed research involving recombinant or synthetic nucleic acid molecules, using the NIH Guidelines as a minimum standard.
3. Occupational Safety and Health Administration (OSHA): Bloodborne Pathogens. This regulation covers occupational exposure to human blood and other potentially infectious material, 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 the Hepatitis B virus and receive annual training. Personnel who work with HIV or Hepatitis B virus in a research laboratory must receive additional training and demonstrate proficiency in working with human pathogens

4. Department of Health and Human Services (CDC) and Department of Agriculture (APHIS): Select Agent and Toxin Regulations. These regulations cover the possession, use, and transfer of biological agents and toxins that affect humans, animals, and plants and which have been determined to be potential bioterrorism agents (known as select agents). Entities and personnel who wish to work with select agents must be registered with the CDC or APHIS before acquiring or having access to select agents. Individuals who require access to select agents require a FBI background check and submittal of fingerprints, and must be approved by the Select Agent Program. These regulations mandate strict requirements for biosafety, emergency planning, and security of select agents and toxins, and requires that laboratories that possess select agents comply with the BMBL (see above) and the OSHA Laboratory Standard (see the University Chemical Hygiene Plan) if select agent toxins are used. Each transfer of a Select Agent must have prior approval of the Select Agent Program through completion of APHIS/CDC Form 2, which requires signature by the Select Agent Responsible Official (University Biosafety Officer) or designated alternate. Accurate inventory records of Select Agents, including transfers, must be maintained. See Chapter 14 of this manual for additional information.
5. Washoe County: Regulations of the Washoe County District Board of Health Governing Solid Waste Management (see Section 080 "Biohazardous Waste"). These regulations include requirements for biohazardous waste storage, treatment, and disposal, including specific requirements for decontamination of biohazardous wastes by autoclaving or treatment with chemical disinfectants.

BIOLOGICAL SAFETY

Introduction

The following sections provide general safety guidelines and procedures for biological safety. This chapter covers the following topics:

For more detailed information and resources, please visit the EH&S Biological Safety web page.

Biosafety Principle

Biological hazards include e.g., pathogenic microbes (human, animal or plant pathogens), toxins, venoms, human blood, certain body fluids, cells/tissues, recombinant deoxyribonucleic acid, and genetically modified agents. Biological safety (i.e., biosafety) or biohazard control is management of biological hazards through proper application of engineered containment and administrative controls. The term containment refers to a series of safe methods for managing infectious agents in the laboratory. The purpose of containment is to reduce or eliminate human and environmental exposure to potentially harmful agents. The primary principle of biosafety is thus containment.

Primary and Secondary Containment

There are two levels of biological containment: primary and secondary. Primary containment protects people and the immediate laboratory environment from exposure to infectious agents. Good microbiological techniques and safety equipment provide sufficient primary containment. Examples of primary barriers include safety equipment such as biological safety cabinets (BSCs), enclosed containers, and safety centrifuge cups. Occasionally, when it is impractical to work in BSCs, personal protective equipment, such as laboratory coats and gloves may act as the primary barrier between personnel and infectious materials.

Secondary containment protects the environment external to the laboratory from exposure to infectious materials. Good facility design and operational practices provide secondary containment. Examples of secondary barriers include work areas that are separate from public areas, decontamination facilities, hand-washing facilities, special ventilation systems, and airlocks.

Elements of Containment

Ultimately, the three key elements of biological containment are:

1) laboratory practices,
2) safety equipment, and
3) facility design.

To ensure minimal exposure, employees must assess the hazards associated with their work and determine how to apply the biosafety principle appropriately.

IMPORTANT:

Employees working with infectious agents or potentially infectious materials must be aware of the hazards associated with their work. Employees must be trained and proficient in biosafety procedures and techniques.

General Biosafety Guidelines

Biohazardous materials require special safety precautions and procedures. Follow the guidelines below when working with infectious agents:

Clothing Guidelines:

- Always wear appropriate Personal Protective Equipment (PPE) such as a laboratory coat, gloves, and a mask, if applicable, when working with infectious agents or infected animals. Add boot/shoe covers and eye protection/face shields when necessary.
- Wear gloves over laboratory coat cuffs.
- Never wear contact lenses when working with infectious agents.
- Do not wear potentially contaminated clothing outside the laboratory area.

