وقاية

هيئة الصحة العامة

PUBLIC HEALTH AUTHORITY

National Laboratory Biosafety and Biosecurity Manual

v1.0

Contents

Abbreviations and Acronyms

Ag	Agriculture (i.e., CL2-Ag, CL3-Ag)
BSC	Biological safety cabinet
BSO	Biological safety officer
CBS	Canadian Biosafety Standard
CL	Containment level (i.e., CL1, CL2, CL3, CL4)
ERP	Emergency response HEPA High-efficiency particulate air
HEPA	High efficiency particulate air
HVAC	Heating, ventilation, and air conditioning
IBC	Institutional Biosafety Committee
LA	Large animal containment zone
LOHI	Laboratory Occupational Health Immunoprophylaxis
LRA	Local risk assessment
PHA	Public Health Authority
PI	Principal investigator
PM room	Post mortem room
PPE	Personal protective equipment
PSDS	Pathogen safety data sheet
RG	Risk group (i.e., RG1, RG2, RG3, RG4)
RA	Risk assessment
SA	Small animal (containment zone)
SOP	Standard operating procedure
SSBA	Security-sensitive biological agent
UPS	Uninterruptible power supply
LAI	Laboratory acquired infection

Definitions

Biosafety: refers to the principles, technologies, and practices designed to prevent accidental exposure to harmful pathogens or toxins.

Biosecurity: encompasses measures aimed at preventing the loss, theft, misuse, diversion, or intentional release of infectious substances.

Biological material: Refers to microorganisms, proteins, nucleic acids,or anything that contains them (e.g., tissue), whether infectious or toxic.

Microorganisms: Are tiny living organisms usually not seen by the naked eye. They include viruses, bacteria, fungi, protozoa, and parasites.

Pathogens: Are a subset of biological material that can cause disease in humans or animals.

Infectious material: Is used throughout to collectively refer to pure cultures or isolates ofpathogens as well as any material that may contain a pathogen (e.g., infected tissue sample) or part of one that retains its pathogenicity.

Microbial toxins: are poisonous substances that are a natural product of the metabolic activities of certain microorganisms (e.g., bacteria, fungi). Intoxication can occur because of exposure through ingestion, inhalation, inoculation, or absorption. There are two types of toxins: endotoxins and exotoxins.

Prions: are small proteinaceous infectious particles that are generally accepted to be the cause of a group of progressive neurodegenerative diseases in humans and animals known as transmissible spongiform encephalopathies (TSEs).

Biotechnology: describes the application of science and engineering to the direct or indirect use of living organisms or parts or products of living organisms in their natural or modified forms.

Recombinant DNA: Genetic material, either natural or synthetic, can be combined to construct novel rDNA.

Genetically Modified Organisms: GMOs are organisms (i.e., plants, animals, or microorganisms) that are created through the alteration of genetic materials in a way that does not occur naturally through mating or natural recombination.

Laboratory Biosecurity: The nation continues to face a challenge in safeguarding public health from potential domestic or international bioterrorism.

Laboratory acquired infection: An infection acquired by laboratory personnel during laboratory work.

1. Scope

This document provides guideline and standards for local facilities in the Kingdom of Saudi Arabia including all facilities that handle and store human pathogens or toxins in Saudi Arabia, such as public health, teaching, research, diagnostic laboratories, and vaccine and therapeutic production plants, and veterinary laboratories. It addresses the safe handling and containment procedures of infectious microorganisms and hazardous biological materials. Notably, the guidelines address any activity where human or terrestrial animal pathogens are handled and ensure adherence to the physical containment and operational practice requirements as well as performance and verification testing requirements for these facilities. This proposed guideline addresses contemporary issues and mitigates work-related risks that may face laboratory personnel.

Newly emerged microorganisms have risen in recent years, requiring additional measures related to the containment and safe storage of those emerging pathogens. Furthermore, the use of infectious agents in various health and research systems was expanded to study and understand those novel and newly emerged pathogens. Therefore, this guideline is fundamental for those facilities handling those pathogens and aids in evaluating and assessing their biosafety and biosecurity programs to ensure safe practice and containment of microbiological agents.

This document aims to: 1) outline the process and conceptual framework for laboratory biosafety and biosecurity risk assessment; 2) detail the national standards for four biosafety laboratory levels for both human and animal laboratory settings; and 3) provide details about the biosecurity in laboratories.

2. Introduction

The containment of microorganisms and hazardous biological materials is essential to maintaining biosafety, particularly in laboratories that handle infectious agents. The primary goal of containment is to protect laboratory workers, the environment, and the public from potential exposure to dangerous microorganisms and biological hazards. This is achieved through a combination of stringent microbiological practices, the use of specialized safety equipment, and the implementation of facility safeguards that are specifically designed to mitigate risk.

Conducting a thorough risk assessment is critical to ensuring effective containment. This process evaluates the potential dangers of handling microorganisms or hazardous materials and determines the most appropriate safety measures. Risk assessments (RA) help determine which microbiological practices, safety equipment, and facility safeguards are necessary to reduce the risk of Laboratory-Associated Infections (LAIs) and other hazards.

By carefully applying these measures, laboratories can create secure working environments that protect personnel and prevent accidental release into the environment. As new biological threats and emerging infectious diseases continue to present challenges, the role of containment in biosafety becomes even more critical to public health and environmental safety.

2.1 Historical insights into laboratory infection incidents

The history of Laboratory-associated infections (LAIs) first appeared around the start of the 20th century. In 1885 it was the first recorded LAI of typhoid fever. And then, thirty years later, Kisskalt published an investigation, making it the first documented report of LAI. Between 1930 and 1978, Pike and Sulkin collectively identified 4,079 LAIs, resulting in 168 deaths. LAIs occurred as a result of the failure of the laboratories to report subclinical and asymptomatic cases. Furthermore, the laboratories did not report the incidents or exposure events, which complicated the quantitative RA.

The most common causes of LAIs are Brucella spp., *Coxiella burnetii*, hepatitis B virus (HBV), *Salmonella enterica* serotype Typhi, *Francisella tularensis*, *Mycobacterium tuberculosis*, *Blastomyces dermatitidis*, Venezuelan equine encephalitis virus, *Chlamydia psittaci*, and *Coccidioides immitis*. The clinical and research laboratories accounted for 17% and 59%, respectively. Twenty years later, Harding and Byers revealed 1,267 overt infections with 22 deaths, and they indicated that clinical (diagnostic) and research laboratories accounted for 45% and 51%, respectively. The difference in the two reports reflects that improvements in containment equipment, engineering controls, and greater emphasis on safety training may contribute to the apparent reduction in LAIs over two decades. Exposure of laboratory technologists to Brucella melitensis on a bench resulted in an attack rate of 31% (8/26) due to transmission through the aerosol route

2.2 Biosafety Risk assessment

Thousands of infectious biological agents and toxins such as *Mycobacterium tuberculosis*, foot-and-mouth disease virus, *Escherichia coli* O157:H7, *Brucella abortus*, *Francisella tularensis* and dengue fever virus are handled and manipulated in a variety of laboratory types for diagnostic, clinical, veterinary, research, and commercial purposes around the world. The type, number, and quantity of these materials are depending on the scope and nature of the work conducted in the laboratory. Each agent and toxin handled is a potential hazard posing a risk to personnel in the institute, and possible to surrounding public and communities beyond the laboratory.

RAs are the basis of all components of a biosafety program; they are critical for identifying the hazards associated with specific tasks or activities involving infectiousmaterial and toxins and for implementing the appropriate mitigation strategies. Developing a functional biosafety program requires an overarching risk assessmentof all the work with infectious material and toxins. In addition, local risk assessments (LRAs) specific to the work area are conducted to identify hazards based on the infectious material or toxin in use and the activities being performed. RAs describing the hazardous properties of well-characterized human pathogens and toxins and recommendations for their safe handling have been developed into technical documents known as Pathogen Safety Data Sheets (PSDSs).

Implementing RA in the laboratory offers more than just reducing and mitigating risks. They also contribute to the following:

1. Ensuring adherence to government regulations
2. Allocating resources efficiently to address risks
3. Identifying necessary training and supervision
4. Advance planning for renovations
5. Evaluating changes in procedures
6. Justifying the need for space and equipment
7. Reviewing emergency response plans
8. Planning for regular maintenance
9. Evaluating interactions and workflow with other laboratories or units

2.3 Risk criteria and containment levels

The primary risk criteria used to define the four ascending levels of containment, referred

to as Biosafety Levels 1 through 4, are:

- Infectivity
- Severity of disease
- Transmissibility
- The nature of the work being conducted
- The origin of the microorganism (indigenous, or exotic).

The four ascending biosafety levels and their agent`s characteristics and containments descriptions are shown in table 1 below.

Table 1: Four ascending biosafety levels.

Biosafety Level	Agents:	Containment:
Biosafety Level 1 (BSL-1)	Well-characterized agents are not known to cause disease in healthy adults consistently.	Standard microbiological practices without particular primary or secondary barriers.
Biosafety Level 2 (BSL-2)	Agents associated with human disease and moderate agents that cause human disease (e.g., Staphylococcus aureus	Enhanced laboratory practices, BSCs, and facilities to handle moderate-risk pathogens.
Biosafety Level 3 (BSL-3)	Indigenous or exotic agents have a known potential for aerosol transmission for agents that may cause severe and potentially lethal infections and that are Indigenous or exotic in origin (e.g., *Mycobacterium tuberculosis*.	Strict laboratory practices, use of BSCs, and controlled access to the laboratory.
Biosafety Level 4 (BSL-4)	Dangerous and exotic agents with a high risk of life-threatening disease, aerosol transmission, or unknown risk of transmission and for which no treatment is available (e.g., Ebola virus).	Maximum containment facilities, including full-body, air-supplied suits, specialized ventilation, and waste management systems.

3. Biological Risk Assessment

Risk is defined as the probability of an unfortunate event or the sum of the likelihood and consequences of a particular dangerous situation. The estimated severity of a given risk can vary between people, as their different previous experiences lead to different perceptions of risk. Whereas biological risk refers to potential harm to individual citizens, the environment, and wider society caused by certain pathogenic microorganisms and their associated operational procedures or experimental activities.

RA means the possibility of quantitatively measuring the impact or loss caused by a particular phenomenon. RA is a process that includes three steps: risk identification, analysis, and assessment, and provides a basis for risk management. RA is an essential process prior starting any laboratory work. For activities demanding Institutional Biosafety Committee (IBC) approval, conducting a RA is crucial in the registration process. The Principal Investigator (PI), lab supervisor, or laboratory director bears the responsibility for spotting potential hazards, evaluating associated risks, and setting safety precautions and standard operation procedures (SOPs) to mitigate risk exposure for employees. These measures should be recorded in a lab-specific biosafety manual accessible to all lab personnel. The IBC will review the PI's risk assessment and may suggest modifications before granting approval.

The biological risk assessment aims to identify the characteristics of all potential infectious agents or material, the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a LAI and the probable consequencesof such an infection. Subsequently, this information should guide the selection of appropriate interventions to mitigate the risk by applying biosafety levels, good microbiological practices, safety equipment, and facility safeguards to prevent LAIs. The risk management process is continuous andshould promote a positive culture through collaboration and inclusion of all stakeholdersto facilitate. Therefore, this process should be integrated into the daily laboratory operation to identify hazards, prioritize the risks, and implement specific measures andprotocols targeting current situations.

Performing a qualitative biological RA relies on subjective professional judgments and is often based on incomplete or uncertain scientific data, leading to inherent limitations and assumptions. Risk perception varies among individuals, and the risk is never zero due to the possibility of human error. Identifying hazards is the first step in a RA process, requiring a thorough approach that gathers information from multiple sources. No single correct approach exists, but options include using a risk prioritization matrix, performing a job hazard analysis, or listing potential issues for procedures or tasks.

3.1. Risk Assessment Process

The following five steps are essential in RA process:

1. Identify the hazards associated with an infectious or biohazardous agent or material, including human pathogens, recombinant viral vectors, and acute biological toxins.

2. Identify the laboratory activities that might cause exposure to the agent or material.
3. Consider the training, competencies and experience of laboratory personnel.
4. Evaluate and prioritize risks (evaluate the likelihood that an exposure would cause a LAI and the severity of consequences if such an infection occurs).
5. Develop, implement, and evaluate controls to reduce the risk for exposure and establish plans for how to deal with an exposure, should it occur.

Each one of the five steps mentioned above is presented in more details as following:

Step 1. Identify the risks linked to an infectious or biohazardous agent or material.

a. Risk of infection based on common transmission routes (e.g., contact with contaminated surfaces, skin cuts, mucous membranes, inhalation of aerosols).
b. Volume and concentration of organisms being used.
c. Intrinsic factors (if the agent is known):
   - Pathogenicity, virulence, and infectivity of the strain;
   - Mode of transmission (which may differ in the lab compared to natural settings);
   - Infectious dose required or LD50 for toxic substances;
   - Genetic modification that may increase risk, such as oncogene expression or siRNA use;
   - Potential formation of replication-competent viruses with viral vectors;
   - Agent form (e.g., cell wall presence, spore vs. vegetative state, conidia vs. hyphae for fungi);
   - Agent's invasiveness (e.g., enzyme production ability);
   - Material origin, like human tissues or cell lines, which may carry pathogens;
   - Availability of vaccines or preventive treatments;
   - Antibiotic resistance.

Step 2. Identifying activities that might cause exposure to the agent or material.

- The facility (e.g., BSL-2, BSL-3, BSL4 open floor plan [more risk] versus separate areas or rooms for specific activities [less risk], sufficient space versus crowded space, workflow, equipment present);
- The equipment (e.g., uncertified BSCs, cracked centrifuge tubes, improperly maintained autoclaves, overfilled sharps containers, Bunsen burners);
- Potential for generating aerosols and droplets.

Aerosols can be generated from most routine laboratory activities but most of the time are undetectable. **The following procedures have been associated with generation of infectious aerosols:**

a. Manipulating needles, syringes and sharps, the following practices are considered:

- Sub-culturing positive blood culture bottles, making smears
- Expelling air from tubes or bottles
- Withdrawing needles from stoppers
- Separating needles from syringes
- Aspirating and transferring body fluids
- Harvesting tissues

b. Manipulating inoculation needles, loops, and pipettes

- Flaming loops
- Cooling loops in culture media
- Sub-culturing and streaking culture media
- Expelling last drop from a pipette or a dropper
- Manipulating specimens and cultures
- Centrifugation
- Setting up cultures, inoculating media
- Mixing, blending, grinding, shaking, sonicating, and vortexing specimens or cultures
- Pouring, splitting, or decanting liquid specimens
- Removing caps or swabs from culture containers, opening lyophilized cultures, opening cryotubes
- Spilling infectious material
- Filtering specimens under vacuum
- Preparing smears, performing heat fixing, staining slides
- Performing serology, rapid antigen tests, wet preps, and slide agglutinations
- Throwing contaminated items into biohazardous waste
- Cleaning up spills

- Use of animals;
- Use of sharps;
- Production of large volumes or concentrations of potential pathogens or agents;
- Improperly used or maintained equipment;

Examples of possible hazards are decreased dexterity or reaction time for workers wearing gloves, reduced ability to breathe when wearing N95 respirators, or improperly fitting personal protective equipment (PPE). More details about droplet and aerosols are presented in section 3.2 below.

- Working alone in the laboratory.

No inherent biologic danger exists to a person working alone in the laboratory; however, the lab director and lab supervisor is responsible for knowing if and when a person is

assigned to work alone. Because assigning a person to work alone is a facility-specific decision, a RA should be conducted that accounts for all safety considerations, including type of work, physical safety, laboratory security, emergency response, potential exposure or injury, and other laboratory-specific issues.

Step 3. Considering the competencies and experience of laboratory personnel.

a. Age (inexperienced employees might be at higher risk)
b. Genetic predisposition and nutritional deficiencies, immune/medical status (e.g., underlying illness, receipt of immunosuppressive drugs, chronic respiratory conditions, pregnancy, nonintact skin, allergies, receipt of medication all are known to reduce dexterity or reaction time)
c. Education, training, experience, competence
d. Stress, fatigue, mental status, excessive workload
e. Perception, attitude, adherence to safety precautions
f. The most common routes of exposure or entry into the body (i.e., skin, mucous membranes, lungs, and mouth).

