BIO SAFETY STANDARDS AND ETHICS - BT242AT

Unit-1: Biohazards, Bio safety levels and cabinets

Introduction: After Covid – 19, we have improved our quality of living when compared to earlier. Importance of hygiene, Personal healthcare, Food Habits and hygiene Keeping these 3 as basic points we have designed the course on Bio safety standard and ethics. As an Engineer of any branch should know about Biosafety levels, Food safety and Bio ethics

Biohazard

Biohazardous materials are any microorganism, or infectious substance, or any naturally occurring, bioengineered, or synthesized component of any such microorganism or infectious substance, capable of causing: Death, disease, or other biological malfunction in a human, an animal, a plant, or another living organism.

This can include medical waste or samples of a microorganism, viruses, or toxins (from a biological source) that can affect human health.

Biohazard Symbol

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 their containment products. It is used in the labeling of biological materials that carry a significant health risk, including viral samples and used hypodermic needles. In Unicode, the biohazard symbol is U+2623 (☣).

Types of biological hazards

- Viruses, such as Coronavirus (COVID-19) and Japanese encephalitis.
- Toxins from biological sources.
- Spores.
- Fungi.
- Pathogenic micro-organisms.
- Bio-active substances

Biohazard examples

Human blood and blood products. This includes items that have been affected by blood and other body fluids or tissues that contain visible blood.

Animal waste. Animal carcasses and body parts, or any bedding material used by animals that are known to be infected with pathogenic organisms.

Human body fluids. Semen, cerebrospinal fluid, pleural fluid, vaginal secretions, pericardial fluid, amniotic fluid, saliva, and peritoneal fluid.

Microbiological wastes. Common in laboratory settings, examples of microbiological wastes include specimen cultures, disposable culture dishes, discarded viruses, and devices used to transfer or mix cultures.

Pathological waste. Unfixed human tissue (excluding skin), waste biopsy materials, and anatomical parts from medical procedures or autopsies.

Sharps waste. Needles, glass slides and cover slips, scalpels, and IV tubing that has the needle attached.

Introduction to Bacteria and virus in human body

- The human body is made of about 100 trillion cells. These cells are quite complex. Most have a nucleus and many special parts.
- Bacteria are much simpler. A bacterium is made of only one cell but has no nucleus. Bacteria are small; each is about 1/100th the size of a human cell.
- Bacteria are like fish swimming in the ocean of your body. As they swim around, they eat and reproduce rapidly. One bacterium can become millions of bacterium in just a few hours.
- Viruses are completely different. A virus is a particle of DNA or RNA with a special cover over it. When virus comes in contact with a living cell, it attaches to it. The virus injects its DNA or RNA into the cell.
- The virus DNA or RNA takes over and uses the cell to make more viruses.

Eventually the cell dies and bursts open spewing millions of new viruses into the body of its victim. Each new virus particle can infect another cell

Bacteria

- Bacteria are microscopic, single-celled organisms that can live anywhere. They are classified as prokaryotes, a simple internal structure that lacks a nucleus. They contain DNA that either floats freely in a twisted, thread-like mass called the nucleoid, or in separate, circular pieces called plasmids. Not all bacteria are harmful, some are necessary for healthy body function.

- Bacteria are classified according to a range of criteria including the nature of their cell walls (i.e. gram positive or negative), their shape (i.e. round, cylindrical or spiral), or by differences in their genetic makeup.

- Most bacteria multiply by a process called binary fission – a single bacterial cell makes a copy of its DNA, doubles its cellular content, and then splits apart pushing the duplicated material out creating two identical 'daughter' cells. Bacteria can introduce variation into themselves by integrating additional DNA, often from their surroundings, into their genome.