Remove contaminated clothing as follows:

- Remove boot/shoe covers.
- Remove gloves by peeling them from the inside out. Do not let the glove to make a snapping sound since that would splash any material on the glove's surface to the air.
- Take off eye protection/face shield.
- Remove overall clothing protection. If clothing protection will be reused, hang in approved, controlled area. Otherwise discard.
- Remove mask/respirator by untying or lifting the straps up and over from the back of your head and away from your face.
- Properly dispose used personal protective equipment in containers lined with a red biohazard bag and marked with the biohazard symbol. These containers must be closeable and prevent leakage during collection, handling, processing, storage, transport, or shipping.

Infectious Agents Handling Guidelines:

- Use mechanical pipetting devices and minimize aerosol production.
- Add disinfectant to water baths for infectious substances.
- Use sealed rotors, sealed buckets, or a guard bowl cover complete with gasket as well as safety centrifuge tubes (tube or bottle carrier with sealable cap or "O" ring cap) for potentially infectious samples or otherwise hazardous samples. Before use, the tubes should be checked for cracks. Always wait at least 10-30 minutes after the centrifuge has completely stopped to allow any aerosolized leakage to settle, especially if the samples are potentially infectious.
- Always use secondary leak-proof containers when transporting samples, cultures, inoculated Petri dishes, and other containers of biohazardous materials.

Working with Sharps Guidelines:

- To avoid accidental sticks, place hypodermic needles directly into the sharps containers and do not recap, bend, break, clip, or remove from disposable syringes.
- Use needle-locking or disposable needle units.
- Do not attempt to treat (decontaminate) sharps yourself for any biohazard.
- Do not allow the containers to become overfilled. They should not be more than ¾ full when picked up.
- Do not force anything into a sharps container. If it is full, start a new one.
- Never put your hands in a used sharps container.
- Do not dispose of these containers with the regular trash or incinerate them. Contact the EH&S Office at 817-272-2185 for disposal!

Personal Hygiene Guidelines:

Do not touch your face when handling biohazardous materials!

Wash your hands thoroughly:

- after working with any biohazard.
- after removing gloves, laboratory coat, and other contaminated protective clothing.
- before leaving the laboratory area.
- before eating, drinking, or applying cosmetics (never eat, drink, or apply cosmetics in the work area!)

Work Area Guidelines:

Ensure that warning signs are posted on laboratory doors if biohazardous agents are handled in the laboratory. These signs should include the universal biohazard symbol and the approved biosafety level for the laboratory. EH&S maintains a register of all laboratories and personnel working with infectious agents and provides appropriate warning signs after receiving Human Pathogen Registration.

- Keep laboratory doors shut when experiments involving biohazardous agents are in progress and limit access to laboratory areas when unoccupied.
- Protect house vacuum system during aspiration of infectious fluids by collecting contaminated fluids into a suitable decontamination solution. An in-line HEPA filter is used to protect the vacuum system from aerosolized microorganisms.
- Decontaminate work surfaces daily and after each spill.
- Decontaminate all potentially contaminated equipment and completely decontaminate equipment before having maintenance or repair work done.
- Transport contaminated materials in leak-proof containers.
- Keep miscellaneous materials (i.e., books, journals, etc.) away from contaminated areas.

Universal Precautions:

Universal precautions is a method of infection control—recommended by the Centers for Disease Control and Prevention (CDC)—in which all human blood, certain body fluids, as well as fresh tissues and cells of human origin are handled as if they are known to be infected with human immunodeficiency virus, hepatitis B virus, and/or other bloodborne pathogens. The universal precautions include specific recommendations for use of gloves, gowns, masks, and protective eyewear when contact with blood or body secretions containing blood is anticipated. (see Exposure Control Plan for Bloodborne Pathogens)