Step 4. Evaluating and prioritizing risks.

Risks are evaluated according to the likelihood of incidence and severity of consequences. The risk evaluation is illustrated as following:

a. Likelihood of occurrence:
   - Almost certain: expected to occur
   - Likely: could happen sometime
   - Moderate: could happen but not likely
   - Unlikely: could happen but rare
   - Rare: could happen, but probably never will

b. Severity of consequences:

Consequences may rely on time and frequency of exposure and on availability of vaccine and appropriate medication. Following are examples of consequences for individual workers:

- Colonization leading to a carrier state
- Asymptomatic infection
- Toxicity, oncogenicity, allergenicity
- Infection, acute or chronic
- Illness, medical treatment
- Disease and sequelae

- Death

Step 5. Developing, implementing, and evaluating controls to minimize the risk for exposure.

a. Engineering controls:

If possible, first isolating and containing the hazard at its source.

- Primary containment: BSC, sharps containers, centrifuge safety cups, splash guards, safer sharps (e.g., auto-retracting needle/syringe combinations, disposable scalpels), and pipette aids
- Secondary containment: building design features (e.g., directional airflow or negative air pressure, hand washing sinks, closed doors, double door entry)

b. Administrative and work practice controls:

- Strict adherence to standard and special microbiological practices
- Adherence to signs and SOPs
- Frequently washing hands
- Wearing PPE only in the work area
- Minimizing aerosols
- Prohibiting eating, drinking, smoking, chewing gum
- Limiting use of needles and sharps, and banning recapping of needles
- Minimizing splatter (e.g., by using lab "diapers" on bench surfaces, covering tubes with gauze when opening)
- Monitoring appropriate use of housekeeping, decontamination, and disposal procedures
- Implementing "clean" to "dirty" work flow
- Following recommendations for medical surveillance and occupational health, immunizations, incident reporting, first aid, post-exposure prophylaxis
- Training
- Implementing emergency response procedures

c. PPE (as a last resort in providing a barrier to the hazard):

- Gloves for handling all potentially contaminated materials, containers, equipment, or surfaces
- Face protection (face shields, splash goggles worn with masks, masks with built-in eye shield) if BSCs or splash guards are not available. Face protection, however, does not adequately replace a BSC. At BSL-2 and above, a BSC or similar containment device is required for procedures with splash or aerosol potential.
- Laboratory coats and gowns to prevent exposure of street clothing, and gloves or bandages to protect non-intact skin

- Additional respiratory protection if warranted by risk assessment

Details about the PPE and safety equipment hazard is presented in section 3.3 below.

d. Job safety analysis:

One way to initiate a risk assessment is to conduct a job safety analysis for procedures, tasks, or activities performed at each workstation or specific laboratory by listing the steps involved in a specific protocol and the hazards associated with them and then determining the necessary controls, on the basis of the agent/organism. An example is precautions beyond the standard and special practices for BSL-2 is indicated in the following circumstances:

- Organisms transmitted by inhalation
- Work with vectors expressing oncogenes or toxins
- Work with large volumes or highly concentrated cultures
- Compromised immune status of staff
- Training of new or inexperienced staff
- Technologist preference

e. Monitoring effectiveness of controls:

- Risk assessment is an ongoing process that requires at least an annual review because of changes in new and emerging pathogens and in technologies and personnel
- Review reports of incidents, exposures, illnesses, and near-misses
- Identify causes and problems; make changes, provide follow-up training
- Conduct routine laboratory inspections
- Repeat risk assessment routinely

Determining hazardous characteristics of agents is based on the probable routes of transmission in the laboratory, infective dose, stability in the environment, host range, and endemic nature. Previous LAI reports are a clear indicator of hazardous agents. The absence of a report does not indicate minimal reporting, and the number of reports for a single agent may indicate the frequency and risk of the agent.

The most likely source of transmission is direct skin contact, eye or mucous membrane, parenteral transmission by syringe needle, sharps or bite of infected animals, the ingestion of liquid suspension of an infectious agent or contaminated hand to mouth,and inhalation of infectious aerosols. Awareness of an agent's transmission route helps identify the potential risk to laboratory workers and the community. For example, respiratory exposure to infectious aerosols is a severe hazard to the person handling the sample and

other occupants. The agents with low infectious dose and environmental *stability, such as Coxiella burnetiid* (10 inhaled infectious particles), can live outside the living cells and culture media and become an aerosol hazard.

A special consideration is required for risk assessment of hazardous characteristics of zoonotic agents. Laboratory animals can shed zoonotic and other infectious agents under study in saliva, urine, or faeces. Previous report of laboratory worker death after ocular exposure to biological material from a rhesus macaque containing Monkey B virus. Experiment animals infected with *Francisella tularensis, Coxiella burnetii*, *Coccidioides immitis*, or *Chlamydia psittaci* rarely infect cagemates. However, these agents cause many LAIs.

The non-endogenous origin of an agent is more concerning because of the potential to transmit and spread from other countries to Saudi Arabia. The importation of human disease agents requires a permit from the PHA.

In the case of risk assessment for an agent with no sufficient information or the hazard of the unknown till final identification and typing of the agent, the specimen contains an unknown agent presenting the hazardous classification correlated with a minimum of BSL-2 containment. The epidemiological and experimental data are the primary source of information needed for risk assessment. Attenuated agents are less hazardous than the wild-type parent pathogens.

Laboratory procedures that carry major hazards are agent concentration, suspension volume, generation of small and large aerosols, airborne particles, and use of sharps. In the case of animals, the hazards are animal bites or scratches. Research shows that the probable source of infections is frequently not known.

3.2. Aerosols and droplets

Aerosols and droplets are a severe hazard in the laboratory because they are ubiquitous, undetected and extremely pervasive, resulting in high exposure risk for laboratory workers. The aerosols generated by procedures are the major source of LAIs. Procedures that use microbial suspension produce aerosols, and the equipment that handles infectious agents, such as pipettes, blenders, centrifuges, sonicates, vortex mixers, cell sorters, and MALDI-TOF, are the potential sources of aerosols. Laboratory procedures

and equipment can generate small airborne particles that pose inhalation and contaminationrisks. Experiments show that the aerosol burden with maximal aeration of the sonic homogenizer is approximately 200 times greater than that with minimal aeration. The hurried worker may operate a sonic homogenizer with maximum aeration; procedures and equipment that generate respirable size particles also generate larger size droplets that settle out of the air rapidly, contaminating hands, work surfaces, and possibly the mucous membranes of the persons performing the procedure Minimizing aerosol generation and assessing droplet contamination risks are crucial to minimize the risk of exposure to infectious agents.

Table 2. Laboratory activities associated with exposure to infectious agents

Routes of exposure/transmission	Laboratory activities/practices
Ingestion/oral	▪ Pipetting by mouth ▪ Splashing infectious material ▪ Placing contaminated material or fingers in mouth ▪ Eating, drinking, using lipstick or lip balm
Percutaneous inoculation/nonintact skin	▪ Manipulating needles and syringes ▪ Handling broken glass and other sharp objects ▪ Using scalpels to cut tissue for specimen processing ▪ Waste disposal (containers with improperly disposed sharps)
Direct contact with mucous membranes	▪ Splashing or spilling infectious material into eye, mouth, nose ▪ Splashing or spilling infectious material onto intact and nonintact skin ▪ Working on contaminated surfaces ▪ Handling contaminated equipment (i.e., instrument maintenance) ▪ Inappropriate use of loops, inoculating needles, or swabs containing specimens or culture material ▪ Bites and scratches from animals and insects ▪ Waste disposal ▪ Manipulation of contact lenses
Inhalation of aerosols	▪ Manipulating needles, syringes, and sharps ▪ Manipulating inoculation needles, loops, and pipettes ▪ Manipulating specimens and cultures ▪ Spill clean-up

3.3. Personal Protective Equipment (PPE) and Safety Equipment Hazards

Certain laboratory activities may involve hazards that require specific PPE, such as safety glasses, laboratory gowns, and gloves. For example,procedures with splash hazards require a mask and a face shield for adequate protection.Inadequate training in PPE use can reduce effectiveness and increase risk to workers.Safety equipment, like biological safety cabinets (BSCs), centrifuge safety cups, andsealed rotors, protects from microbial aerosols and droplets. Promptly addressing malfunctioning safety equipment is crucial, as issues like poor placement, reducedairflow, and improper user technique can increase safety risks.

3.4. Facility Control Hazards

Facility safeguards help prevent the accidental release of an agent from the laboratory. Directional airflow is dependent on the operational integrity of the laboratory's heating, ventilation, and air conditioning (HVAC) system. HVAC systems require careful monitoring and periodic maintenance to sustain operational integrity. Loss of directional airflow may compromise safe laboratory operation. BSL-4 containment facilities provide more complex safeguards requiring significant design and operation expertise. Risk assessment may support the need to include additional facility safeguards in constructing new or renovating old facilities.

The appropriate biosafety level and additional laboratory precautions must be chosen, along with a thorough understanding of the practices, safety equipment, and facility safeguards. The biosafety plan should address the Select Agent or Toxin risk and consider PHA guidelines. Individuals in the laboratory may differ in their susceptibility todisease, and equipment deficiencies should be corrected before starting work with an agent. Institutions must address risk perception by setting risk tolerance limits and performance expectations.

The safety of laboratory workers and the public depends on the workers themselves.The laboratory director or principal investigator should ensure that the workers haveacquired technical proficiency in microbiological practices and safety equipment.

Laboratory workers must be proficient in microbiological practices, identify hazards, use safety equipment, and obtain assistance. Evaluating workers'safety practice training, experience, and willingness to follow safety procedures is crucial for safe laboratory work.

The risk assessment should identify laboratory workers' knowledge, competency, and practice deficiencies. Carelessness and fatigue are serious concerns, and their adverse effects on safety have been well documented. Training, experience, knowledge of the agent and procedure hazards, good habits, caution, attentiveness, and concern for coworkers' health are prerequisites for laboratory staff to reduce the risks associated with hazardous agents. Laboratory directors or principal investigators should consider using competency assessment(s) to train and retrain new staff to the point where aseptic techniques and safety precautions become second nature.

3.5. Risk Communication

Maintaining a safety culture relies on effective communication with all stakeholders and reporting risk indicators, such as incidents and near misses, in a nonpunitive manner. An appropriate organizational and governance structure is essential to ensure compliance with biosafety, biocontainment, and laboratory biosecurity regulations and tocommunicate risks. The principal investigator or equivalent facility personnel are primarily responsible for communicating hazards and risks in the laboratory.

Staff should be able to report issues, including incidents and near misses, without fearof reprisal.

Additionally, laboratory staff, Institutional Biosafety Committees, and laboratory animal veterinarians should identify and communicate biological risks associated with laboratory work. Biosafety officers and other safety personnel can coordinate the institution's safety program and assist in developing risk communication documents, SOPs, biosafety manuals, hazard control plans, and emergency response plans. Changes in the biosafety program that promote a safety culture should be effectively communicated across the institution using multiple communication routes to ensure that all staff are informed. Good communication practices include messages from leadership, risk management documents, IBCs or equivalent resources, and other committee reviews as necessary.

3.6. Enhancing a Safety Culture Among Workers through Risk Assessment

Biological materials are stored between when they are collected and when. RA must address all realistic risks to protect people, the community, and the environment. Changes in research, staff, and regulations will require reconsiderationof all factors. RA is an

ongoing process, and everyone plays a role in its success. The goal is to develop good habits and procedures through training and competency checks with support from leadership. Effective risk communication is crucialfor identifying hazards and successful implementation. While policies and plans are essential outcomes of the risk assessment process, the accurate measure of success will be establishing, reinforcing, and maintaining a safety culture and promoting communication about risks between management and staff. Regularly reviewing hazards, prioritizing risks, conducting multidisciplinary reviews, andestablishing risk mitigation measures demonstrate the institution's commitment to a safeworking environment. The approach to risk assessment outlined needs to be fixed and benefits from active participation by all stakeholders. Aim for ongoing evaluation and adjustments to protect everyone from potential exposure to biological materials in laboratories and facilities.

3.7. Advancement of National Biosafety Measures

From 1944 to 1969, organizations such as WHO and CDC developedseveral national biosafety guidelines. In 1974, the CDC published the Classification of EtiologicAgents Based on Hazard, establishing ascending levels of containment that correspond to risks associated with handling infectious microorganisms with similar hazardouscharacteristics.

During the late 20th century, improvements in personal protective equipment (PPE), laboratory design, and biosafety protocols significantly reduced the incidence of LAIs. Despite that, these were the 1976 Ebola virus infection in a laboratory worker in England and the 1978 smallpox infection in a Birmingham laboratory. Biosafety in Microbiological and Biomedical Laboratories (BMBL): The BMBL manual, first published by the US CDC and NIH in 1984, provides comprehensive guidelines for laboratory safety and is regularly updated. In the 21st century, the focus on bioterrorism and emerging infectious diseases further emphasized the importance of laboratory safety. Therefore, stricter regulations and more sophisticated biosafety practices were applied. Genetic engineering and synthetic biology have also emerged with new challenges and risks associated with LAIs, necessitating updated biosafety protocols and training.

Globally, organizations like the WHO and the International Health Regulations (IHR) framework promote international cooperation in managing laboratory biosafety and biosecurity.

4. Biosafety

A biosafety program's main goal is to contain potentially hazardous biological effectively agents and toxins. This involves using a combination of primary and secondary barriers, facility practices and procedures, and safety equipment, including PPE, to manage the risks associated with handling and storing hazardous materials in a laboratory setting. The overall objective of containment is to minimize the risk of staff exposure and the unintentional release of hazardous biological agents or toxins into the community. It is crucial to make well-informed decisions about the combination of containment measures required to address biosafety risks at a facility. These decisions should be based on a comprehensive biosafety RA and ongoing to address evolving risks in the laboratory environment.

Management and leadership, with support from the facility's biosafety professionals and other health and safety personnel, are responsible for performing and reviewing the RA using the best available information. They must evaluate the risks and select appropriate mitigation measures. All individuals within the institution are accountable for conducting their work to ensure the successful implementation and performance of the safety measures identified in the RA and review.

4.1. Biosafety Program:

Biosafety involves consistently applying safety measures to minimize or preventharm to laboratory personnel, building occupants, the public, the animal population, and the environment resulting from exposure to infectious material, infected animals, or toxins handled in a containment zone.

A biosafety program comprises institutional plans and policies for the safe handling and storage of infectious materials and toxins, and to prevent the release of infectious materials from containment zones. Key elements of the biosafety program are but limited to a comprehensive training program, a medical surveillance program, an emergency response plan (ERP), SOPs adhering to safe work practices, and a biosecurity plan. A functional and practical biosafety program covers all components relevant to specific laboratory work areas, large-scale production areas, or animal work areas. It involves essential safety measures such as good microbiological practices, suitable primary containment equipment, and proper physical design of containment zones. With

increasing public awareness, there's a focus on preventing the misuse of pathogens and toxins, highlighting the importance of integrating biosecurity into every biosafety program.

4.2. Safety Equipment (Primary Barriers)

The primary barrier, also known as the primary containment, refers to the physical measures at the hazard level to protect personnel, the surrounding community, and the environment from potential exposure to hazardous biological agents and toxins. This can include safety equipment such as BSCs, enclosed containers, and other biosafety controls. There are three primary types of BSCs (Class I, II, III) used in laboratory facilities, and the selection of the appropriate BSC should be based on the risks identified for each respective laboratory.

Other primary containment devices are rotors and centrifuge safety cups designed to contain aerosols, droplets, and leakage of hazardous biological agents andtoxins that may occur during certain activities like centrifugation. Sealed containers provide containment for transfers between laboratories within and between facilities within a laboratory, depending upon risk assessment. The appropriate primary containment device should be selected based on the risks identified for activities likely to produce aerosol droplets or potentially leak hazardous biological agents and toxins.