- Bacteria can cause harm by directly invading and damaging tissue, others produce powerful chemicals known as toxins, which damage cells. Antibiotics are commonly used to treat bacterial infections, but some strains of
- Bacteria have become resistant to antibiotics, making them difficult to treat. Antibiotic resistance can be transferred between bacterial strains through conjugation
- Bacteria size will varies from 0.05–0.2 µm

Bacteria	**Name of infection or illness**
Salmonella enterica	Diarrheal illness
Vibrio cholerae	Cholera
Shigella dysenteriae	Dysentery
Mycobacterium tuberculosis	Tuberculosis (TB)
Pseudomonas aeruginosa and Burkholderia cepacia	Pneumonia
Legionella pneumophila	Legionnaire's disease
Chlamydia psittaci	Psittacosis
Staphylococcus aureus	Skin infections, endocarditis and osteomyelitis

Leptospira	Leptospirosis, also called Weil's disease

This is a common microscopic organism, which can multiply rapidly and build-up, causing infection. Most bacteria are harmless. Many types grow naturally on the human body and mucus membranes. Bacteria can be found on all workplace surfaces, in the soil and growing in various substances used by workers. Bacteria needs certain conditions to replicate, such as: Warmth, (5 to 60C), Moisture, Food (Nutrients, including foodstuffs, biological metalworking fluids and more), pH (7), Time (20 minutes).

Viruses

- Viruses are different to bacteria as they are the smallest microbes.
- Size of the viruses ranges from 20nm to 400 nm
- A virus is a core genetic material, either Deoxyribonucleic acid (DNA) or Ribonucleic acid (RNA), covered by a protective coat of protein.
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- Viruses can cause several diseases in more advanced cell structures. Examples include: chicken pox, cold, COVID 19, ebola, flu, hepatitis, herpes simplex virus (HSV), measles, mumps, polio, rabies, rubella, smallpox.

Toxins from biological sources

- Toxins are a subset of poison produced by living organisms. Poisons are any substance that can cause harm to an organism if enough has been absorbed, this can be either through ingestion, inhalation, or direct contact.
- Many organisms produce toxins either as a defence mechanism or for predation, they tend to be produced by bacteria, fungi, plants, insect, and animals.

The five most deadly toxins are:

- botulinum toxin A, from the bacteria clostridium botulinum
- tetanus toxin A, from the bacteria clostrifium tetani
- diphtheria toxin, from the bacteria corynebacterium diphtheriae
- muscarine, from the mushroom amanita muscaria
- bufotoxin, from the common toad genus bufo.
- Toxins can present in a variety of workplace settings. Venomous insects such as bees and wasps can nest in any number of workplace buildings and can potentially sting workers. In many countries, snakes and spiders are highly venomous, so can be a threat to workers.

Anaphylaxis

A severe allergic reaction which can be fatal. This usually occurs in response to almost any foreign substance. Common triggers include:

- toxins from insect bites
- Stings
- Food
- Medicines.

Anaphylaxis is a common medical emergency and a life-threatening acute hypersensitivity reaction. It can be defined as a rapidly evolving, generalized, multi-system allergic reaction. Without treatment, anaphylaxis is often fatal due to its rapid progression to respiratory collapse.

The following biological agents are extremely diverse in both form and function. Cell lines: cultures of cells from plants or animals grown in a laboratory. Powerful immune reactions usually prevent cells from another individual from colonising human hosts. However, colonisation may occur if the hosts have a suppressed immune system or are injected with cells derived from their own tissues or tissues from very close relatives. Genetically modified organisms (GMOs): produced artificially in a laboratory through the genetic manipulation of organisms. In theory it is possible that organisms could be produced with novel properties that may be harmful to human health or the environment. Viruses: agents containing genetic material, ie deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), and a protein coat. They vary in size from about 30–400nm and can only replicate using the mechanisms of a host cell. Examples include the influenza virus, Covid-19 and the Zika virus.

Long-term diseases, also called chronic diseases

Local diseases target one part (Lungs) of the body. An example of a local disease is hepatitis B (HBV). This can be transmitted into the body through contact with infectious body fluids such as blood, vaginal secretions or semen.

Systemic diseases affect many parts of the body or the whole body. They can start as a local disease and progress to systemic disease. For example, pneumonia may begin in one lung or both lungs but then spread throughout the body into a potentially life-threatening condition.

Parasitic diseases are infectious diseases caused or transmitted by a parasite. A common example of parasitic disease is toxoplasmosis. Infection usually occurs from:

eating undercooked contaminated meat, exposure to infected cat poo, or mother-to-child transmission in pregnancy.

Cancer

Carcinogens are substances that can cause cancer. Cancer is an uncontrolled growth of abnormal cells in the body. Some new cases of cancer could be attributed to agents such as human papillomavirus (HPV), helicobacter pylori, hepatitis B and C viruses.

