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.

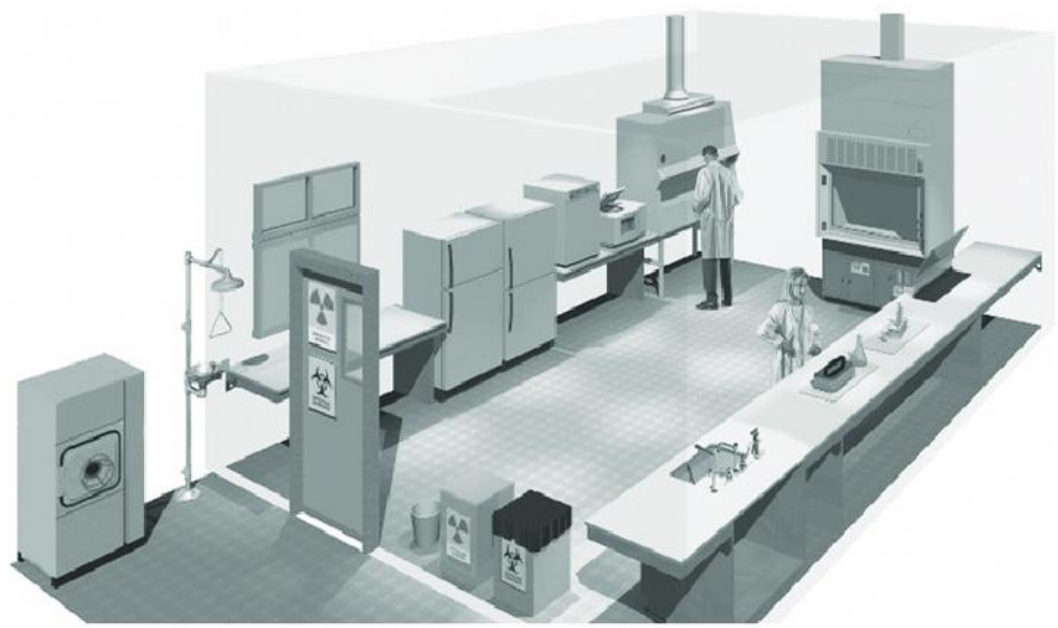
1. The lowest level, biosafety level 1 (BSL-1) laboratory is essentially a teaching laboratory that may include research involving well-characterized agents not known to consistently cause any disease in immunocompetent adult humans, and pose minimal potential hazard to laboratory personnel and the environment. 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

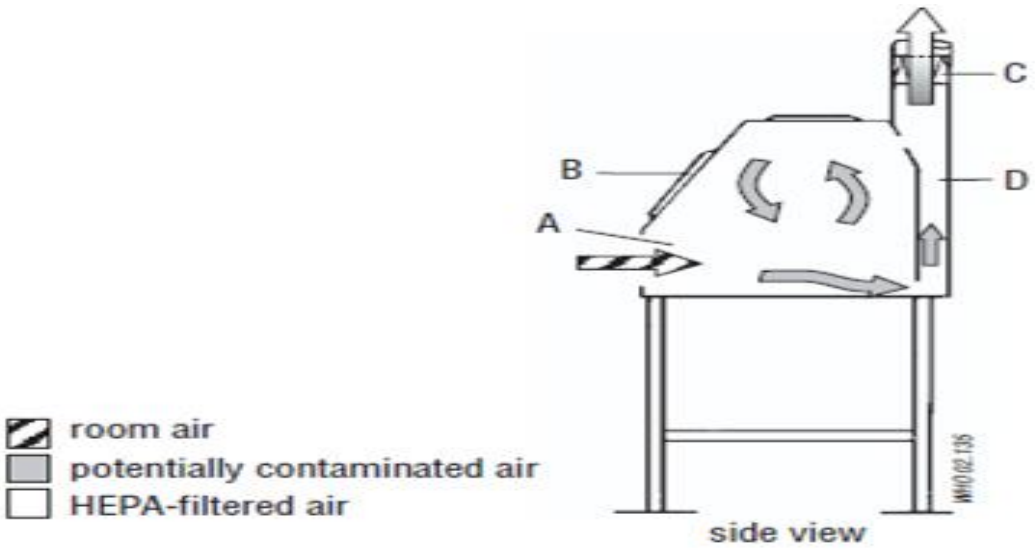


Figure 2: Schematic diagram of a Class I biological safely cabinet. (A) Front opening; (B) Sash; (C) Exhaust HEPA filter; (D) Exhaust plenum

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.

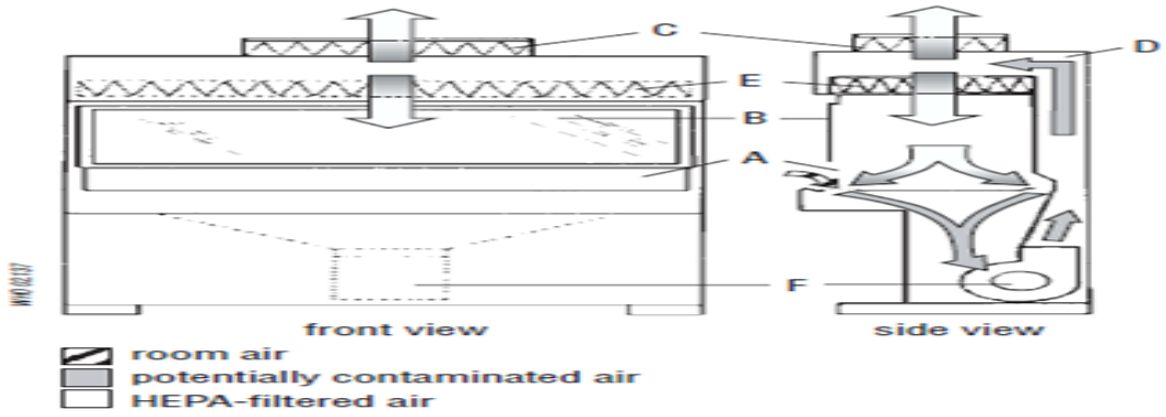


Figure 3**: Schematic representation of class-II biological safety cabinet**. a) Front opening; b) Sash; c) Exhaust HEPA filter; d) Rear plenum; f) Supply HEPA filter d) Blower

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.

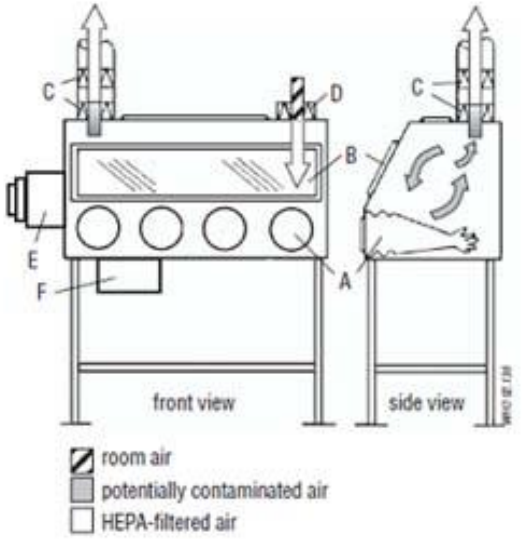


Figure 4: **Schematic representation of class-III biological safety cabinet (glove box**). a) Glove ports of arm-length; b) Sash; c) double exhaust HEPA filter; d) double ended autoclave or pass through box; f) chemical dunk tank

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