PPE ensures laboratory personnel's safety. It serves as a vital barrier against a wide range of hazards, including physical, electrical, heat, noise, and chemical dangers, as well as biological and airborne risks. PPE encompasses a variety of gear such as gloves, coats, gowns, footwear,respirators, face shields, safety glasses, goggles, and ear plugs, all of which play a crucial role in safeguarding against potential threats. When used in conjunction with other biosafety controls like biological safety cabinets, PPE becomes the frontline defense, especially in settings where such cabinets are not accessible. This includes scenarios such as fieldwork, resource-limited environments, animal studies, necropsy, and various laboratory operations. Careful consideration and selection of appropriate PPE based onspecific risks in each laboratory setting is paramount to ensuring the well-being of personnel and the integrity of the research environment.

Secondary barriers may become primary in situations like working with large animals.

In such cases, where traditional primary barriers (e.g., BSC) are lacking, the facility becomes the primary barrier. In these cases, the facility may require additional engineering controls and precautions (e.g., HEPA filtration on the exhaust air) to mitigate the risks posed to personnel, the surrounding community, and the environment.

4.3. Facility Design and Construction (Secondary Barriers)

The laboratory facility is designed to provide secondary containment for hazardous biological agents and toxins. This is to ensure the safety of personnel, the surrounding community, and the environment from potential exposure to these hazards.In situations where there is a risk of infection through aerosol or droplet exposure, additional levels of secondary containment and multiple primary barriers may be used along with other controls to minimize the risk of exposure to personnel and to prevent unintentional release of hazardous agents into the environment. Design features aimed at achieving these goals may include, but are not limited to, the following:

- Ventilation strategies to ensure containment of the hazards;
- Effluent decontamination systems and
- Specialized building/suite/laboratory configurations, including:
- Controlled access zones to support the separation of the
- laboratory from office and public spaces;
- Anterooms; and
- Airlocks.

Design engineers may refer to specific ventilation recommendations in the national guidelines for the Laboratory Design Guide. Please note that depending on the laboratory facility, design professionals may need to follow international design recommendations andrequirements such as:

- The NIH Design Requirements Manual (DRM);
- The WHO Laboratory Biosafety Manual;
- World Organization for Animal Health (OIE) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals; and/or
- Other similar national or international design reference documents.

4.3.1. Facility Practices and Procedures

Establishing and maintaining facility-specific best practices and procedures for a successful biosafety program is crucial. Personnel need training on safe practices and

procedures. Management is responsible for providing appropriate training to personnel for the biosafety program. Adherence to documented laboratory best practices and procedures is critical, as failure to follow the established procedures could result in accidental exposure or release of hazardous biological agents. All facilities should develop and implement a biosafety program that identifies hazards and specifies risk mitigation strategies. Management holds overall responsibility for work conducted in the laboratory facilities. If safety practices are inadequate to minimize risks associated with a particular hazardous biological agent, additional risk mitigation measures may be necessary. Safety best practices and other components of the overall biosafety program must be developed and implemented.

4.4. Biosafety Levels

The four primary BSLs for laboratories are described previously. They include facility design features, safety equipment (primary and secondary barriers), facility practices and procedures, and personal protective equipment. Selecting the appropriate combinations based on a comprehensive biosafety risk assessment specific to the facility is essential. This assessment should document the properties of the biological agents and toxins to be used, potential host characteristics, potential routes of infection, and the laboratory work practices and procedures to be conducted.

This section does not encompass all biological agents and toxins capable of causing human disease. When working with well-defined organisms, identifying appropriate biosafety controls should be based on a comprehensive risk assessment. However, if information suggests significant alterations in virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors, an adjustment to the biosafety controls may be necessary. For example, handling large volumes or high concentrations of a biological agent or toxin may require additional practices outlined in international guidelines stated above. Similarly, procedures that produce large amounts of aerosols may also require additional biosafety controls to reduce the likelihood of exposure to personnel and the unintentional release of a biological agent or toxin into the surrounding community or environment.

Additionally, it is crucial to understand that the four Biosafety Levels should not be confused with Agent Risk Groups as described in the National Institutes of Health Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH

Guidelines). An agent's Risk Group (RG) is essential during the biosafety risk assessment process. Biological agents and toxins are assigned to their relevant Risk Groups based on their ability to cause disease in healthy human adults and spread within the community.

4.4.1. Biosafety Level I

BSL-1 practices, safety equipment, and facility requirements are suitable for undergraduate and secondary educational training laboratories and other labs working with well-defined and characterized strains of biological agents that are unknown to cause disease in healthy adult humans. These agents may include *Bacillus subtilis*, *Naegleria gruberi*, and infectious canine hepatitis virus. BSL-1 involves standard microbiological practices and procedures with no specific primary or secondary barriers other than a door, a handwashing sink, and non-porous work surfaces that are easily clean and decontaminated.

4.4.2. Biosafety Level II

BSL-2 standard includes practices, safety equipment, and facility specifications for laboratories involving a wide range of biological agents and toxins thatcan cause human diseases such as hepatitis B virus, human immunodeficiency virus (HIV), *Salmonella, and Toxoplasma.* These agents and toxins can be handled safely on an open bench with good practices and procedures if the potential for creating splashes and aerosols is low. Work with human, animal, or plant-derived specimens (such as blood, body fluids, tissues, or primary cell lines) where a biological agent or toxin may be unknown can usually be conducted under conditions associated with BSL-2. Personnel working with human-derived materials should refer to the OSHA Bloodborne PathogensStandard for specific required precautions. The main routes of exposure for personnel working with these types of biological agents and toxins are accidents, including exposure via the skin, mucous membranes, and ingestion of potentially infectious materials. Caution should be taken with contaminated needles and other sharp materials.

Primary containment equipment is also recommended when high-risk infectious agents are suspected in human, animal, or plant-derived specimens. The appropriate personal protective equipment should be selected based on the risks identified for each respective laboratory. Secondary barriers should include those previously mentioned for BSL-1. Waste decontamination capabilities should be available to reduce the potential of environmental contamination, and laboratory spaces should be separated from office and

public spaces to reduce the risk of exposure to other personnel.

4.4.3. Biosafety Level III

BSL-3 standards apply to laboratories where work involves using indigenous or exotic biological agents capable of respiratory transmission and causingsevere and potentially lethal infections. Examples of such biological agents include *Mycobacterium tuberculosis, St. Louis encephalitis virus, and Coxiella burnetii*. Personnel working with these agents are primarily exposed through accidental percutaneous or mucosal contact and inhalation of infectious aerosols. At BSL-3, there is a heightened focus on using primary and secondary barriers to protect personnel, the community, and the environment from exposure to infectious aerosols. All manipulation of infectious materials is carried out within a BSC or other primarycontainment device, and no open vessel work is done on the bench. When a procedurecannot be performed within a BSC, a combination of personal protective equipment andother primary containment strategies is implemented based on a risk assessment. Therotors and centrifuge safety cups are loaded and unloaded within the BSC or another containment device. Secondary barriers for BSL-3 laboratories include those mentioned for BSL-1 and BSL-2 laboratories, enhanced ventilation strategies to ensure inward directional airflow and controlled access zones to limit access to only laboratory-approved personnel. These areas may include anterooms, airlocks, exit showers, and exhaust HEPA filtration.

4.4.4. Biosafety Level IV

BSL-4 practices, safety equipment, and facility specifications are designed for laboratories dealing with dangerous and rare biological agents that can cause life-threatening diseases. These agents can be transmitted via aerosols and do not have available vaccines or therapies. Examples of such agents include the Marburg virus and the Congo-Crimean hemorrhagic fever virus. Until sufficient data are available to confirm a lower level of containment, biological agents closely related to those requiring BSL-4 containment should be handled at this level. Personnel working with these biological agents are primarily at risk of exposure through accidental contact with skin,mucous membranes, or inhalation of infectious aerosols. To protect against exposure toinfectious aerosols, laboratory workers work in a Class III or a Class II BSC with a full-body, air-supplied positive-pressure personnel suit. Additionally, BSL-4 laboratoriesshould have the same secondary barriers as previous Biosafety Levels. The facility should be a separate

building or an isolated zone with specialized ventilation systems and waste management to prevent the release of hazardous biological agents into the environment.

4.5. Biosafety Cabinets

The terms BSC and biosafety cabinet have been extensively used to describe a variety of containment devices equipped with HEPA filter(s), designed to provide personnel or both personnel and product protection from pathogenic materials. The terms should only be applied to those devices that meet the requirements of Class I, II, or III specifications, based on their construction, airflow velocities and patterns, and their exhaust systems. The BSC is a fragile, precision piece of equipment intended for protecting the user from airborne aerosols that may cause infection. Table 3 represents the different types of BSCs.

Table 3. The different types of BSCs.

Class	Inflow Velocity (m/s)	Recycle Air (%)	Exhaust Air (%)	Control Plenum Surrounded by	Exhaust Alternatives	Biosafety Level
Type I	US: 0.38 EN: 0.70	0	100	Outside Air	Inside room/Hard duct	1,2 & 3
Type II A1	US: 0.38 EN: 0.40	70	30	Outside Air	Inside room/Thimble duct	1,2 & 3
Type II A2	US: 0.50 EN: 0.40	70	30	Negative Plenum	Inside room/Thimble duct	1,2 & 3
Type II B1	US: 0.50 EN: 0.40	30	70	Negative Plenum	Hard duct only	1,2 & 3
Type II B2	US: 0.50 EN: NA	0	100	Negative Plenum	Hard duct only	1,2 & 3
Type III	Closed: * >0.5"WC	0	100	Negative Plenum	Inside room/Hard duct	1,2,3 & 4

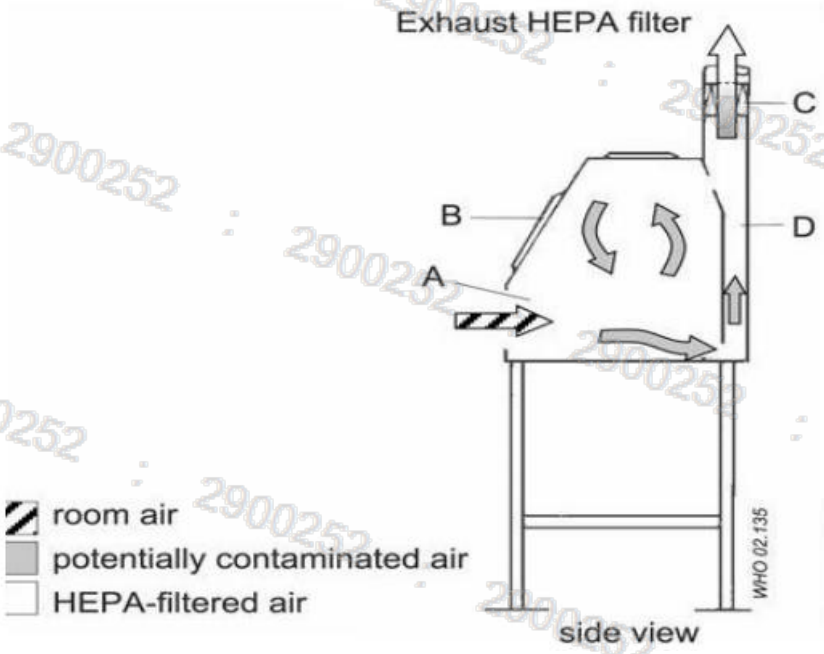
*Pressure differential between chamber and environment

4.5.1. Class I Biological Safety Cabinets

The Class I cabinet has the most basic and rudimentary design of all biological safety cabinetry available today. A stream of inward air moving into the cabinet contains aerosols generated during microbiological manipulations. It then passes through a filtration system that traps all airborne particles and contaminants. Finally, clean, decontaminated air is exhausted from the cabinet. The filtration system usually consists of a pre-filter and a High

Efficiency Particulate Air (HEPA) filter. Although the Class I (figure 2) cabinet protects the operator and the environment from exposure to biohazards, it does not prevent samples being handled in the cabinet from coming into contact with airborne contaminants that may be present in room air. Naturally, there is a possibility of cross-contamination that may affect experimental consistency. Subsequently, the scope and application of Class I cabinets is limited and it is largely considered obsolete. All Class I biological safety cabinets are suitable for work with microbiological agents assigned to biosafety levels 1, 2 and 3. Figure 1 below shows the airflow of Class I BSCs.

Figure 1. Schematic representation of a Class I biological safety cabinet. A: front opening, B: sash, C: exhaust HEPA filter, D: exhaust plenum.



4.5.2. Class II Biological Safety Cabinets

Like Class I safety cabinets, Class II cabinets have a stream of inward air moving into the cabinet. This is known as the inflow and it prevents the aerosol generated during microbiological manipulations to escape through the front opening. However, unlike Class I cabinets, the inflow on Class II cabinets flows through the front inlet grille, near the operator. None of the unfiltered inflow air enters the work zone of the cabinet, so the product inside the work zone is not contaminated by the outside air. A feature unique to Class II cabinets is a vertical laminar (unidirectional) HEPA-filtered air stream that descends downward from the interior of the cabinet. This continuously flushes the cabinet interior of airborne contaminants

and protects samples being handled within the cabinet from contamination and is known as the downflow.

The differences between the various Class II cabinets available lie primarily with the percentage of air exhausted to that of air re-circulated from the common air plenum. In addition, different Class II cabinets have different means of cabinet exhaust. Some cabinets may exhaust air directly back to the laboratory, while others may exhaust air through a dedicated ductwork system to the external environment. Despite these differences, all Class II cabinets, like Class I cabinets, protect both the operator and environment from exposure to biohazards. In addition, Class II cabinets also protect product samples from contamination during microbiological manipulations within the cabinet interior and are all suitable for work with agents assigned to biosafety levels 1, 2 and 3.

4.5.3. Class II Type A (A1/A2) Biological Safety Cabinets

The Class II Type A biological safety cabinet is the most common Class II cabinet. It is also the most common safety cabinet of all the different types available. It has a common plenum from which 30% of air is exhausted, and 70% re-circulated to the work area as the downflow.

Type A cabinets exhaust air directly back to the laboratory, and they may contain positive pressure contaminated plenums. When toxic chemicals must be employed as an adjunct to microbiological processes, these cabinets should not be used. Exhaust HEPA filtration only removes airborne aerosols including biohazards, and not chemical fumes.

The Class II Type A1 (figure 2) has the positively-pressurized contaminated plenum bordering the ambient environment, and therefore is less safe than the Class II Type A2 (figure 3) that has a negative pressure surrounding the positively pressurized contaminated plenum. In case there is a leakage on the positive plenum, the leaking aerosol will be pulled by the negative pressure back to the positive plenum, and it will not leak out. Because of the safety issue, the Type A1 design is now considered obsolete. In the A2 cabinet, about 70% of air from the positive plenum is recirculated as downflow, and the remaining 30% is discharged to the lab through the exhaust filter.

A Class II A2 cabinet must have a minimum inflow velocity of 100 ft/min, allowing it to be used for volatile chemicals adjunct to microbiological studies, if properly exhausted outdoors via a canopy exhaust connection (figure 3). In other respects, the specifications are identical to those of a Type A1.

Figure 2. Schematic representation of a Class II (left) A1 biological safety cabinet. A: front opening, B: sash, C: exhaust HEPA filter, D: supply HEPA filter, E: common plenum, F: blower and (right) e A2 BSC (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) positive pressure common plenum; (F) negative pressure plenum. Note: The A2 BSC should be canopy connected to the exhaust.