Psychological conditions

There is a possible connection between infections and the development of disorders such as schizophrenia, depression and bipolar disorder. A theory is that infection may influence the brain with infective agents, altering the central nervous system.

Toxins (long-term)

Some toxins can have long-term effects. They affect people in different ways, from mild illness to death. For example, there are several types of toxins produced by harmful algae, which in large quantities can form toxic blooms. These can be responsible for causing:

respiratory irritation and distress, diarrhoea, vomiting, numbness, dizziness, paralysis, death.

Allergies

Allergies are long-term conditions that are not life threatening but cause localised tissue inflammation. Some biological hazards can cause hypersensitivity, which is an overreaction by the immune system to an allergen. Examples include:

pollens from plants, viruses, bacteria, animals and birds.

Biosafety

The application of knowledge, techniques and equipment to prevent personal, laboratory and environmental exposure to potentially infectious agents or biohazards

Objectives of studying biosafety:

to reduce the potential exposure of the laboratory worker, persons outside of the laboratory, and the environment to potentially infectious agents

Classification of infective microorganisms by risk group (WHO)

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

Basis for the Classification of Biohazardous Agents by Risk Group

Risk Group 1 (RG-1)	Agents that are not associated with disease in healthy adult humans.
Risk Group 2 (RG-2)	Agents that are associated with human disease which are rarely serious and for which preventive or therapeutic interventions are often available.
Risk Group 3 (RG-3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).
Risk Group 4 (RG-4)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk)

Risk group classification

Risk Group	Individual risk	Community risk
1	no, low	no, low
2	moderate	low
3	high	low
4	high	high

Biosafety Principles

Containment

Laboratory biosafety practices are based on the principle of containment of biological agents to prevent exposure to laboratory workers and the outside environment. Primary containment protects the laboratory workers and the immediate laboratory environment from exposure to biological agents. Primary containment is achieved through good microbiological technique and the use of safety equipment and personal protective equipment. Secondary containment protects the environment outside the laboratory, and is provided by facility design and operational procedures.

Laboratory Practice and Technique

The use of good microbiological technique is the most important element of containment. Personnel working with biological agents must be aware of hazards, and must be trained to safely handle and dispose of these materials. Although we are all responsible for our own safety, the Principal Investigator is responsible for ensuring that persons working in their laboratory are adequately trained.

This Biosafety Manual has been developed to provide general policies and procedures when working with biological agents at the University of Nevada, Reno. Each individual laboratory must supplement this manual with laboratory specific policies; procedures and training that will minimize the specific risks present in the laboratory.

Safety Equipment

Safety equipment includes biological safety equipment, safety centrifuge cups, and other engineered controls designed to minimize exposure to biological agents. Biological safety cabinets (BSCs) are the most important safety equipment for protection of personnel and the laboratory environment, and most BSCs also provide product protection. Safety equipment is most effective at minimizing exposure when workers are trained on the proper use of such equipment, and the equipment is regularly inspected and maintained.

Personal Protective Equipment

Personal protective equipment includes safety eyewear, lab coats, gloves, and other protective equipment, and is used to supplement the containment provided by laboratory practices and safety equipment. Personal protective equipment is considered the least desirable containment method because its failure results in direct exposure of personnel to the biological agent.

Facility Design

Facility design features include physical separation of laboratories from public access, specially designed ventilation systems (to prevent airborne biological agents from migrating outside the laboratory), and autoclaves. These design features protect personnel working outside the immediate laboratory, as well the outside environment.

Biosafety levels (BSL)

According to the World Health Organisation (WHO), "biosafety is a strategic and integrated approach to analysing and managing relevant risks to human, animal and plant life and health and associated risks for the environment.

A biosafety level (BSL), or pathogen/protection level, is a set of biocontainment precautions required to isolate dangerous biological agents in an enclosed laboratory facility. In the United States, the Centers for Disease Control and Prevention (CDC) have specified these levels of containment range from the lowest biosafety level 1 (BSL-1) to the highest at level 4 (BSL-4).