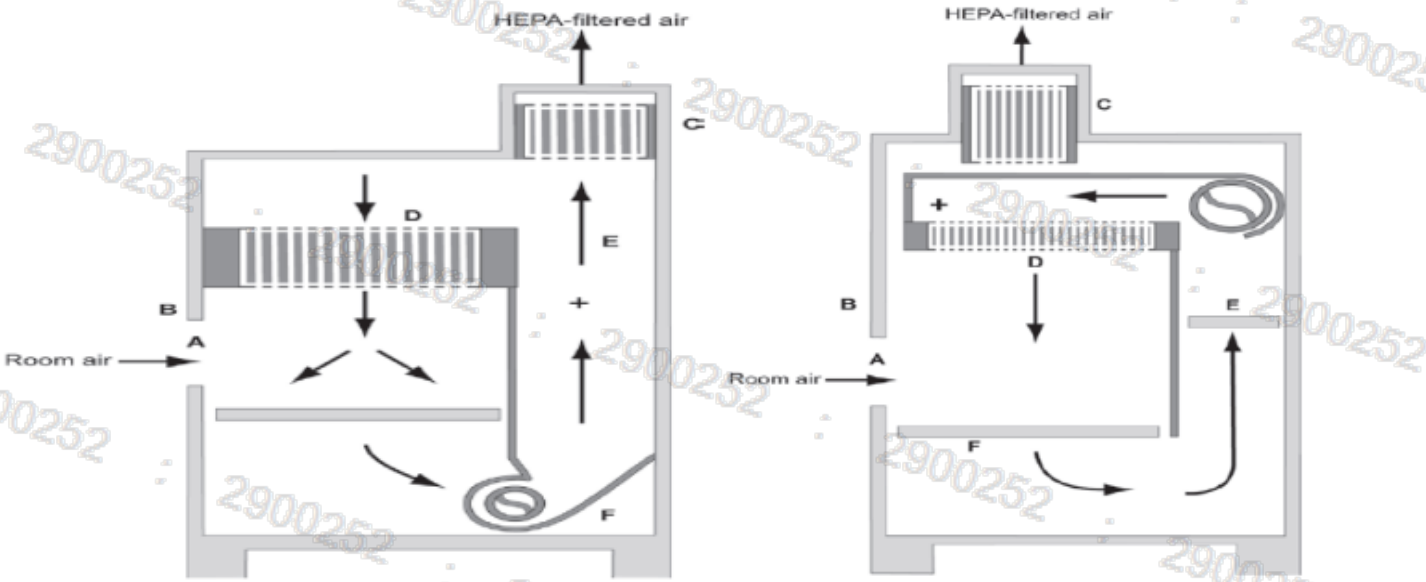
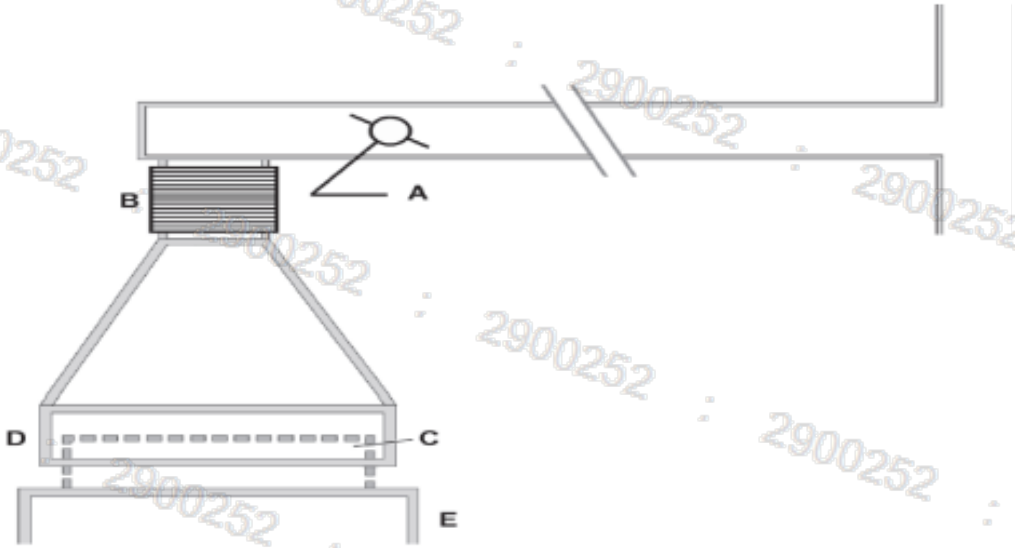


Figure 3. Canopy (thimble) unit for ducting a Class II, Type A BSC (A) balancing damper; (B) flexible connector to exhaust system; (C) cabinet exhaust HEPA filter housing; (D) canopy unit; (E) BSC. Note: There is a 1" gap between the canopy unit (D) and the exhaust filter housing (C), through which room air is exhausted.



4.5.4. Class II Type B Biological Safety Cabinets

The main difference between Type A and Type B cabinet (table 5) is: Type B cabinets must be operated with an external blower and it exhausts air to the external environment via a dedicated ductwork system. Without the external blower, the cabinets internal blower will blow the air (and microbiological agents) inside the work zone through the front opening, towards the operators face, creating a dangerous situation. This cabinet is not self-balancing, in the sense that its own blower can only create downflow, and the cabinet relies on the external blower to create inflow.

On all Type B cabinets, environmental protection may be enhanced by installing a scrubbing system between the exhaust of the cabinet and the final exhaust point outside the building to neutralize the chemical fumes present in exhaust air.

Although Type B cabinets are commonly used when chemicals are involved in your work processes, they theoretically provide an increased level of safety as compared to other Type A cabinets. By exhausting air directly to the external environment, they provide an additional "fail-safe" in the event that the regular exhaust HEPA filtration ceases to function.

- Class II Type B1 Biological Safety Cabinets

The Class II Type B1 (figure 4 left) biological safety cabinet was originally specified by the American National Cancer Institute. It has a common plenum from which 70% of air is exhausted, and 30% re-circulated to the work area as the downflow.

Type B1 cabinets also have a dedicated exhaust feature that eliminates re-circulation when work is performed towards the back within the interior of the cabinet. Toxic chemicals employed as an adjunct to microbiological processes should only be employed if they do not interfere with work when re-circulated in the downflow.

- Class II Type B2 Biological Safety Cabinets

In the Class II Type B2 (figure 4 right) cabinet all inflow and downflow air is exhausted after HEPA filtration to the external environment without recirculation within the cabinet. Type B2 cabinets are suitable for work with toxic chemicals employed as an adjunct to microbiological processes under all circumstances since no re-circulation occurs. In theory, Type B2 cabinets

may be considered to be the safest of all Class II biological safety cabinets since the total exhaust feature acts as a fail-safe in the event that the downflow and / or exhaust HEPA filtration systems cease to function normally. However, Class II Type B2 cabinets are, in practice, difficult to install, balance and maintain. Table 4 shows the comparison between Class II type A2 and B2 BSCs.

Figure 4. The Class II BSC. (left) type B1 BSC (classic design) (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) negative pressure dedicated exhaust plenum; (F) blower; (G) additional HEPA filter for supply air. Note: The cabinet exhaust needs to be hard connected to the building exhaust system whereas (right), The Class II, Type B2 BSC (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) negative pressure exhaust plenum. Note: The carbon filter in the exhaust system is not shown. The cabinet needs to be hard connected to the building exhaust system.

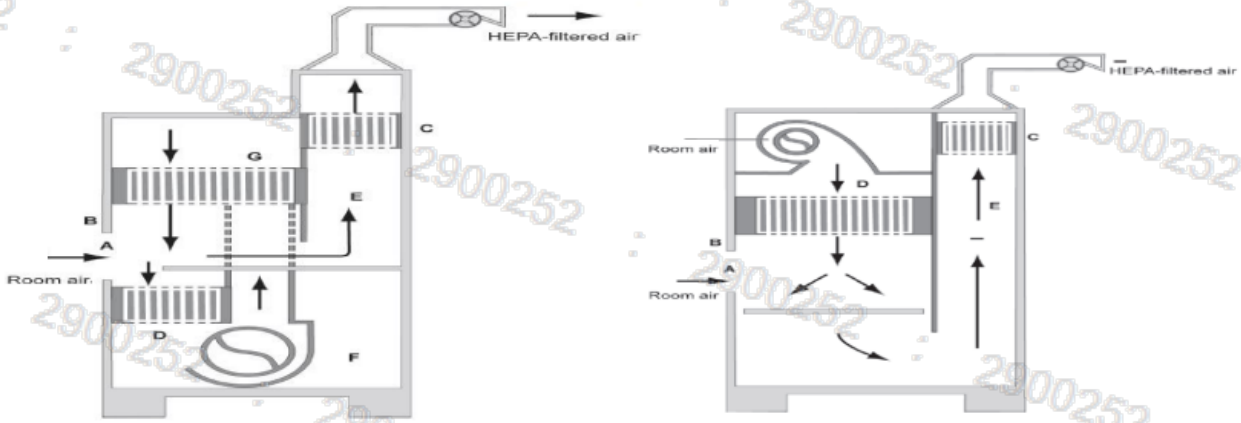


Table 4. Comparison between Class II, type A2 and B2 BSCs.

Characteristic	Class II, Type B2 BSC	Class II, Type A2 BSC
Airflow pattern	No recirculation within work area. 100% total flow is exhausted.	Approximately 70% of air is recycled and approx. 30% exhausted
Exhaust system type	Must be direct ducted as per NSF	Can have three types of exhaust: 1.Recirculating — filtered exhaust into room 2.Thimble-type duct 3.Direct duct (only EN12469, NSF 49 does not allow direct duct for A2 type BSC)
Inflow velocity	≥100 FPM (NSF 49)	≥ 100 FPM (NSF 49) ≥ 0.40 m/s (EN12469)
Downflow velocity	Not defined	Not defined (NSF 49) 0.25–0.50 m/s (EN12469)
Recognized by EN12469	No	EN12469 only recognizes 1 type of Class II BSC, which is very similar in design to an NSF 49 A2 type BSC

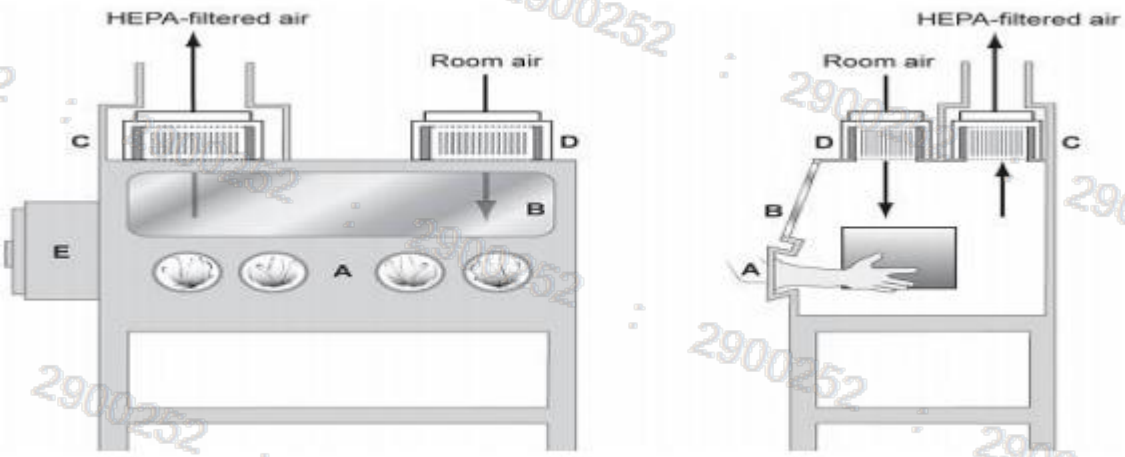
4.5.5. Class III Biological Safety Cabinets

The Class III biological safety cabinet provides an absolute level of safety, which cannot be attained with Class I and Class II cabinets. All Class III cabinets are usually of welded metal construction and are designed to be gas tight. Work is performed through glove ports in the front of the cabinet. During routine operation, negative pressure relative to the ambient environment is maintained within the cabinet. This provides an additional fail-safe mechanism in case physical containment is compromised.

On all Class III cabinets (figure 5), a supply of HEPA filtered air provides product protection and prevents cross contamination of samples. Exhaust air is usually HEPA filtered and incinerated. Alternatively, double HEPA filtration with two filters in series may be utilized. Materials are transferred into the cabinet using a pass-through unit installed at the side of the work area. Class III cabinets usually exhaust air back to the laboratory; however, air may also be exhausted via a dedicated ductwork system to the external environment. When a dedicated ductwork system is employed, they are also suitable for work employing toxic chemicals as an adjunct to microbiological processes.

All Class III biological safety cabinets are suitable for work with microbiological agents assigned to biosafety levels 1, 2, 3 and 4. They are frequently specified for work involving the most lethal biological hazards.

Figure 5. The Class III BSC. (A) glove ports with O-ring for attaching arm-length gloves to cabinet; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) double-ended autoclave or passthrough box. Note: A chemical dunk tank may be installed which would be located beneath the work surface of the BSC with access from above. The cabinet exhaust needs to be hard connected to an exhaust system where the fan is generally separate from the exhaust fans of the facility ventilation system. The exhaust air must be double HEPA-filtered or HEPA-filtered and incinerated.



Warning: In case of power failure, infectious particles are no longer trapped to the HEPA filter and flow back to the open front of the BSC, constituting a major biohazard for personnel.

4.6. Animal Facilities

Four primary BSLs describe activities involving hazardous biological agents and toxin work conducted with animals. These levels are designated as Animal Biosafety Levels (ABSL) 1, 2, 3, and 4, and they provide increasing protection to personnel, the surrounding community, and the environment. Additionally, another Biosafety Level called Animal Biosafety Level 3-Agriculture (ABSL-3Ag) specifically addresses activities involving hazardous biological agents and toxins. ABSL-3Ag laboratories are designed so that the laboratory building itself serves as a primary barrier to prevent the unintentional release of these high-consequence agents into the environment.

4.7. Clinical Laboratories

Clinical laboratories regularly handle unknown specimens and specimens that may be infected with multiple pathogens. As a result, the occupational risks in a clinical laboratory setting are different from those in research or teaching laboratories. Most public and animal health clinical laboratories utilize BSL-2 facilities,engineering, and biosafety practices. Personnel in clinical diagnostic laboratories may need to be made aware of the infectious agents or other hazards in the specimens they handle and process.

4.8. Laboratory Biosecurity

Biosafety protects personnel, the community, and the environment from theaccidental release of dangerous biological agents and toxins. On the other hand, laboratory biosecurity focuses on preventing the theft, loss, or misuse of hazardous biological agents, equipment, and information for malicious purposes. A comprehensive containment strategy should encompass biosafety and laboratory biosecurity to address the facility's risks effectively. More details about the laboratory biosecurity is shown in section 5 below.

4.9. Biosafety Program Management

A biosafety program is designed to prevent infections, intoxications, and illnesses among personnel and the community and to protect the environment from harm by preventing the release of regulated materials from containment. The level of detail and complexity of the biosafety program will depend on the nature of the organization (i.e., size, structure,and complexity) and the activities performed within it. An effective biosafety program requires a strong commitment and involvement by all institution`s levels, including senior management, supervisors, and personnel. The day-to-day management of the biosafety program can be determined internally, and responsibilities can be delegated accordingly. The biosafety program management in each biosafety level is illustrated in table 5.

Table 5. Biosafety Program Management

Biosafety Program Management	
A biosafety program is to be developed, documented, implemented, kept up to date, and evaluated and improved as necessary.	All Biosafety levels
A biosafety representative (i.e., BSO) with the knowledge appropriate for the containment levels and regulated materials handled to be designated for the oversight of biosafety and biosecurity practices, including: a) Verifying the accuracy and completeness of applications for legislative documents, as applicable; b) Communicating with the PHAC and the CFIA, as applicable; c) promoting and monitoring compliance with applicable legislation (including the HPTA, HPTR, HAA, and HAR), conditions of license and terrestrial animal pathogen import permits, the biosafety manual, and SOPs, which includes, but is not limited to: i. arranging and documenting appropriate biosafety and biosecurity training for personnel, as applicable; ii. conducting periodic inspections and biosafety audits and reporting the findings, as applicable; and iii. Informing the license holder and/or terrestrial animal pathogen import permit holder, as applicable, in writing of any non-compliance by a person working with regulated materials that that person is not correcting after they have been made aware of it; c) Assisting in the development and maintenance of SOPs and the biosafety manual; and d) Assisting with internal investigations of incidents.	All Biosafety levels
Where non-indigenous terrestrial animal pathogens are handled or stored, changes in program intent and changes to the facility's physical structure, equipment, or SOPs that could affect biocontainment must be submitted to the CFIA for approval before implementation.	BSL3, BSL4
An overarching risk assessment is to be conducted, documented and updated.	All Biosafety levels
A biosecurity risk assessment is to be conducted, documented and kept up to date.	All Biosafety levels
An LRA will be conducted, documented, and updated on regulated materials activities.	All Biosafety levels
A biosafety manual to be developed, documented, implemented, followed, kept up to date, and communicated and made available to authorized personnel. A biosafety manual includes a description of the: • Institutional biosafety policies, programs, and plans; • Physical design and operation of the containment zone and systems; • Overarching, biosecurity, and local risk assessments; • Biosecurity plan;	All Biosafety levels

• Medical surveillance program; • Training program; • ERP and incident reporting procedures; • Housekeeping program; • Facility and equipment maintenance program for components of the containment zone, including integrity testing of primary containment devices and • SOPs for safe work practices specific to the containment zone.	
A biosecurity plan is to be developed, documented, implemented, followed, evaluated, kept up to date, communicated, and made available to authorized personnel. A biosecurity plan describes: • Risks associated with activities and assets with dual-use potential; • Physical security; • Personnel security; • Accountability and defined accountability measures for regulated materials; • Inventory control; • Incident and emergency response; • Information management, and • Biosecurity training and awareness.	All Biosafety levels
A medical surveillance program based on an overarching risk assessment and LRAs to be developed, documented, implemented, followed, and updated.	All Biosafety levels
SOPs for operational practices and performance and verification testing are to be developed, documented, implemented, followed, kept up to date, communicated, and made available to authorized personnel.	All Biosafety levels

4.10. Training Program

Training is a core element of biosafety and biosecurity and is essential to the success of the biosafety program. Personnel must be knowledgeable about the hazards inthe work environment and the practices, tools, and equipment that can protect them from exposure to regulated materials and prevent their release from containment. The training program may encompass education (i.e., theoretical knowledge), training (i.e., practical skill development), and supervision of personnel until they have demonstrated knowledge of the information and proficiency in the procedures on which they were trained. The training program is detailed in table 6 below.

Table 6: Training Program

Training Program
A training needs assessment to be conducted, documented, kept up to date, and reviewed annually.
Based on a training needs assessment, a training program is to be developed, documented, implemented, kept up to date, and evaluated and improved as necessary. A training program includes training on: • SOPs and relevant elements of the biosafety manual; • Potential hazards associated with the regulated materials handled; • Signs and symptoms of diseases associated with the regulated materials handled; • Necessary precautions to prevent exposure to, or the release of, regulated materials handled; • Necessary precautions to prevent biosecurity incidents involving regulated materials or related assets (e.g., sensitive information); • Relevant physical design and operation of the containment zone and containment systems; • Correct use and operation of laboratory equipment, including primary containment devices; • Restraint and handling techniques for work involving animals, and • Emergency response procedures (including annual refresher training).
Visitors, maintenance and janitorial staff, contractors, and others who require temporary access to the containment zone must be trained and accompanied by authorized personnel for their anticipated activities.
Personnel must demonstrate knowledge of the relevant elements of the biosafety manual and proficiency in the procedures on which they were trained before engaging in unsupervised activities with regulated materials and regulated animals.

4.11. Biosafety level 1

4.11.1. Introduction

BSL-1 laboratories provide a fundamental framework for the safe handling of Risk Group 1 (RG1) biological materials. These microorganisms are not known to cause disease in healthy adults, and the safety measures at this level are designed to protect laboratory personnel and the environment from any minimal risks posed by these agents. BSL-1 containment practices serve as the foundation for higher-level biosafety protocols, ensuring that both personnel and environmental safety are prioritized. The implementation of biosafety in BSL-1 laboratories is primarily achieved through the integration of structural and operational controls. The physical design of the laboratory, including functional workspaces and suitable infrastructure, is complemented by basic operational practices such as the adherence to standard microbiological techniques. These measures are critical in minimizing the risk of accidental exposure or contamination.

BSL-1 containment practices are foundational to all biosafety systems and typically include standard microbiological techniques performed on open benchtops. Facilities are designed to be functional yet do not require specialized containment equipment, such as biosafety cabinets or advanced PPE. This makes BSL-1 laboratories ideal for educational and research environments, where routine procedures involve non-pathogenic organisms. Personnel in BSL-1 facilities must be trained in the specific procedures and organisms they are handling. Oversight is provided by scientists with expertise in microbiology or related disciplines, ensuring adherence to safety protocols even though the biological materials present minimal risk.

4.11.2. Biosafety level 1 guidelines in the KSA for RG1 biological materials

The WHO has established a risk-based approach to biosafety, which aligns with Saudi Arabia's public health priorities. The WHO's Laboratory Biosafety Manual emphasizes that each laboratory should tailor its biosafety practices according to local RAs, ensuring that safety measures correspond to the real risks posed by the biological agents in use. This approach allows countries to develop sustainable and context-specific biosafety frameworks that maximize resource use while ensuring public and environmental safety.

For the KSA, ensuring the safe operation of BSL-1 laboratories is essential for supporting public health and research initiatives. These laboratories are crucial for non-pathogenic microbiological studies, vaccine production, and other biomedical applications that require a low-level containment. By adopting international standards, such as those recommended by the CDC and WHO, Saudi Arabia ensures that its biosafety regulations align with global best practices, thereby protecting both personnel and the broader community.

In summary, BSL-1 laboratories, while handling minimal-risk agents, play a key role in public health and research. The adoption of internationally recognized biosafety guidelines ensures that these laboratories operate safely and efficiently, in line with the Public Health Authority's commitment to maintaining high standards of health and safety across the Kingdom. According to international guidelines, safety measures in BSL-1 laboratories rely on strict operational practices. Further details are included in the table 7 below.

Table 7. Detailed BSL-1 laboratory facilities and practices.

Feature	Description
Laboratory Separation	Work areas, including laboratory, large-scale production, and animal work areas, are separated from public and administrative spaces by lockable doors. This ensures controlled access to areas where biological materials are handled, limiting potential exposure to unauthorized personnel.
Dedicated Workstations	Paperwork and computer workstations are located in separate, dedicated areas away from benches or spaces where RG1 biological materials or animals are handled. This separation helps prevent contamination of sensitive materials, ensuring a safer workflow.
Access Control	Doors control access to laboratories, restricting entry to authorized personnel only. Access is granted based on clearance levels appropriate for the work being conducted with RG1 biological materials.
Personal Protective Equipment (PPE) Storage	Designated areas for PPE storage are provided within the lab. PPE worn during work with RG1 materials is stored separately from personal clothing and other items to prevent cross-contamination.
Handwashing Stations	Sinks for handwashing are conveniently located within the laboratory. Personnel are required to wash their hands frequently, especially after handling RG1 biological materials or removing gloves.
Eyewash Stations	Eyewash stations are installed in laboratories, easily accessible in case of accidental exposure to hazardous materials. These stations must be kept clear and functional at all times.
Cleanable Surfaces	Floors, walls, benchtops, and furniture in the laboratory are designed to be easily cleanable, non-absorbent, and resistant to damage from disinfectants and decontamination procedures. This ensures a safe working environment that can be effectively sanitized.
Non-Porous Furniture	Furniture, including chairs used in the laboratory, are covered with non-porous materials. This allows for easy cleaning and decontamination after exposure to RG1 biological agents. Carpets and rugs are prohibited in the laboratory to prevent contamination.
Windows and Pest Control	Laboratory windows that open to the exterior are fitted with screens to prevent entry of pests. These screens are part of the essential pest control measures that ensure a clean and safe environment for laboratory work.
Illumination	Adequate lighting is provided for all activities conducted in the laboratory, ensuring visibility without causing glare or reflections that might interfere with the safety of personnel.
Work Area Separation	Laboratory work areas, particularly those dealing with RG1 materials, are kept separate from animal housing rooms. This prevents cross-contamination between biological materials and animal areas.
Animal Escape	Animal cages and rooms are designed with secure containment

Prevention	features to prevent the escape of animals during handling. This reduces the risk of contamination or exposure to biological agents.
Cold Storage for Carcasses	Cold storage, such as freezers or refrigerators, is available for temporarily storing animal carcasses near post-mortem (PM) rooms. This reduces the decay rate and minimizes contamination risks in adjacent work areas.
Durable Floors and Walls	Floors and walls in animal work areas are built to withstand the demands of frequent decontamination and high-pressure cleaning. They are also designed to support the weight of large animals and equipment.
Cage-Washing Areas	Dedicated areas for washing animal cages are provided to ensure thorough cleaning and reduce the risk of contamination. These areas are equipped with necessary cleaning tools and disinfectants.
Containment Devices	While biosafety cabinets (BSCs) and other containment devices are not typically required for BSL-1 labs, they may be employed in cases where procedures present a potential risk of exposure.
Protective Clothing	Personnel are required to wear laboratory coats, gowns, or uniforms to prevent contamination of personal clothing. These garments are removed and stored appropriately within the lab to avoid cross-contamination.
Eye and Face Protection	When procedures present a risk of splashing biological agents, protective eyewear is worn to protect personnel. Eye and face protection is either decontaminated or properly discarded after use.
Training and Supervision	Laboratory supervisors ensure that all personnel are adequately trained on safety protocols, including the proper handling of RG1 materials. Supervisors enforce institutional safety policies to maintain a safe working environment.
Personal Hygiene	Personnel must wash their hands after handling RG1 biological materials and after removing gloves. Long hair is tied back or restrained to avoid accidental contamination, and jewelry is removed or covered.
Clean Workspaces	Laboratory areas are maintained free of clutter and unnecessary materials to ensure that workspaces can be easily decontaminated. Materials not required for ongoing experiments are stored appropriately.
Access Restrictions	Access to laboratory areas is limited to authorized personnel and approved visitors. Signage indicating biosafety level, PPE requirements, and the presence of biological agents is prominently displayed.

4.12. Biosafety level 2

4.12.1. Introduction

BSL-2 is an advancement from Biosafety Level 1, designed for laboratories handling

biological agents with RG2 that are associated with human diseases and pose moderate risks to laboratory personnel and the environment. In comparison to BSL-1, BSL-2 incorporates additional safety measures to mitigate these risks. Laboratory access is restricted to authorized personnel during active procedures to ensure the containment of hazardous agents. Furthermore, all experimental activities that have the potential to produce infectious aerosols or splashes must be conducted within BSCs or similar containment devices to prevent exposure and environmental contamination.

BSL-2 laboratories are designed for working with moderate-risk biological agents that are associated with human disease. According to international standards set by the U.S. CDC and the WHO, BSL-2 practices are essential to protect laboratory personnel, the environment, and public health. In the KSA, compliance with these international guidelines ensures the safe handling of biological agents while addressing local regulations and public health objectives. BSL-2 is particularly critical in areas like public health laboratories, hospitals, and research institutions handling infectious agents such as *hepatitis, influenza,* and *Salmonella*.

4.12.2. Biosafety level 2 guidelines in the KSA for RG2 biological materials

In the KSA, the PHA adopt these international guidelines. BSL-2 laboratories must therefore align with local policies on occupational health, waste management, and environmental protection while implementing global standards on laboratory safety. Key components of BSL-2 include controlled access, the use of BSCs, proper training of personnel, and stringent decontamination procedures to ensure containment of infectious agents.

The following table outlines the essential biosafety features and practices for BSL-2 laboratories, with specific emphasis on international guidelines and their application within the KSA. According to international guidelines, safety measures in BSL-2 laboratories rely on strict operational practices. Further details are included in the table 8 below.

Table 8: Detailed Biosafety Level 2 (BSL-2); Laboratory Facilities and Practices

Feature	Description
Access Control	Laboratory access is restricted to authorized personnel when work is conducted. Doors must remain closed and locked. Signage at the laboratory entrance indicates containment level, PPE requirements, and contact details for emergency personnel.
Training and Supervision	Personnel are trained in handling RG2 agents, including risk assessments, proper use of PPE, and emergency response procedures. Supervisors ensure compliance with biosafety protocols and keep records of training.
Biosafety Cabinets (BSCs)	Class II BSCs are mandatory for procedures generating aerosols or involving infectious agents. These cabinets provide a barrier between personnel and biological materials, ensuring containment and preventing contamination of the laboratory environment.
Hand Hygiene	Handwashing sinks with hands-free controls are available near the exit. Personnel are required to wash their hands thoroughly after handling RG2 materials, removing gloves, and before leaving the laboratory.
Personal Protective Equipment (PPE)	Personnel must wear laboratory coats, gloves, and protective eyewear when handling infectious materials. In procedures where splashes are possible, face shields are recommended. All PPE must be decontaminated or disposed of according to local guidelines.
Handling of Sharps	International standards dictate the use of safety-engineered sharps devices to minimize the risk of injuries. Sharps are immediately disposed of in puncture-resistant containers. Bending or recapping needles is strictly prohibited.
Decontamination	Work surfaces are disinfected after each procedure and any potential spill. Contaminated equipment is autoclaved or chemically disinfected before maintenance. All biological waste is autoclaved before disposal, in line with local regulations.
Waste Management	Biological waste, including cultures, stocks, and contaminated materials, must be decontaminated prior to disposal. Autoclaving and incineration are the preferred methods. Waste is labelled and disposed of according to Saudi waste management regulations.
Personal Clothing and Belongings	Personnel must store personal clothing and belongings separately from PPE and laboratory materials. Changing areas should be provided to ensure that lab coats or other protective clothing do not come into contact with personal items.
Signage and Hazard Communication	Biohazard signs must be posted at the entrance of the laboratory, clearly indicating the risk group, containment level, and required PPE. Emergency contact information and specific entry/exit procedures must also be included.
Vacuum Systems	Vacuum lines are protected with liquid disinfectant traps and in-line HEPA filters to prevent contamination. These filters must be regularly replaced in

	accordance with local and international guidelines.
Mechanical Pipetting	Mouth pipetting is prohibited. Mechanical pipetting devices must be used for all procedures involving liquids, to prevent accidental ingestion or aerosolization of hazardous agents.
Laboratory Surfaces and Furniture	Surfaces, including benchtops and floors, must be non-absorbent, smooth, and easily cleanable. All furniture should be covered with non-porous materials. Carpets and rugs are not allowed in BSL-2 laboratories.
Ventilation and Airflow	The laboratory must maintain an inward airflow to prevent the escape of airborne contaminants. Air is HEPA-filtered and not recirculated. Saudi Arabia's biosafety regulations may require additional verification and monitoring devices for airflow.
Eyewash Stations	Eyewash stations must be located in accessible areas of the laboratory and kept operational at all times, ensuring immediate access in case of chemical or biological exposure.
Spill Management	Spills involving RG2 materials must be contained and disinfected immediately. Spill procedures must be clearly posted in the laboratory, and personnel should be trained to handle emergency decontamination and clean-up.
Emergency Response	Each BSL-2 laboratory must have a detailed emergency response plan that includes procedures for managing exposure incidents, spills, and equipment malfunctions. This plan should comply with both international and Saudi safety regulations.
Pest Control	Windows are fitted with screens to prevent pests from entering the laboratory. Pest control measures should align with Saudi environmental health regulations to prevent contamination.
Respiratory Protection	Respirators may be required for specific procedures depending on risk assessments. The institution must provide a respiratory protection program for personnel, including fit-testing and training in proper use.
Decontamination of Equipment	All laboratory equipment that comes into contact with biological materials must be decontaminated before repair, transport, or disposal. The method of decontamination must comply with international standards and be verified.
Primary Containment Devices	BSCs and other primary containment devices must be regularly inspected and certified. Their use is required for any procedure involving high concentrations or volumes of RG2 materials. Certification must be done annually or according to Saudi regulations.

4.13. Biosafety Level 3

The BSL-3 laboratories are designed to work safely with microorganisms that can cause serious human disease. In addition to biological hazards, employees may be exposed to a range of chemical, radiological, and physical hazards. Therefore, it is incumbent upon each employee to understand the potential dangers inherent in biological diagnosis and research to work in a manner that minimizes risk to them and to others. A BSL-3

laboratory has special engineering and design features. Table 9 below shows the standard and practices, safety equipment, and facility specifications are recommended for BSL-3.

Table 9: Detailed BSL-3; Laboratory Facilities and Practices.