Each biosafety level (BSL-1 through BSL-4) is defined based on the following:

- Risks related to containment
- Severity of infection
- Transmissibility
- Nature of the work conducted within the lab
- Origin of the microbe
- Agent in question
- Route of exposure

Clarification of Biosafety Level

Biosafety Level 1 (BSL-1)

The lowest of the four biosafety levels, biosafety level 1 (BSL-1) applies to laboratory settings in which personnel work with low-risk microbes that pose little to no threat of infection in healthy adults — for example, a BSL-1 laboratory might work with a nonpathogenic strain of E.coli. BSL-1 labs typically conduct research on benches, do not use special contaminant equipment, and do not need to be isolated from surrounding facilities.

Safety protocols for biosafety level 1 labs which require only standard microbial practices include:

- Mechanical pipetting (no mouth pipetting allowed)
- Safe sharps handling
- Avoidance of splashes or aerosols
- Daily decontamination of all work surfaces when work is complete
- Regular handwashing
- Prohibition of food, drink, and smoking materials
- The use of personal protective equipment (PPE), such as goggles, gloves, and a lab coat or gown
- Biohazard signs

BSL-1 labs also require immediate decontamination after spills. Infectious materials should also be decontaminated prior to disposal, generally through the use of an autoclave.

Biosafety Level 2 (BSL-2)

Biosafety level 2 (BSL-2) covers all laboratories that work with agents associated with human diseases — that is, pathogenic or infectious organisms — that pose a moderate health hazard. Common examples of agents found in a BSL-2 lab include equine encephalitis viruses, HIV, and staphylococcus aureus (staph infections).

BSL-2 labs are required to maintain the same standard microbial practices as BSL-1 labs, as well as enhanced measures due to the potential risk the aforementioned microbes pose. Personnel working in biosafety level 2 laboratories are expected to take even greater care to prevent injuries, such as cuts and other breakage to the skin, as well as ingestion and mucous membrane exposures.

In addition to the safety protocols established for BSL-1 labs, BSL-2 labs are subject to the following safety controls:

- The use of PPE, including lab coats, gloves, eye protection, and — in some cases — face shields
- All procedures that could cause infection from aerosols or splashes must be performed within a biological safety cabinet.
- Decontamination of infectious materials prior to disposal, generally through the use of an autoclave
- Self-closing, lockable doors
- Access to a sink and eyewash station
- Biohazard warning signs

Access to a biosafety level 2 lab is far more restrictive than to a biosafety level 1 lab. Outside personnel, or those with an increased risk of contamination, are often restricted from entering the area while work is underway.

Biosafety Level 3 (BSL-3)

Once again building on the two prior biosafety levels, a biosafety level 3 (BSL-3) laboratory typically conducts research into or work on microbes that are either indigenous or exotic and can cause serious or potentially lethal disease through inhalation. Common examples of microbes found in BSL-3 labs include yellow fever, West Nile virus, and the bacteria that causes tuberculosis.

Microbes found within biosafety level 3 settings are so serious that work is often strictly controlled and registered through the appropriate government agencies. Laboratory personnel are also under medical surveillance and may require immunizations for the microbes they work with.

Common safety controls within a BSL-3 lab include:

- The use of PPE, including goggles and gloves; respirators may also be required

- The use of solid-front wraparound gowns, scrub suits, and/or coveralls is often required
- Access to a hands-free sink and eyewash station available near the exit
- Sustained directional airflow to draw air into the laboratory from clean areas toward potentially contaminated areas (exhaust air cannot be recirculated)
- Self-closing set of locking doors with access away from general building corridors

Access to a BSL-3 laboratory is restricted and controlled at all times.

Biosafety Level 4 (BSL-4)