Feature	Description
Leadership responsibilities	**The Laboratory Director / supervisor is responsible for:** **A.** Communicating experimental design through written or verbal instructions. **B.** Ensure the safety of all employees under his/her management. **C.** Ensure that laboratory personnel receive appropriate training regarding their duties, handle and contain hazardous agents and animals, the necessary precautions to prevent exposures, and exposure evaluation procedures. **D.** Ensure his/her employees receive adequate medical surveillance and are provided with the equipment to safely perform their duties and the appropriate record is maintained. **E.** Ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents. **F.** Make sure employees understand the proper post-exposure procedures for any hazardous agents or chemicals where they are at risk of exposure. **G.** Investigate, follow up, and report any potential exposures immediately to the Environmental Health & Safety (EHSRM) Department and the PHA. **H.** Report any significant changes in employee status affecting the use and possession of biological agents or toxins as soon as he/she becomes aware of such changes. **I.** Report all incidents involving exposure, theft, and security breaches to the Environmental Health & Safety (EHSRM) Department. **J.** Maintain an accurate inventory of infectious agents, chemicals, and radioactive materials in his/her laboratories. **K.** Immediately notify EHSRM and the Security Office when an authorized person will terminate employment, transfer to another laboratory, or be reassigned to other areas not requiring BSL-3 access. **L.** Responding to and correcting any nonconformities as a result of inspections/evaluations performed by the Environmental Health and Safety department.
The Environmental Health & Safety (EHSRM) Department responsibilities	**The EHSRM is responsible for:** **A.** Communicating experimental design through written or verbal instructions **B.** Ensuring the safety of all employees under his/her management. **C.** Ensuring that employees are properly trained to safely handle and contain hazardous agents and animals used in the course of their duties. **D.** Ensuring his/her employees receive adequate medical surveillance and are provided with the equipment to safely perform their duties. **E.** Making sure employees understand the proper post-exposure procedures for any hazardous agents or chemicals where they are at risk of exposure. **F.** Investigating, follow up, and reporting any potential exposures immediately

	to the biosafety officer and PHA as appropriate. **G.** Reporting any significant changes in employee status affecting the use and possession of biological agents or toxins as soon as he/she becomes aware of such changes. **H.** Reporting all incidents involving exposure, theft, and security breaches to the head of EHSRM. **I.** Maintaining an accurate inventory of infectious agents, chemicals, and radioactive materials in his/her laboratories. **J.** Immediately notifying EHSRM and the security office when an authorized person will terminate employment, transfer to another laboratory, or be reassigned to other areas not requiring BSL-3 access. **K.** Responding to and correcting any deficiencies as a result of inspections/evaluations performed by the Environmental Health and Safety department.
Authorized Personnel	The names of those currently authorized to work in the BSL-3 are on file at the security department. Only authorized users are permitted to work in the BSL-3. In order to become authorized, potential BSL-3 users must: **I.** Have attended a bloodborne pathogen training session in the past year. **II.** Being certified to handle pathogenic materials at BSL-3 laboratory. **III.** Be vaccinated (according the PHA vaccination list). **IV.** Provide a baseline infectious sample. **V.** Attend a BSL-3 code of practice training session with certified team of BSL-3 **VI.** Read, understand, accept, and adhere to all rules in BSL-3 laboratory code of practice.
Permitted work	The BSL-3 pathogens that may currently be used in the BSL-3 facility are posted at the entrance to the BSL-3 facility. Work that does not require level 3 containment (for example, the manipulation of fixed or non-infectious materials, cultivation of uninfected cell lines, storage of chemicals, and the making of buffers and media) should be conducted elsewhere in external labs whenever possible.
Precautions and Safety Concerns	**A. DO NOT eat, drink, mouth pipette, or smoke in the BSL-3 facility** **B. Sharps and Glass** The major causes of infection of laboratory personnel by human retroviruses are scratches, cuts, and needle-stick injuries. Because of this, the following are not allowed in the BSL-3 facility without proper safety training and precautions and prior authorization from the laboratory manager: • Hypodermic needles • Razors or scalpel blades • Glass pipettes • Pasteur pipettes • Laboratory glassware. All media and solutions used inside the facility are to be contained in plastic bottles ONLY. Plastic and/or disposable alternatives must be used for all glassware items which have such suitable alternatives. Any other items which might pose some danger (e.g., damaged apparatus) must be disposed of

immediately.

C. Precautions to prevent genetic recombination

Each BSL3 research proposal must be reviewed and approved by the BSL3 Facility, The Laboratory Director /laboratory supervisor, and Safety Manager prior to initiation. Special consideration should be given to address the risk and control of genetic recombination that may result from the handling, manipulation, or storage of existing viral agents or human materials. To prevent the chance of genetic recombination, experiments using different types of biological agents (such as different viruses) should be conducted in separate BSCs and stored in separate incubators.

D. PPE

I. Before entering the BSL-3 facility, users must wear a powered air-purifying respirator system at the main entry.

II. BSL-3 users must wear the provided double gloves at all times while in the facility. Users must also wear disposable protective cover all.

III. The first pair of gloves, ones that should be long enough to tuck a disposable protective cover all under the latex, is provided in the main entry. The second pair of gloves is provided inside the facility and should be changed frequently, particularly when the user changes stations (e.g., when the user is moving from a biosafety cabinet to the microscope or the computer or when the user is answering the phone).

IV. Minor skin abrasions should be protected by applying bandages under the first pair of gloves. Users should not work in the BSL-3 laboratory if they have skin abrasions, cuts, or conditions that seriously impair the integrity of the skin. BSL-3 users should also not use petroleum jelly or other agents that weaken glove latex.

E. Serological surveillance

I. Serum samples should be obtained at least once a year and analysed for seroconversion.

II. Results should be reported to individual workers in a timely manner.

III. Procedures that maintain strict confidentiality should be adopted.

IV. All personnel working in the BSL-3 facility are required to sign a consent form to have a serum sample drawn prior to working in the BSL-3 facility and then re-drawn every 6 months thereafter. Confidential testing for HIV status and seroconversion of the drawn samples is at the discretion of the user; that is, the samples may be immediately tested, or they may be stored, untested, after being drawn for testing at a later time, if necessary.

F. Re-evaluation and Safety Meetings

I. Meetings for the re-evaluation of the BSL-3 code of practice will be called on occasion. All authorized BSL-3 facility users must attend these meetings in order to maintain their authorization.

II. All authorized BSL-3 users will be required to attend regular safety meetings and review laboratory policies and procedures. Notification of such meetings

	will be before the meeting by enough time.
Equipment and Supplies	**I.** The BSL-3 facility is equipped with a multitude of items necessary for autonomous work inside the laboratory. There are many laptops and micro-centrifuges, biological safety cabinets, tissue culture incubators, microscopes, computers, refrigerators, -80C freezers, fridges and a liquid nitrogen storage tank that can be found in various areas around the BSL-3 laboratory. Some of this equipment is explained in more detail in the sections to follow. All common-use equipment inside the facility should be treated carefully and used with regard to all other persons authorized to work within the BSL-3 facility. **II.** Equipment must be decontaminated before repair, maintenance, or removal from the laboratory. Spaces between benches, cabinets, and equipment must be accessible for cleaning. Equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually. **III.** Supplies for tissue culture and for other required work inside the BSL-3 facility can be found in various designated areas around the laboratory and on the supply carts near each of the biosafety cabinets. General supplies will be kept stocked by the BSL-3 laboratory manager. The supply cart near each biosafety cabinet must be stocked with all necessary items by the current user. All personal supplies must be removed from the cart when work in the biosafety cabinet is finished. These supplies should be stored in the user's drawer space. **IV.** The laboratory manager should be notified if stocks within the BSL-3 itself are running low or are out, if a BSL-3 user needs a specific item that they cannot find within the facility, or if equipment within the facility needs service or repair. **V.** Each BSL-3 facility user must contact the laboratory manager to review equipment and supply charges in order to determine a method of payment or repayment for the facility's use.

Laboratory access	**I.** Access to the laboratory is strictly limited to authorised personnel who have undergone specialised training (i.e. certification) in handling infectious agents and have been approved to work in the BSL-3 lab. **II.** The main entry to the BSL-3 facility is controlled by a card-key and biometric access system. A light on the door-lock control will change from red to green to indicate that the door may be opened. In the event that two users are entering or exiting the BSL-3 facility at the same time, both users are required to perform the aforementioned procedures. **II.** The internal entry door to the BSL-3 facility is controlled by negative pressure and will not open until the external door is fully closed. The entryway between the two doors is supplied with Personal protective clothing and storage lockers. Authorized users may enter the BSL-3 facility on a 24-hour basis.
Special practices	1. All persons entering the laboratory are advised of the potential hazards and meet specific entry/exit requirements by institutional policies. Only persons whose presence in the facility or laboratory areas is required for scientific or support purposes are authorized to enter. 2. All persons who enter operational laboratory areas are provided with information on signs and symptoms of disease, receive occupational medical services, including medical evaluation, surveillance, and treatment, as appropriate, and are offered available immunizations for agents handled or potentially present in the laboratory. 3. The laboratory supervisor is responsible for ensuring that laboratory personnel demonstrate proficiency in standard microbiological practices and techniques for working with agents requiring BSL-3 containment. 4. A system is established for reporting and documenting near misses, laboratory accidents, exposures, and unanticipated absences due to potential Laboratory-associated infection and for the medical surveillance of potential laboratory-associated illnesses. 5. Incidents resulting from exposure to infectious materials are immediately evaluated per institutional policy. According to institutional policy, all such incidents are reported to the laboratory supervisor, institutional management, and appropriate safety, compliance, and security personnel. Appropriate records are maintained. 6. Biological materials that require BSL-3 containment are placed in a durable, leak-proof, sealed primary container and then enclosed in a non-breakable, sealed secondary container before removal from the laboratory. Once removed, the primary container is opened within a BSC in BSL-3 containment unless a validated inactivation method is used. The inactivation method is documented in-house, and viability testing data is used to support the method. 7. All procedures involving the manipulation of infectious materials are conducted within a BSC or other physical containment device when possible. No work with open vessels is conducted on the bench. Performing a procedure within a BSC or other physical containment device is impossible. In that case, a combination of personal protective equipment and other administrative and/or engineering controls, such as centrifuge safety cups or sealed rotors, are used based on a risk assessment. Loading and unloading of the rotors and centrifuge safety cups occur in the BSC or another containment device. 8. Laboratory equipment is routinely decontaminated after spills, splashes, or

	other potential contamination and before repair, maintenance, or removal from the laboratory: 9. Equipment or material damaged by high temperatures or steam is decontaminated using an effective and verified method, such as a gaseous or vapor. 10. A method for decontaminating all laboratory waste is available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, or other validated decontamination method). 11. Decontamination of the entire laboratory is considered when there has been gross contamination of the space, significant changes in laboratory usage, major renovations, or maintenance shutdowns. The appropriate materials and methods are selected based on a risk assessment. 12. Decontamination processes are verified on a routine basis.
Laboratory facilities	1. The BSL-3 Lab is separated from areas that are open to unrestricted traffic flow within the building. 2. The BSL3 Lab access is restricted. The BSL-3 Lab doors are lockable in accordance with institutional policies. 3. Access to the BSL-3 Lab is through two consecutive self-closing doors. A clothing change room and/or an anteroom may be included in the passageway between the two self-closing doors. 4. Laboratories have a sink for handwashing. The sink is hands-free or automatically operated and should be located near the exit door. If a laboratory suite is segregated into different zones, a sink is also available for handwashing in each zone. 5. An eyewash station is readily available in the BSL-3 Lab. 6. The BSL-3 Lab is designed, constructed, and maintained to facilitate cleaning, decontamination, and housekeeping. The following specifications are considered: - Carpets and rugs are not permitted. - Spaces between benches, cabinets, and equipment are accessible for cleaning. - Seams, floors, walls, and ceiling surfaces are sealed. Spaces around doors and ventilation openings are capable of being sealed to facilitate space decontamination. - Floors are slip-resistant, impervious to liquids, and resistant to chemicals. - Flooring is seamless, sealed, or poured with integral cove bases. 7. BSL-3 Lab furniture can support anticipated loads and uses; which includes: - Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. - Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant. 8. All windows in the laboratory are sealed. 9. Illumination is adequate for all activities and avoids reflections and glare that could impede vision. 10. Vacuum lines in use are protected with liquid disinfectant traps and in-line HEPA filters or their equivalent. Filters are replaced as needed or are on a replacement schedule determined by a risk assessment. Vacuum lines not

	protected as described are capped. The placement of an additional HEPA filter immediately prior to a central vacuum pump is considered. 11. A ducted mechanical air ventilation system is required. This system provides sustained directional airflow by drawing air into the laboratory from "clean" areas toward "potentially contaminated" areas. The laboratory is designed such that under failure conditions, the airflow will not be reversed at the containment barrier. This includes: - A visual monitoring device that confirms directional airflow is provided at the laboratory entry. Audible alarms to notify personnel of airflow disruption are considered. - The laboratory exhaust air is not re-circulated to any other area in the building. - The laboratory exhaust air is dispersed away from occupied areas and from building air intake locations or the exhaust air is HEPA filtered. 12. BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness. The BSL-3 BSCs should be: - Installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, heavily travelled laboratory areas, and other possible airflow disruptions. - Connected to the laboratory exhaust system by either a canopy connection (Class IIA only) or directly exhausted to the outside through a hard connection (Class IIB, IIC, or III). Class IIA or IIC BSC exhaust can be safely recirculated back into the laboratory environment if no volatile toxic chemicals are used in the cabinet. - Certified at least annually to ensure correct performance, or as specified. 13. Equipment that may produce infectious aerosols is used within primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters are tested annually and replaced as needed. 14. The facility is constructed to allow decontamination of the entire laboratory when there has been gross contamination of the space, significant changes in usage, major renovations, or maintenance shutdowns. The selection of the appropriate materials and methods used to decontaminate the laboratory is based on the risk assessment. Facility design consideration is given to means of decontaminating large pieces of equipment before removal from the laboratory. 15. Based on the RA and applicable PHA guidelines, enhanced environmental and personal protection may be necessary. These laboratory enhancements may include one or more of the following: - An anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities - Gas-tight dampers to facilitate laboratory isolation - Final HEPA filtration of the laboratory exhaust air - Laboratory effluent decontamination - Containment of other piped services - Advanced access control devices, such as biometrics.

	16. When present, HEPA filter housings have gas-tight isolation dampers, decontamination ports, and/or bag-in/bag-out (with appropriate decontamination procedures) capability. All HEPA filters are located as near as practicable to the laboratory to minimize the length of potentially contaminated ductwork. The HEPA filter housings allow for leak testing of each filter and assembly. The filters and housings are certified at least annually. 17. The BSL-3 facility design, operational parameters, and procedures are verified and documented prior to operation. Facilities are tested annually or after significant modification to ensure operational parameters are met. Operational experience modifies verification criteria as necessary. 18. Appropriate communication systems (e.g., voice, fax, CCTV cameras, and computer) are provided between the laboratory and the outside. Provisions for emergency communication and emergency access or egress are developed and implemented.

4.14. Biosafety level 4

The PHA upholds stringent biosafety and biosecurity standards to mitigate risks posed by high-consequence pathogens. These BSL-4 guidelines were developed to support the safe handling, containment, and decontamination of life-threatening biological agents that may cause severe human or animal disease, and for which effective treatment options are often unavailable. The PHA's dedication to biosafety reflects not only a commitment to public health but also to global standards for laboratory safety and biosecurity, aligning with regulations from institutions such as the WHO, CDC, and other international bodies.

The evolution of biosafety measures has been shaped by lessons from LAIs and global efforts to control infectious diseases. Historical data reveal the impact of containment failures, which have underscored the critical need for strict biosafety protocols. The establishment of the four-tier Biosafety Levels system reflects a continuous effort to manage risks posed by various classes of pathogens. BSL-4 laboratories, representing the highest containment level, incorporate advanced engineering controls, facility design, and stringent operational practices to safeguard personnel, prevent accidental release, and control potential aerosolized transmission. The following standard and practices, safety equipment, and facility specifications are recommended for BSL-3.

The PHA emphasizes a culture of biosafety that prioritizes stringent compliance, routine

training, and an integrated response framework. The guidelines outline essential BSL-4 practices, such as full-body positive-pressure suits, HEPA-filtered ventilation systems, rigorous waste management protocols, and regular drills to prepare personnel for emergency scenarios. These measures ensure that BSL-4 facilities operate under the highest containment standards, safeguarding laboratory personnel and the surrounding community. Through strict adherence to these BSL-4 guidelines, the PHA advances national efforts to prevent LAIs and accidental or intentional release of pathogens, reinforcing Saudi Arabia's role in the global biosecurity network. The following standard and practices, safety equipment, and facility specifications are recommended for BSL-4. According to international guidelines, safety measures in BSL-4 laboratories rely on strict operational practices. Further details are presented in the table 10 below.

Table 10: Detailed BSL-4; Laboratory Facilities and Practices.

Feature	Description
Access Control	1. Only personnel with specialized training and clearance are granted access to BSL-4 laboratories. 2. Entry and exit procedures are meticulously controlled, including interlocked doors and decontamination showers. 3. Regular risk assessments ensure that only those fully competent in handling high-risk pathogens operate within the facility. 4. Access records are rigorously maintained and reviewed for compliance.
Training and Competency	1. Personnel must complete extensive biosafety training, encompassing pathogen handling, PPE usage, and emergency response. 2. Training program is a critical component of the safety culture at BSL-4 facilities, reinforcing skills necessary to prevent accidental exposures. 3. Competency is verified through regular assessments and re-certifications.
Risk Assessment and Management	1. BSL-4 facilities conduct comprehensive risk assessments to evaluate potential hazards associated with high-risk pathogens. 2. Assessments include identification of pathogen characteristics, modes of transmission, and potential exposure scenarios. 3. Tailored containment strategies are implemented and reviewed periodically to ensure they address emerging risks effectively.
PPE	1. Full-body positive-pressure suits equipped with HEPA-filtered air supply are mandatory in BSL-4 Suit Laboratories.

	2. These suits protect personnel from exposure to airborne pathogens and are rigorously inspected for integrity before each use. 3. PPE protocols include double gloves and eye protection, which are selected based on specific risk assessments with certain pathogens might need further protection.
Primary Containment - Safety Equipment	1. All pathogen handling in BSL-4 laboratories occurs within Class III Biological Safety Cabinets (BSCs) or while personnel wear positive-pressure suits in Suit Laboratories. 2. Primary containment devices are equipped with dual HEPA filters and airtight seals to prevent pathogen escape. 3. Maintenance of these devices is conducted routinely to ensure optimal functionality and containment.
Laboratory Facilities - Secondary Containment	1. BSL-4 facilities are isolated with advanced containment barriers, including inward airflow systems, HEPA-filtered exhausts, and airlock mechanisms. 2. The laboratory structure is designed to withstand internal pressure changes and prevent airborne pathogens from escaping. 3. Backup power systems ensure uninterrupted containment even during emergencies.
Decontamination and Waste Management	1. Decontamination protocols in BSL-4 laboratories are stringent, with waste materials subjected to autoclaving or chemical disinfection before disposal. 2. Surface and equipment decontamination follow a structured schedule to minimize contamination risks. 3. Solid and liquid waste disposal meets international standards, ensuring no hazardous materials exit the containment zone.
Emergency Response and Spill Management	1. Detailed emergency response protocols address potential spills, containment breaches, and equipment failures. 2. Personnel participate in regular emergency drills, and response procedures include immediate containment, area lockdown, and decontamination. 3. All protocols are aligned with national and international guidelines to ensure effective crisis management and updated regularly.
Documentation and Record-Keeping	1. All BSL-4 operations, including access records, training logs, waste management, and decontamination cycles, are meticulously documented. 2. Records are reviewed periodically for compliance with biosafety standards and are essential for auditing and accountability. 3. Documentation supports a transparent and traceable safety program within BSL-4 facilities.

4.15. Work Practices

The implementation of safe work practices is essential for protecting individuals from exposure to regulated materials and ensuring these materials remain contained. Basic microbiological laboratory practices are the cornerstone of all safety procedures involving biological materials. Within containment zones where these materials are manipulated or stored, safe practices include the correct use and maintenance of containment systems, biosafety equipment (e.g., BSCs and centrifuges), as well as maintaining an organized workspace (e.g., cleanliness, minimizing clutter) to promote effective containment and safety. The work practices for BSL-2, BSL-3 and BSL-4 (human and agriculture) are represented in table 11. The following table shows symbols and combinations.

Table 11: Work practices for BSL-2, BSL-3 and BSL-4.

Work practice	BSL2	BSL2-Ag	BSL3	BSL3-Ag	BSL4
Procedures to be followed to prevent personnel exposure to regulated materials and the spread of contamination during tasks.	✓	✓	✓	✓	✓
Traffic and workflow patterns are to be established and followed to prevent the spread of contamination.	✓	✓	✓	✓	✓
The containment zone is to be kept clean, and the presence of the following is to be minimized: a) obstructions, b) materials that are in excess or not required, and c) items that cannot be easily decontaminated.	✓	✓	✓	✓	✓
Contact of the face or mucous membranes with items contaminated or potentially contaminated with regulated materials to be prevented.	✓	✓	✓	✓	✓
Hair that may become contaminated when working in the containment zone must be restrained or covered.	✓	✓	✓	✓	✓
Sharp and glass objects are to be strictly limited and avoided when suitable alternatives can be used.	✓	✓	✓	✓	✓
Use of needles and syringes is to be strictly limited. Bending, sharing, re-capping, or removing needles from syringes to be avoided	✓	✓	✓	✓	✓

and, if necessary, performed only as specified in SOPs					
Verification of small in-line filter assemblies associated with vacuum pump systems to be performed at a frequency based on use.	✓	✓	✓	✓	✓
Verification of primary containment devices to be performed at a frequency based on use.	✓	✓	✓	✓	✓
The operation of containment and life safety systems is to be verified daily.					✓
Integrity of positive-pressure suits to be verified at a frequency determined by an LRA.					✓
Personnel to doff activity-specific **PPE** to minimize contamination of the skin, hair, and personal clothing (where worn) after completing work activities and when **PPE** may have become contaminated.	✓	✓	✓	✓	✓
Primary containers of regulated materials may be opened only at the containment level to which the PHAL and the SFDA have assigned the material and activities.	✓	✓	✓	✓	✓
Primary containers of regulated materials removed from the containment zone are to be stored in a labelled, leak-proof, impact-resistant secondary container and kept either in locked storage equipment or within an area with limited access.	✓	✓			
Primary containers of regulated materials are removed from the containment zone to be stored in a labelled, leak-proof, impact-resistant secondary container. They are kept in locked storage equipment and within an area with limited access.	**P**	**P**	✓	✓	
Security sensitive Biological Agent`s (SSBAs') primary containers must be removed from the SSBA zone and stored in a labelled, leak-proof, impact-resistant secondary container. The secondary container must be kept in locked, non-movable storage equipment.	**S**	**S**	✓	✓	
RG4-regulated materials are to be stored within the containment zone.					✓
Regulated materials to be inactivated with a validated and routinely verified method before removal from the containment zone for use at a lower level.			✓	✓	✓
Procedures must be established to prevent a leak, drop, spill, or similar event during Storage or the movement of regulated materials.	✓	✓	✓	✓	✓

A BSC or other primary containment device to be used for activities with open vessels, based on the risks associated with a) the inherent characteristics of the regulated material, b) the potential to produce infectious aerosols or aerosolized toxins, c) the handling of high concentrations of regulated materials; and d) the handling of large volumes of regulated materials. [Not required when inoculating or collecting samples from regulated animals housed in an animal cubicle.]	✓	✓			
All activities involving open vessels of regulated materials are to be performed in a BSC or other primary containment device. [Not required when inoculating or collecting samples from regulated animals housed in an animal cubicle.]			✓	✓	✓
BSCs and other primary containment devices must be located and operated to minimize airflow disruption.	✓	✓	✓	✓	✓
Centrifugation of regulated materials that are primarily infectious or transmitted by inhalation is to be carried out in sealed safety cups, or rotors unloaded using a mechanism that prevents release.	✓	✓			
Centrifugation of regulated materials to be carried out in sealed safety cups or rotors unloaded in a BSC or other primary containment device using a mechanism that prevents their release.	**P**	**P**	✓	✓	✓
Large scale production and processing of regulated materials to be performed within process equipment, a closed system, or other primary containment device. **[Not required** for CL2 SA zones.]	•		✓		✓
Collecting samples, adding materials, or transferring fluids during large scale activities to be performed to prevent the release of regulated materials. [**Not required** for **CL2** SA zones.]	•		✓		✓
A mechanism is needed to prevent, detect, and respond to pest control issues.	✓	✓	✓	✓	✓
A mechanism is to be implemented to maintain water seals in drainage traps.			✓	✓	✓

5. Biosecurity

5.1. Introduction

Biosafety and biosecurity concepts work together, often enhancing one another. Effective biosafety programs in laboratories frequently fulfil key security requirements for biological materials, and vice versa. However, it is crucial to distinguish between these two concepts, especially in environments dealing with infectious and hazardous materials or toxins.

Biosafety refers to the principles, technologies, and practices designed to prevent accidental exposure to harmful pathogens or toxins. In contrast, **biosecurity** encompasses measures aimed at preventing the loss, theft, misuse, diversion, or intentional release of infectious substances. This distinction aligns with definitions provided by the WHO and the ABSA.

Both biosafety and biosecurity are built upon risk assessment and performance-based approaches, enabling facilities to comply with regulatory standards through both physical and operational measures. Conducting risk assessments within these programs helps determine appropriate control levels. Biosafety focuses on implementing proper laboratory procedures and practices to prevent exposure and occupationally acquired infections. Meanwhile, biosecurity is centred on protecting biological materials and sensitive information from unauthorized access.

Additionally, both programs place significant emphasis on evaluating personnel qualifications. The biosafety program ensures employees are trained and skilled to perform their tasks safely, supported by technical expertise documentation. Staff are expected to follow correct materials management procedures and display professional responsibility for handling research materials responsibly.

Biosafety protocols require restricting laboratory access during active work, whereas biosecurity measures ensure that access to both laboratory and biological materials is controlled and monitored. Effective management and tracking of inventories for biological stocks and sensitive materials are essential components of both programs.

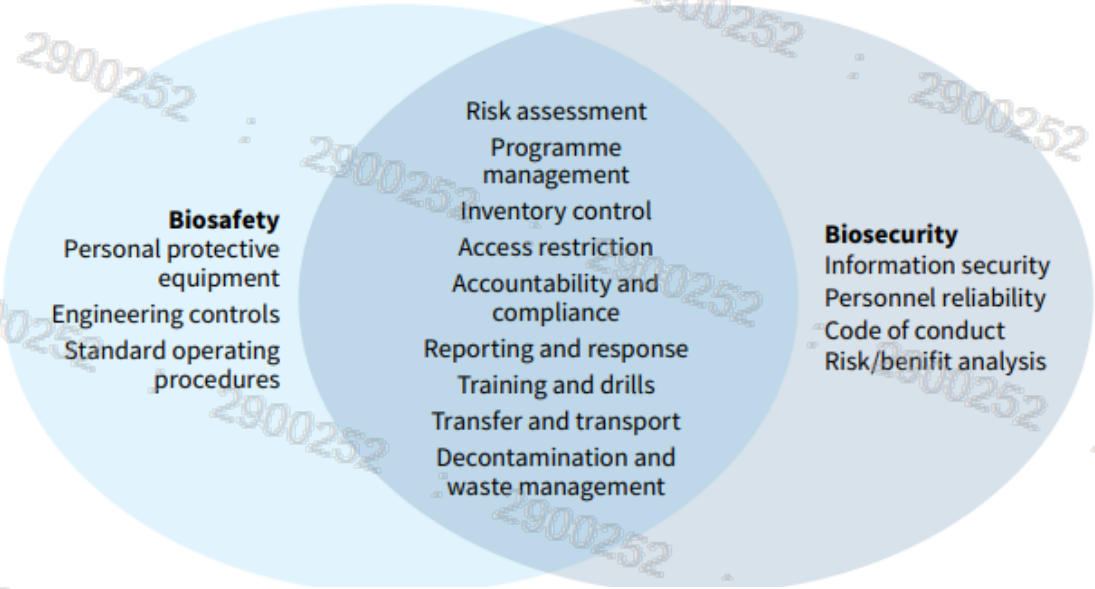
For biosafety, shipping infectious materials includes appropriate packaging, containment, and transportation protocols. On the other hand, biosecurity emphasizes that transfers of such materials are controlled, tracked, and documented according to their risk level. Both programs must work collaboratively with laboratory personnel to establish practices and procedures that meet biosafety and biosecurity goals without obstructing research, clinical, or diagnostic activities.

The primary gal of biosecurity measures is to protect biological resources from incidents such as theft, misuse, or unauthorized access. Such incidents, if involving high-risk materials, could result in severe or even catastrophic consequences, including economic impacts and heightened public fear and concern.

Laboratory biosecurity is deeply intertwined with biosafety, complementing each other to ensure secure and safe lab operations (figure 6). The term "biological risk management" represents the integrated biosafety and biosecurity efforts at both institutional and national scales. The risk assessment framework in the laboratory biosafety manual aids institutions in creating and implementing effective, region-specific measures to manage biosafety risks associated with working with biological agents.

These guidelines aim to guide the establishment of a robust biosecurity program. It is important to note that in this context, the term "biosecurity" differs from "agricultural biosecurity," which focuses on protecting livestock and the food supply from disease through preventive measures that limit the entry and spread of pathogens within animal or plant populations.

Figure 6. Spectrum of biological risk management: examples of overlapping elements of biosafety and laboratory biosecurity.



5.2. Risk management approach to laboratory biosecurity

It involves the following steps:

1. Identifying the agents that require biosecurity measures to prevent loss, theft, diversion, or intentional misuse
2. It ensures that the protective measures and associated costs are proportionate to the level of risk posed by different agents and toxins. This requires identifying and prioritizing the risks and allocating resources accordingly.

The program's components should be tailored to the specific site and founded on the organization's assessment of threats and vulnerabilities. Implementing biosecurity program elements should be determined based on risk assessment needs. These components should not be seen as "minimum requirements" or "minimum standards" for a biosecurity program. The biosecurity program should be incorporated into relevant institutional policies and plans.

5.3. Biosecurity risk assessment

The initial step in developing a biosecurity program involves conducting an appropriate risk assessment. This assessment should be reviewed annually and updated to reflect any changes, such as the introduction of new pathogens or facility expansions. The complexity of the program should be proportional to the level of risk associated with the pathogens or toxins present.

5.3.1. The key elements included in a biosecurity risk assessment are as follows:

1. Identify and prioritize assets:

The first step in the biosecurity risk assessment is identifying all relevant assets. Assets include pathogens, infectious material, toxins, equipment, data, and personnel. It is essential to maintain an inventory of assets, especially higher-risk material (i.e., security-sensitive biological agents [SSBAs], Risk Group 3 [RG3], and Risk Group 4 [RG4]). Best practice suggests that other factors, such as the concentration, quantity, and state of the material, should also be included in the inventory. This helps to determine the potential for intentional misuse of pathogens or toxins.

The impact of loss on facility operations should also be considered. Pathogens and toxins with dual-use potential (i.e., where the inherent qualities of a pathogen or toxin allow for its use in legitimate scientific applications, as well as for intentional and malicious misuse as a biological weapon to cause disease in humans or animals) are of the most significant biosecurity concern. These human pathogens and toxins have been determined to have potential misuse. They are identified as "prescribed pathogens," "prescribed toxins," and SSBAs.

Assets are prioritized according to their biosecurity risk based on several critical factors, including the consequences of malicious use, the material's ease of use, and the impact of material loss on the facility.

5.3.2. Access control and monitoring:

Identifying threats and vulnerabilities from both internal (insider threat) and external (outsider threats) sources. Potential threats include disgruntled employee, former staff, terrorist groups, and opportunistic criminals. A comprehensive risk assessment can help evaluate and prevent access, damage, or misuse of assets.