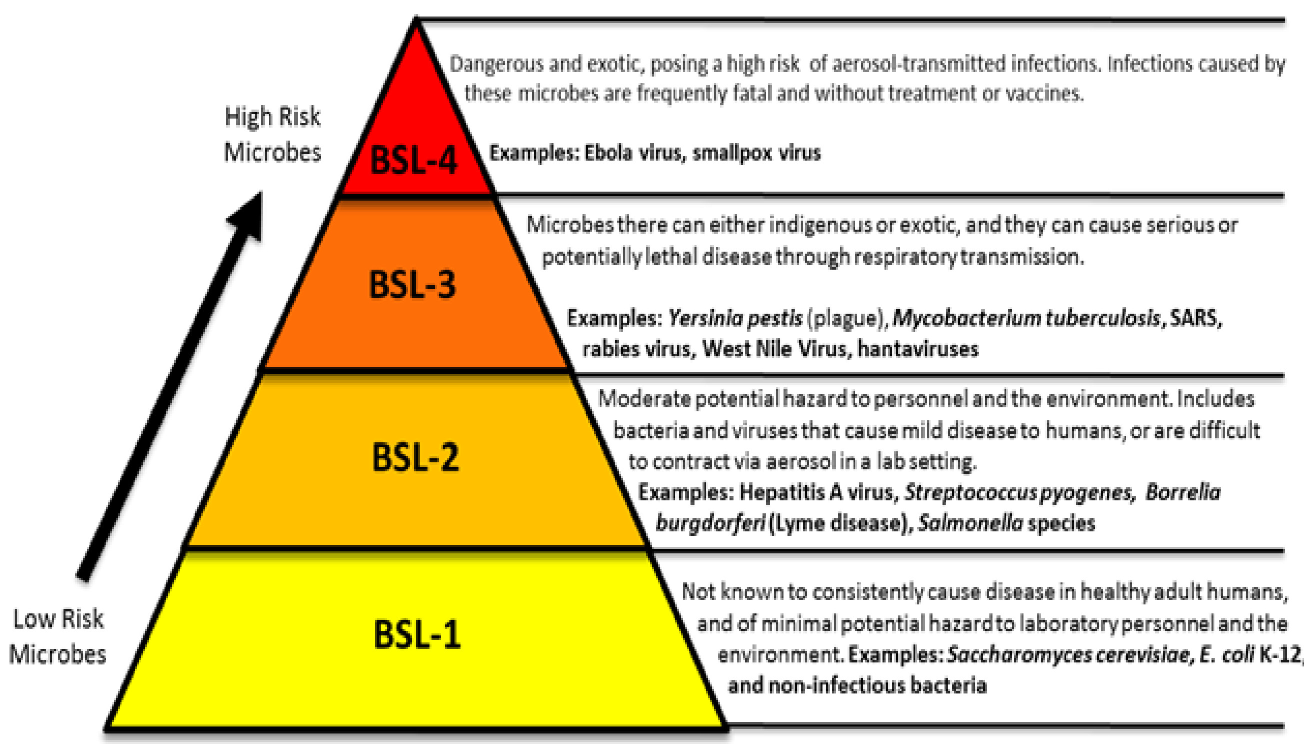
Biosafety level 4 (BSL-4) labs are rare; however, a small number exist in the U.S. and around the world. As the highest level of biological safety, BSL-4 labs work with highly dangerous and exotic microbes, such as the Ebola and Marburg viruses. Infections caused by these types of microbes are often fatal and come without treatment or vaccines.

In addition to biosafety level 3 considerations, biosafety level 4 laboratories must follow these safety protocols:

- Personnel must change clothing before entering the facility and shower upon exiting
- All materials must be decontaminated before leaving the facility
- Personnel must wear the PPE from lower BSL levels, as well as a full-body, air-supplied, positive pressure suit
- Access to a Class III biological safety cabinet

BSL-4 labs are extremely isolated, often located in an isolated and restricted zone of a building or in a separate building entirely. BSL-4 labs also feature a dedicated supply of exhaust air, as well as vacuum lines and decontamination systems.

Biosafety Level	BSL-1	BSL-2	BSL-3	BSL-4
Description	· No Containment · Defined organisms · Unlikely to cause disease	· Containment · Moderate Risk · Disease of varying severity	· High Containment · Aerosol Transmission · Serious/Potentially lethal disease	· Max Containment · "Exotic," High-Risk Agents · Life-threatening disease
Sample Organisms	E.Coli	Influenza, HIV, Lyme Disease	Tuberculosis	Ebola Virus
Pathogen Type	Agents that present minimal potential hazard to personnel & the environment.	Agents associated with human disease & pose moderate hazards to personnel & the environment.	Indigenous or exotic agents, agents that present a potential for aerosol transmission, & agents causing serious or potentially lethal disease.	Dangerous & exotic agents that pose a high risk of aerosol-transmitted laboratory infections & life-threatening disease.
Autoclave Requirements	None	None	Pass-thru autoclave with Bioseal required in laboratory room.	Pass-thru autoclave with Bioseal required in laboratory room.