5.3.3. Determining risk levels and mitigation strategies:

The biosecurity risk level is determined by analysing the risk associated with each asset or group of assets with similar characteristics combined with each threat. The highest biosecurity risks are events with the most significant consequences, even if they are unlikely, followed by events with moderate consequences that are more likely to occur.

Risks can be mitigated using physical security measures, enhanced personnel screening, a clear accountability framework for pathogens and toxins, effective incident and emergency response protocols, and information security measures. Assets can be managed according to risk levels as follows:

a. Assets at low risk of unauthorized access need minimal management and control measures.
b. Assets at medium or high risk of unauthorized access require moderate management and risk mitigation.
c. Assets that are at very high risk require extensive management and controls.

Senior management within the organization is responsible for determining the acceptable levels of risk for identified scenarios (risk tolerance) and the resources available to mitigate the risks. Possible mitigation strategies for identified vulnerabilities should be outlined, and preventive measures should be implemented to counter the identified risk. It may be found that identified risks are already controlled through existing biosafety and biosecurity measures. Any risks that have not been mitigated or deemed acceptable should be documented along with an explanation of the decision. Mitigation strategies developed to protect against unacceptable risks can be used to create a biosecurity plan that will complement the biosafety program.

5.3.4. Risk statements and risk registers

It is recommended to develop risk statements and risk registers, as they are crucial tools used in the risk management process. Risk statements help to identify and document biosecurity risks during the risk assessment, providing an accurate picture of the potential negative impacts of specific events. A risk statement can be structured to read: "If [event] occurs, the consequences could result in [negative impact]." A risk register is a project management tool that documents the results of risk analysis and response planning. It includes a list of all identified risk statements and their risk levels in a format that can be quickly reviewed, modified, and updated.

5.4. Biosafety and Biosecurity Plan

A biosecurity plan must be available in any facility handling infectious materials or toxin and should be developed based on the outcomes of the risk assessment, addressing internal and external threats. Collaboration with facility staff members, including scientific directors, principal investigators, laboratory personnel, administrators,

information technologists, occupational health and safety personnel, security personnel, and engineering staff is crucial. It's also essential to involve personnel responsible for the overall facility security, as specific biosecurity measures may already be part of an existing security program. Involving local law enforcement may also be appropriate. Regular reviews and updates of the biosecurity plan are essential to ensure the accuracy and practicality of the measures, policies, and procedures. Depending on the risks associated with a pathogen or toxin, management can designate an individual who is accountable, knowledgeable, and responsible for the security of the materials under their control.

5.5. The physical security

The physical security components of a biosecurity plan aim to prevent unauthorized access to sensitive materials and assets, safeguarding against external threats. Adequate physical security measures are crucial to reduce the risk of unauthorized entry into containment zones and access to infectious materials or toxins in the facility. A thorough assessment of the premises, building, containment zones, and storage areas should be conducted to evaluate security measures. Security barriers, such as locked doors and windows, controlled access systems, and secure containers, should be utilized to enhance the security of a containment zone and limit access to authorized personnel only. These barriers can be implemented at various levels, including property perimeter, building, containment zone, and pathogen- or toxin-specific levels. Other aspects to consider include access points to the containment zone, access control measures, methods to detect unauthorized access, additional security barriers, and upkeep of security barriers. Following a biosecurity risk assessment, a facility-specific biosecurity plan should be developed, implemented, regularly reviewed, and improved as necessary. Integrating the components of the biosecurity plan into the overall biosafety program can minimize duplication and establish a more effective biosafety management system. The biosecurity plan should cover several elements and, at minimum, illustrate that the risks associated with each component have been evaluated. It should also outline the strategies in place or newly implemented to mitigate these risks.

5.6. Personnel Suitability and Reliability

When hiring, managers should screen candidates to ensure they have the necessary credentials, skills, and personal traits for the job and are the best fit before accessing pathogens, toxins, or other sensitive assets. While academic credentials and prior experience may demonstrate scientific ability, they only sometimes measure an individual's suitability to handle or access pathogens and toxins. Therefore, it is essential to establish policies and procedures for personnel suitability and reliability to address the risk of potential insider threats.

Clear and documented criteria for training, experience, competency, and other suitability requirements for personnel with access to pathogens or toxins should be in place. Pre-appointment screening of employees is crucial in determining personnel suitability, and procedures may also be needed to approve and grant visitor access. Additionally, an ongoing reliability assessment program should verify that an individual's access to pathogens and toxins is justified based on the established suitability criteria.

The ongoing assessment program also aims to identify insider threats from personnel previously deemed suitable for access. Factors that may affect an employee's ability to perform their duties safely and securely include participation in criminal activities, immigration or financial concerns, significant changes in behaviour, attitudes, demeanour, or actions (such as withdrawal, anger, unexplained absences, signs of substance use), or willful non-compliance with policies and legislation. Implementing programs to identify and assist employees experiencing problems may help reduce these risks.

5.7. The materials (or forms of materials) are subject to accountability measures

Pathogens and toxins accountability procedures are implemented to track and document regulated infectious materials in long-term Storage (i.e., more than 30 days) within the containment zone or organization. The purpose is to make these materials easily accessible when needed and promptly identify any missing items. Establishing effective inventory measures for pathogens and toxins can help prevent insider threats. The level of detail of the inventory system is determined based on the associated risks of the pathogens, toxins, and other infectious materials being handled and stored. For example, when high-risk pathogens like SSBAs, RG3, or RG4 are in long-term Storage,

the inventory must be more detailed than for Risk Group 2 (RG2) pathogens. This ensures that specific samples of pathogens, toxins, and other regulated infectious materials can be easily identified, located, or promptly identified if missing or stolen. Additionally, the biosecurity plan should include provisions for maintaining accountability during shipping, receiving, monitoring, and Storage of packages containing pathogens, toxins, and regulated infectious materials.

All policies for the transportation of biological materials should include accountability measures for moving materials within an institution (e.g., between laboratories, during shipping and receiving activities) and outside the facility (e.g., between institutions or locations). Transport policies should address the need for appropriate Documentation, material accountability, and control procedures for pathogens in transit between locations. Transport security measures should be instituted to ensure proper authorizations have been received and adequate communication between facilities has occurred before, during, and after transporting pathogens or other potentially hazardous biological materials. Personnel should be adequately trained and familiar with regulatory and institutional procedures for proper containment, packaging, labelling, Documentation, and transport of biological materials.

5.8. Information security

Policies should be created to manage sensitive information linked to the laboratory biosecurity program. In this context, "sensitive information" refers to data related to the security of pathogens, toxins, or other vital infrastructure details. This could include facility security plans, access codes, new technologies or methods, inventories of agents, and storage sites.

The goal of an information security program is to maintain data integrity, safeguard information from unauthorized access, and ensure confidentiality levels are adequate. Facilities should establish rules for correctly identifying, marking, handling, securing, and storing sensitive information, including electronic files and removable media like CDs, external hard drives, and USB flash drives. The information security program should be customized to suit the business environment's requirements, support the organization's mission, and address identified threats. Controlling access to sensitive information is essential.

The information management and security policies should outline how sensitive information is classified, handled, collected, documented, transmitted, accessed, and ultimately destroyed. Controlling access to sensitive information, containment zones, and associated areas is essential to mitigate the risk of unauthorized access by external threats. Information protection should be commensurate with the risk associated with the material and tailored to address the organization's needs and mission while mitigating identified threats.

Clear policies or protocols for basic information technology security, such as strong user passwords, discouraging or limiting the use of unsecured wireless connections, and utilizing a virtual private network (VPN) to communicate between several offices, are general considerations for information security. Develop a policy for electronic files and removable electronic media (e.g., CDs, computer drives). The use and control of mobile electronic devices (e.g., tablets, personal data storage devices) and digital cameras should be considered as vulnerabilities to information security, as they can be easily hidden from sight and are capable of storing or transferring information on removable media that can be stored separately.

5.9. Documentation and reporting requirements

A biosecurity plan should include effective documentation system that have incident and emergency response elements integrated into the overall biosafety program. This can incorporate these elements into the ERP and establishing a mechanism to remove unauthorized individuals. It is crucial to report all biosecurity-related incidents, such as missing pathogens or toxins, unauthorized entry or access to sensitive information, and loss of keys or passwords. Reporting these incidents to the biological safety officer (BSO) for proper documentation, investigation, and necessary reporting is encouraged. Depending on the nature of the incident, the BSO may need to report it to local law enforcement and may be obliged to report it to the PHA based on licensing conditions. It is also essential to maintain records, update them at specified intervals, and adhere to timelines for record maintenance.

5.10. Accident, Injury, and Incident Response Plans

Laboratory security policies should address situations where emergency responders or public safety personnel need to enter the facility due to accidents, injuries, safety issues,

or security threats. In an emergency, human life, the safety and health of lab employees, and the surrounding community should be the top priority over biosecurity concerns. Facilities must work with medical, fire, police, and other emergency officials to prepare for emergency and security breach responses.

SOPs should be created to reduce the risk of responding personnel being exposed to potentially hazardous biological materials. Lab emergency response plans should be coordinated with relevant facility-wide or site-specific security plans. These plans should also cover events like bomb threats, natural disasters, severe weather, power outages, and other emergencies that could pose security threats. Reporting and communication are crucial parts of a biosecurity program, so it's essential to establish a "chain of notification" before an actual event occurs.

5.11. Security Clearance

Security clearance is crucial for screening employees to minimize the risk of insider threats in Canadian facilities authorized to handle activities involving severe human or animal pathogens. Individuals must possess a valid security clearance to access facility areas where these activities are authorized. This clearance involves thorough background and credit checks using law enforcement and intelligence databases. Those without this clearance can only access authorized areas of a facility.

5.12. Laboratory Occupational health and immunoprophylaxis

Laboratory workers are at high risk of exposure to hazardous pathogens and toxins in clinical and biomedical research. Occupational health and immunoprophylaxis programs' main goals are to ensure the safety of laboratory workers and the provision of required medical support services.

The program covers various aspects, including risk assessment, vaccination, training, and health surveillance. Building a solid collaboration among healthcare providers, safety specialists, principal investigators, employers, and workplace personnel is crucial. Organizations require regulatory compliance, OSHA, and risk assessment to ensure all personnel receive occupational care. The organizations should provide appropriate safety training tailored to the potential risk of exposure to hazardous materials. The LOHI program is updated, evaluated, and reinforced annually based on changes in job responsibilities and after recognized and suspected exposures and an

annual review of occupational injury and illness reports to revise exposure prevention strategies through raising personnel awareness of biohazards in the workplace and closely monitoring affected employees.

5.13. Laboratory Standard Operational Procedures

Laboratory SOPs should include a printed summary of the recommended medical response to specific exposures in laboratory SOPs, a guide for immediate response in the workplace, and provide the injured worker with the necessary information for the treating facility.

The medical provider's description of the injury should include the potential infectious agent and the mechanism and route of exposure (percutaneous, splash to mucous membranes or skin, aerosol, etc.). It is crucial to include the time of the incident, personal protective equipment used at the time of the injury, and prior first aid provided (e.g., nature and duration of cleaning and other aid, time that lapsed from exposure to treatment). The worker should provide a medical history relevant to the risk of infection or treatment complications. First aid should be repeated if the initial adequacy is in question.

5.14. Risk Assessment and Management

1. Conduct comprehensive risk assessments to identify potential hazards in the laboratory. Vigilance for potential occupational exposures in unexplained illnesses among workers or visitors in biohazardous worksites
2. Implement safety measures such as engineering controls, administrative controls, and PPE to mitigate identified risks.
3. Develop and maintain a laboratory safety manual outlining SOPs for handling hazardous materials.

5.15. Personal Protective Equipment

1. Ensure laboratory workers can access and use appropriate PPE, including gloves, lab coats, safety goggles, and face shields.
2. Provide training on the proper use, maintenance, and disposal of PPE.

5.16. Training and Education

1. Offer regular training on laboratory safety practices, including chemical, biological, and radiological safety.
2. Provide training on handling infectious agents and procedures for spill response and emergency situations.
3. The laboratory workers should be aware of the potential hazards of material and

injuries associated with their work and medical care for exposure to human pathogens

4. Employees and healthcare providers should receive regular emergency medical support training emphasizing the importance of adequacy and timeliness of wound cleansing or other responses after exposure to prevent infection.

5.17. Health Surveillance

1. Conduct baseline health assessments for new laboratory employees.
2. Implement ongoing health surveillance to monitor the health of laboratory workers and detect early signs of occupational illnesses.
3. Provide medical surveillance for those working with particularly hazardous substances or biological agents.

5.18. Ergonomics and Physical Safety

1. Promote ergonomic practices to reduce the risk of musculoskeletal injuries.
2. Ensure safe lifting practices and proper Storage of heavy items to prevent injuries.

5.19. Mental Health and Well-being

1. Create a supportive work environment to reduce stress and promote mental health.
2. Offer access to mental health resources and counselling services.
3. Follow the CDC Recommendations for Immunoprophylaxis.

5.20. Vaccination Programs

Ensure laboratory workers are vaccinated against common and occupationally relevant diseases, including:

5.20.1. Hepatitis B: Essential for workers handling human blood and body fluids.

5.20.2. Influenza: Recommended annually to reduce the risk of flu outbreaks.

5.20.3. Tetanus: Important for those working with animals or potential exposure to soil.

5.20.4. COVID-19: To prevent the spread of the virus within the laboratory.

5.20.5. Measles, Mumps & Rubella (MMR): recommended for clinical laboratory professionals.

5.20.6. Diphtheria-Tetanus-Pertussis vaccine (DTP): recommended for healthcare workers.

5.20.7. Meningococcal conjugate vaccine: (MenACWY) protects against four types of meningococcal bacteria (types A, C, W, and Y).

5.20.8. Pre-Exposure Prophylaxis (PrEP):

1. Protocols for Biohazard Exposures to respond to biohazard exposures, covering first aid, post-exposure prophylaxis, diagnostic tests, and expert

medical evaluation sources. The protocols should be shared with potential healthcare providers, including local hospital emergency departments, following local and national public health recommendations in exceptional cases.

2. The non-immune workers should be informed about risks and provided the vaccination by responsibilities
3. Consider implementation of PrEP for workers at high risk of exposure to specific pathogens, such as those working with HIV.

5.20.9. Post-Exposure Prophylaxis (PEP):

1. The occupational program should establish clear protocols for reporting and managing exposures to infectious agents.
2. During the post-exposure risk assessment and treatment decision process, the clinician should explain the risk to the worker and address all the inquiries
3. After exposure to infectious agents, it is crucial to collect serum specimens at appropriate intervals and over time and practice proper Storage and management of serum samples.
4. The workers should have immediate access to PEP for exposures to specific pathogens, such as HIV or hepatitis B.
5. Seroconversion's serial serological titre and significance should be monitored over the recommended time for each infectious agent.

5.20.10. Health Education and Training: Laboratory workers should know the importance of immunizations and adhere to recommended vaccination schedules. They should also receive information on the risks of occupational infections and preventive measures.

5.20.11. Monitoring and Record Keeping:

1. It is crucial to maintain accurate records of employees' immunization status and for adverse reactions to vaccines and manage them appropriately. The booster doses should be administered promptly as needed.

6. References

1- Biosafety in Microbiological and Biomedical Laboratories, 6th Edition. 2020. Centres for Disease Control and Prevention National Institutes of Health.

2- Canadian Biosafety Handbook 2nd Edition. 2016. Government of Canada. ISBN: 978-1-100-25773-0.

3- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs). Licence: CC BY-NC-SA 3.0 IGO.

4- Pathogen Safety Data sheets. 2024. Government of Canada. https://www.canada.ca/en/public-health/services/laboratory-biosafety biosecurity/pathogen-safety-data-sheets-risk-assessment.html

5- Zuo, K.; Wu, Z.; Zhao, C.; Liu, H. A Historical Study on the Scientific Attribution of Biosafety Risk Assessment in Real Cases of Laboratory-Acquired Infections. Laboratories 2024, 1, 87-102.

6- Laboratory biosecurity guidance. Geneva: World Health Organization; 2024. Licence: CC BY-NC-SA 3.0 IGO.