Biological Safety Cabinets (BSC)
A biosafety cabinet (BSC) is a box-type air purification negative pressure safety device that can prevent the escape of aerosols containing dangerous or unknown biological particles during the experimental operation process. Biological safety cabinets are used to protect the operator, the laboratory environment, and experimental materials from exposure to infectious aerosols and splash when operating infectious experimental materials such as primary cultures, bacterial strains, and diagnostic specimens.

Biosafety Cabinet Application

As for biosafety cabinet application, a biosafety cabinet is widely used in scientific research, teaching, clinical testing, and production in the fields of microbiology, biomedicine, genetic engineering, and biological products. The biosafety cabinet is the basic safety protection equipment in the first-level protective barrier of laboratory biosafety.

Biosafety Cabinet Principle

The biosafety cabinet working principle is mainly to suck the air in the biological safety cabinet outward to keep the biosafety cabinet in a negative pressure state, and to protect the staff through vertical airflow. The outside air is filtered by an air filter (high-efficiency particulate air filter, HEPA filter) and then enters the biosafety cabinet to avoid contamination of the processed samples. The air in the biological safety cabinet also needs to be filtered by a HEPA filter before being discharged into the atmosphere to protect the environment.

BSCs offer three levels of protection:

1. Personnel- HEPA filters and an air curtain shield users from biohazardous aerosols produced inside the chamber.
2. Sample Protection- Recirculating and unidirectional HEPA-filtered air guards against contamination of samples by contaminated lab air.
3. Lab/ Environmental Protection- HEPA-filtered exhaust from the top of the cabinet safeguards against contaminating the lab environment with biohazardous aerosols inside the chamber.

Biosafety Cabinet Types

According to the difference in the level of biological safety protection, biological safety cabinets can be divided into three types: first level, second level, and third level.

1. Class I Biosafety Cabinet

Class I biosafety cabinets protect workers and the environment without protecting samples. The airflow principle is the same as that of the laboratory general kitchen. The difference is that the exhaust port is equipped with a filter. All types of biosafety cabinets use filters on the exhaust and air inlets. The first-class biological safety cabinet has no fan itself and relies on the fan in the external ventilation pipe to drive the airflow. Since it cannot protect test objects or products, it is less used at present.

2. Class II biosafety cabinets

Class II biosafety cabinets are currently the most widely used type of biosafety cabinet. The class II biological safety cabinet can be divided into four levels according to the inlet air velocity, exhaust mode, and circulation mode: Type A1, Type A2, Type B1, and Type B2. All Class II biosafety cabinets provide protection for workers, the environment, and products. If you want to buy these types of biosafety cabinets, you can buy them from Drawell which is a biosafety cabinet manufacturer in China.

The minimum air velocity of the front window of the class II A1 biosafety cabinet or the measured average value shall be at least 0.38m/s. 70% of the air is recirculated to the work area through the HEPA filter, and 30% of the air is filtered out through the exhaust port.

The minimum air velocity of the front window of the class II A2 type biosafety cabinet or the measured average value shall be at least 0.5m/s. 70% of the air is recirculated to the work area through the HEPA filter, and 30% of the air is filtered out through the exhaust port.

Class II B-type biosafety cabinets are all safety cabinets connected to the exhaust system. The fan connected to the exhaust duct of the safety cabinet is connected to the emergency power supply. The purpose is to maintain the negative pressure of the biosafety cabinet in case of power failure, to prevent dangerous gas from leaking out of the laboratory. The minimum or measured average velocity of the front window airflow shall be at least 0.5m/s (100fpm).

70% of the gas in the class II B1 type biosafety cabinet is removed through the HEPA filter at the exhaust port, and 30% of the gas is recirculated to the working area through the HEPA filter at the gas supply port.

The class II B2-type biosafety cabinet is a 100% full-row safety cabinet without internal circulating air flow, which can provide biological and chemical safety control at the same time, and can operate volatile chemicals and volatile nuclear radiation as additives for microbial experiments.

3. Class III biosafety cabinets

Class III biosafety cabinets are designed for laboratories with a biological safety protection level of 4. The biosafety cabinet is completely airtight. The staff operates through gloves connected to the cabinet, and the test products enter and exit the safety cabinet through the double-door transfer box to ensure no contamination. The class III biosafety cabinet is suitable for high-risk biological experiments, such as experiments related to SARS and the Ebola virus.